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Short Communication

THREE-DIMENSION STRUCTURAL CONFIGURATION OF OLR1 PROTEIN REVEALED POLYMORPHISM ASSOCIATED WITH MILK QUALITY IN RIVER BUFFALOES

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ABSTRACT

Gene based variations are consequential only if are in the vicinity or within some functionally significant regions of 3-dimensional encoded protein. Information regarding significant structural attributes of many proteins is underway and based on data of polymorphisms identified in different parts of the genes. Oxidized Low Density Lipoprotein Receptor-1 (OLR1) gene was reported to be significantly associated with high fat content in milk. Oxidized Low Density Lipoprotein Receptor-1 (OLR1) is a key cell surface receptor for oxidized low density lipoprotein. In this study, protein structural attributes of OLR1 were constructed by homology modelling and functionally significant regions were identified, which might have a role in fatty acid synthesis. This OLR1 is a 270-residue protein in bovine carrying 52-amino acid-long signal peptide. A Pro-His substitution was noted at 17th position, which enhanced OLR1 protein hydrophilicity for enhanced attachments. A transmembrane helix was identified between 35-67th residue and another functionally significant variant was identified at position 181, which was part of Domain-3. This domain is a functionally active part of the receptor in fat synthesis. Results of the study, illustrated that mutations in functionally significant parts of the OLR1 protein affect the high fat content in the buffalo milk.

Key words: OLR1 protein, Homology modeling, Mutations, Functional variants, Buffalo.

INTRODUCTION

Among different species of livestock, buffalo stands out as an efficient converter of poor quality roughages into highly valuable products as milk and meat (Kataoka *et al*. 2000). Buffalo contributes about 68% of total milk produced in Pakistan followed by contribution of cattle with 27% and sheep/goat/camel (5%) (Afzal, 2010). Along with increased overall production per animal, quality of buffalo milk is also an important factor making it the most preferred milch species in Pakistan. Buffalo milk is a richer source of fat, which is low in cholesterol. It also contains higher level of calcium as compared to the milk of other species (Han *et al* 2012). This variability in the milk yield and quality has raised the concept about genetic basis of the trait. Animals with higher fat in milk might have some better performing genes. Identify these polymorphisms associated with milk quality would be significant for marker assisted selection programs for breed improvement. OLR1 gene has been reported to affect long chain fatty acid composition of milk. For this purpose, present research was conducted to genetically explore the variations in the OLR1 gene, which is a main contributor in the fat synthesis during milk production (Heyen *et al*. 1999; De Koning *et al*. 2001; Rodriguez-Zas *et al*. 2002; Olsen *et al*. 2002; Awad *et al*. 2010; Schopen *et al*. 2011). Nili Ravi buffalo breed was selected as representative of river buffaloes and blood samples were collected from animals with higher fat %age, which is the proportion of milk, by weight, made up by butterfat (more than 8%). PCR amplification and sequencing of amplicons provided polymorphic sites. Then three-dimensional protein model for OLR1 protein was predicted by Homology Modeling and position of non-synonymous mutations was located. Two variations were found in functionally significant regions: one in signal peptide of the protein and second one in transmembrane helix of the protein. Another variation was located in the Domain-3, which impart its role in fat synthesis during milk production. These amino acid substitutions need to be further investigated on larger population group so that their association with milk quality and yield can be verified. Then these polymorphisms can be selected as genetic markers for selection of animals with superior dairy potentials.

MATERIALS AND METHODS

Sampling Strategy: Animals true representatives of Nili Ravi buffalo breed were selected from different livestock farms as Buffalo Research Institute (BRI) Pattoki and Livestock Experimental Station (LES) Bahadurnagar. Milk of animals was analyzed for fat content and blood sampling was performed only on animals with fat more than 8%. A total of 98 animals were selected and blood was collected in EDTA coated vaccutainer.

DNA extraction, amplification and sequencing: DNA was extracted by organic method of extraction reported by Maryam *et al* (2012). Primers covering exonic regions only were used and amplification was performed by polymerase chain reaction (PCR). DNA sequencing was performed on purified PCR products.

Bioinformatics Analysis: Results of sequencing of 98 Nili Ravi animals were compared with *Bos Taurus* (AC_000162.1) using Multiple Alignment Tool (ClustalW) and total of fifteen SNPs were identified. Firstly, 3 dimensional protein structures were developed by swiss Model (http://swissmodel. expasy.org) indicating all the mutations of the amino acid sequence. Standard models for protein were taken from RCSB-PDB (www.rcsb.org/pdb/ explore. do? structureId). Predicted and reported protein models were then analyzed for location of mutations and their possible role in function of that protein. For this purpose, PyMol (http://www.pymol.org), JMol (http:// jmol.sourceforge.net), Phyre (http://www.sbg.bio.ic.ac.uk/phyre2/phyre2_output), Scooby (http://www.ibi.vu.nl/ programs/scooby) and Predisi (http://www.predisi.de) were used.

RESULTS

For the purpose of analysis of OLR1 protein in river buffaloes, standard three-dimensional protein model of OLR1 was retrieved from RCSB-PDB No-1YXJ (www.rcsb.org/pdb/explore.do?structureId). Mutated sequence OLR1 protein, carrying the identified polymorphisms was developed by homology modelling. The three-dimensional model of protein was then further analyzed for secondary structure information, location of mutation sites, functional significance of mutations, nature of protein and signal peptide cleavage site of protein etc.

Compared with *Bos Taurus*, a total of 15 polymorphisms were identified in Nili Ravi (Table-1). Four were intronic and remaining were found in exonic region. Out of these 11 exonic SNPs, P17H, S57Y, A68G, K89E, R181T were non-synonymous and were changing the amino acid.. Out of these, P17H was in the signal peptide of the protein (Fig-1). S57Y was found in the transmembrane helix identified between 35-67 residues (Fig-2). Residues A68G and K89E were not found in some functionally significant region but R181T was found as part of Domain-3 of the OLR1 protein (Fig-3) which was strongly associated with fat synthesis. OLR1 is a type-II membrane protein. Domain3 (144-256 residue) is associated with Steroidogenesis, which is C-type Lectin domain and is conserved. Location of Domain3 has been mentioned in the structure (Fig-3). Residue-181 was found in this region. This was an Arginine to Threonine change. In figure-4, positions of alpha helices and beta sheets have been mentioned. Alpha helices have been mentioned in green colour and beta sheets are blue in colour.

A 33 residue long transmembrane helix was also identified in the OLR1 molecule by using Phyre2. This helix is between 35-67 amino acids (Fig-2). S57Y was found in this region. This is a Serine to Tyrosine substitution. Both amino acids were polar and hydrophobic, so there was not much expected change in signaling of OLR1. P17H was found to be the most significant variation as far as the function of OLR1 was concerned as this was a non-polar to polar and hydrophobic to hydrophilic substitution, which might have effect on protein signaling.

Table 1. Single Nucleotide Polymorphisms identified in OLR1 gene

SNPs	Position	Wild type	Mutant	Amino acid Substitution
P17H	107080902	C	A	Proline to Histidine
80942C>A	107080942	C	A	Glycine
S57Y	107084380	C	T	Serine to Tyrosine
A68G	107084386	C	G	Alanine to Glycine
84396A>C	107084396	A	C	Leucine
K89E	107084402	A	C	Lycine to Glutamine
84454T>C	107084454	T	C	Leucine
84484A>C	107084484	A	C	Glutamine
84569C>T	107084569	C	T	-
89859T>C	107089859	T	C	-
90332T>C	107090332	T	C	-
90355T>C	107090335	T	C	-
90986G>A	107090986	G	A	Arginine
91007G>A	107091007	G	A	Arginine
R181T	107091125	G	C	Arginine to Threonine

Substitution	Group	Effect
Pro-His	NonPolar-Polar	Hydrophobic- Hydrophilic

MTVDDPKGMKDQLDQKHNGKTAKGFVSSWRWYPAAVTLGVLCLGLLVTVI
LLILQLYQVSDLIKKQQGNITIIQEDILEGQILAQRRSEESAQESQKELKEMIET

Cleavage site

LAIIKLDEKSKKLMELIIRQNLNLQEVLKEAANYSGPCPQDWLWIIEENCYQF
SSGSFNWEKSQENCLSLDAHLLKINSTDELEFIQQMIAHSSFPFWMGLSMRK
PNYSWLWEDGTPLTPHLFRIQGAVSRMYPSGTCAYIQRGIVFAENCILIAFSIC
QKKANLLRAQ

Fig-1. Signal Peptide of *OLR1*

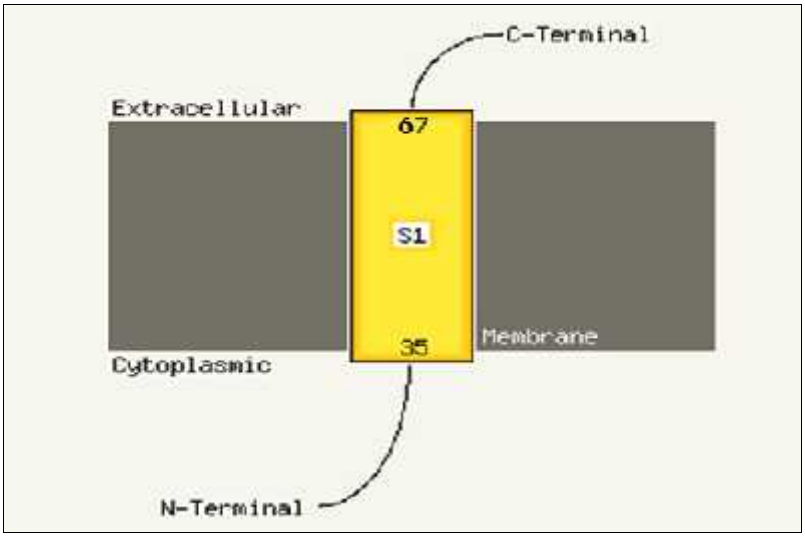

Fig 2. Transmembrane Helix of *OLR1*

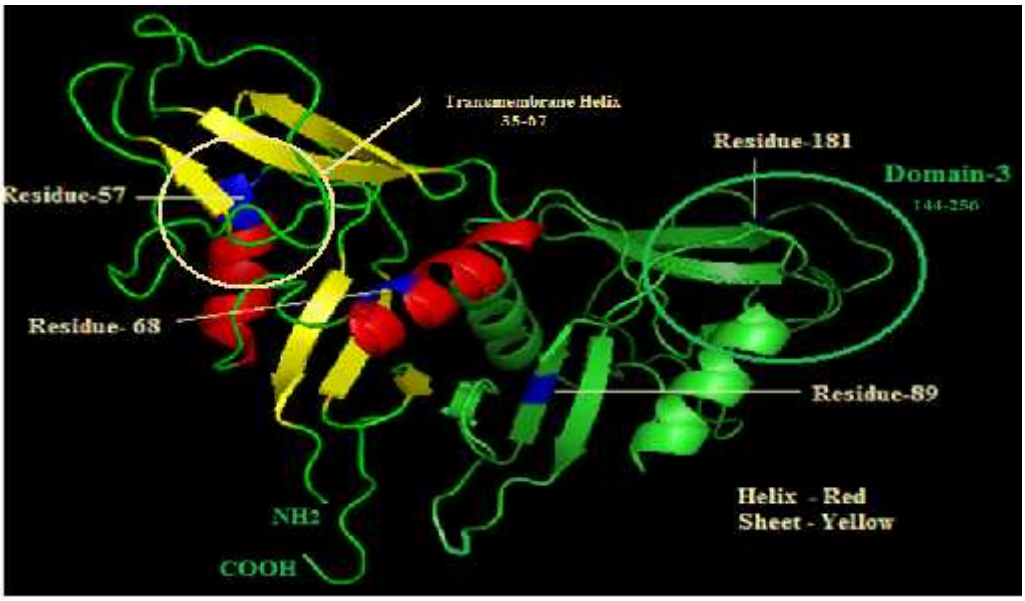

Fig-3. Structural Attributes of *OLR1* Protein

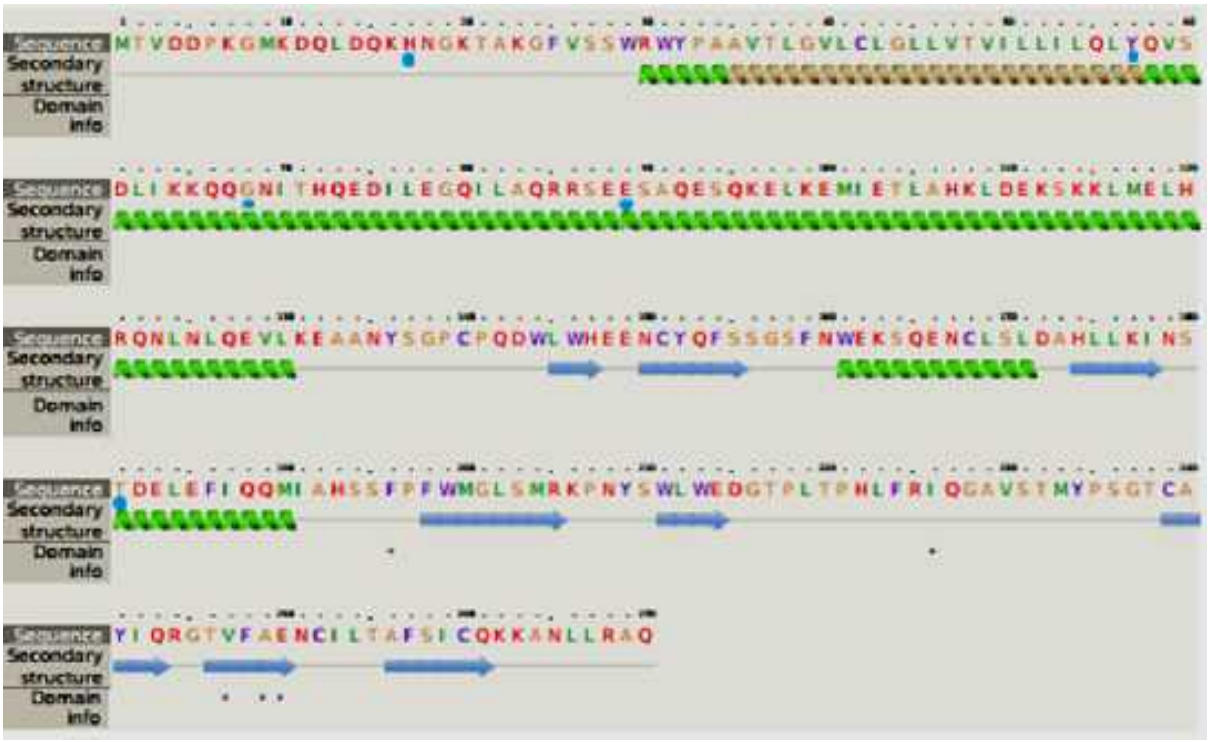

Fig 4. Secondary Structure information of *OLR1*

DISCUSSION

This study was planned to evaluate the effect of candidate genes on the milk fat yield and %age in Nili-Ravi buffalo breed of Pakistan. OLR1 is involved in fatty acid transport and binds and degrades the oxidized form of low-density lipoprotein. Khatib *et al*. (2006) identified OLR1 as a functional and positional candidate gene for milk-fat percentage and milkfat yield, and showed association of a SNP in the 3¢-UTR of OLR1 (OLR1g.8232C>A) with milk-fat percentage and milk-fat yield in a population of North American Holstein cattle. In present study, a total of fifteen polymorphisms were identified. Out of these 15, four were intronic and remaining eleven were exonic. From these eleven polymorphisms, five were synonymous and were not changing any amino acid. Remaining six were non-synonymous. Ratio of transition and transversion is 1.15:1. Chen *et al* (2000) reported that OLR1 is a type-II membrane protein. Domain3 (144-256 residue) is a conservative C-type Lectin domain, which is associated with steroidogenesis. (Kataoka *et al*. 2000, Schennink *et al*. 2009). Polymorphism R181T was found in this region. It is an Arginine to Threonine change. A 33-residue long transmembrane helix was also identified in the OLR1 protein molecule. This helix is between 35-67 amino acids. A Serine to Tyrosine substitution was found in this region. Previously no reports are available on the three-dimensional protein configuration of the OLR1 protein. So this is the first report on the location of identified polymorphisms in the functionally significant regions of the protein and might aid in our understanding about the structure verses function information of OLR1 protein. Identified functional variants can serve as important markers in future breeding programs for selection of superior river buffaloes with better milk fat content.

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