SNP EXPLORATION IN THE OXIDISED LOW DENSITY LIPOPROTEIN RECEPTOR 1 (OLR1) GENE IN Bubalus bubalis


ABSTRACT

Single nucleotide polymorphisms (SNP) are the most common type of DNA sequence polymorphisms and of mutation in vertebrates. Keeping in view the involvement of the oxidised low density lipoprotein receptor 1 (OLR1) gene in lipid metabolism and the higher milk fat percentages in Bubalus bubalis, the present study was carried out to explore the SNPs in OLR1 intron I in two breeds of buffalo viz. Jaffarabadi and Surti by gene cloning and sequencing. 3 SNPs were detected and all the SNPs identified followed the breed lineage.

Keywords: single nucleotide polymorphisms, buffalo, oxidised low density lipoprotein receptor 1, breed specific SNPs

INTRODUCTION

India possesses eleven recognized breeds of buffalo. Jaffarabadi is the heaviest breed and Surti is one of the smallest breeds, both having a high milk fat percentage (>8%). The most abundant type of variation in human and cattle genomes is the single nucleotide polymorphism, or SNP, where a single base pair has been changed. Millions of SNPs have been found in humans, and there are over 600,000 in cattle with more being discovered every day (Meuwissen et al., 2001).

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 et al., 1998). The bovine OLR1 gene encodes 270 AA and has 72% identity to the human protein and was initially identified in bovine aortic endothelial cells. (Sawamura et al., 1997). The genomic sequence of bovine OLR1, recently released by Baylor College of Medicine, contains five exons (GenBank accession no. NW_215807). It has been shown that oxLDL and its lipid constituents have numerous damaging effects on secretory activities of the endothelium, including induction of apoptosis (Imanishi et al., 2002). In addition to binding oxLDL, OLR1 removes aged and apoptotic cells from blood circulation (Oka et al., 1998). Several QTL affecting milk production traits have been reported on bovine chromosome 5 near OLR1 (Khatkar et al., 2004). The role of OLR1 in lipid metabolism and the results of previous whole genome scan studies prompted the investigation of OLR1 as a candidate gene affecting milk composition traits, and a study conducted in Holstein dairy cattle concluded that allele C of SNP 8232 in 3' UTR in OLR1 gene had significant effects on fat yield and fat percentage (Khatib et al., 2004).
al., 2006). A separate study conducted in Italian brown Swiss involving the genotyping for a SNP at position 8232 in \textit{OLR1} (NW_215807:g.8232C>A) revealed a frequency of 0.95 for the g.8232C allele (Khatib \textit{et al}., 2007). Another study conducted in Dutch Holstein-Friesian cattle population on the \textit{OLR1} gene revealed that \(O_{LR\_g.8232C>A}\) had a significant effect (P < 0.05) on milk-fat percentage (Schennink \textit{et al}., 2009).

Since intronic regions include the promoters of a gene, it is necessary to check for the mutations in these regions as they can alter the expression levels of a gene. Khatib \textit{et al}., 2006 identified 5 SNPs in intron 4 but none of them displayed significant association with milk fat percentage.

Based on the aforementioned studies indicating the role of \textit{OLR1} in lipid metabolism, its correlation with milk fat percentage and the role of intron in gene expression, the study of SNP exploration in this gene (Intron I) in two breeds of \textit{Bubalus bubalis}, both having milk fat \(\geq 7\%\), was initiated.

**MATERIALS AND METHODS**

**Sampling and DNA extraction**

Ten blood samples from both the breeds of buffalo, Jaffarabadi (from the Cattle Breeding Farm, Junagadh, Gujarat) and Surti (from the Reproductive Biology Research Unit, Anand Agricultural University, Anand, Gujarat) were collected at random from non-related animals. Genomic DNA was extracted by the phenol:chloroform method. The concentration and the purity of the DNA obtained were assessed by spectrophotometry and electrophoresis in 0.8% agarose gel, respectively.

**PCR amplification**

The genomic DNA from each individual was amplified by PCR. A pair of primers designed using the reference sequence (Accession no. NC_007303.3) of \textit{OLR1} of \textit{Bos taurus} (Forward:AGAAAACATCAATGCCTGGGT & Reverse: CACCACAAGGCAGAGAGT) was used to amplify a gene segment of 1290 bp. Approximately 30 ng of genomic DNA was amplified in 25 µl PCR reaction consisting of 2X Master mix (MBI Fermentas). Thermal cycling was performed on Veriti (Applied Biosystems) with an initial denaturing step at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C at 60 seconds, primer annealing at 56°C for 75 seconds, and primer extension at 72°C for 45 seconds. The PCR products were confirmed on a 1.5% agarose in 0.5% TBE buffer, visualized under ultraviolet light and eluted (Qiagen gel extraction kit) for purification of the PCR product.

**Ligation and transformation**

The purified PCR products were ligated in pTZ57R/T vector using InstAclone™ PCR product cloning kit (Cat no. K1213, MBI Fermentas). Ligation of the PCR fragment was confirmed by PCR using M13 specific primers targeting M13 sequences contained in pTZ57R vector. Ligated recombinant vector was transformed in competent \textit{E. coli} (DH5-\text{a}) cells. Recombinant clones were selected by blue-white screening. Recombinant plasmids were isolated and used for cycle sequencing, and that were later processed for sequencing using the universal M13 primers.

**Sequencing and SNP detection**

Extension products obtained from cycle sequencing reaction were purified by vacuum manifold using AcroPrep™ 96 filter plates, (Cat. no. 5033, Omega 3K, Pall Corporation, USA). Cycle sequencing products were run on an ABI-
PRISM automated DNA sequencer and raw data were collected by 310 Data Collection Software (Version 3.1.0). Sequences obtained by the sequence analyzer were curated and vector sequences were removed using the Viecescreen (NCBI) programme. These filtered sequences were then subjected to local alignment with Genebank database sequences using BLASTn protocol available at NCBI.

A consensus sequence of OLRI Intron I of buffalo was generated and used as a reference sequence. Twenty sequences were compared by multiple sequence alignment by using a multiple sequence alignment tool, Bioedit (v 7.0.7.1). SNP detection was done by multiple sequence alignment of consensus sequences of *Bubalus bubalis* in Bioedit (v 7.0.7.1) and MEGA4.

**RESULTS AND DISCUSSION**

Three SNPs were detected within the 1290bp fragment (172 to 1362bp) of OLRI intron I gene in four breeds of *Bubalus bubalis* (Table 1). The study has not yet been conducted in *Bubalus bubalis*; however, SNP exploration in this gene has been conducted in Bos taurus but intron I has not yet been investigated for SNPs both in either cattle or buffalo.

### Table 1. SNPs in OLRI Intron I of *Bubalus bubalis* (Reference Sequence-GQ478035).

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Sample ID</th>
<th>Nucleotide position</th>
<th>Base change</th>
<th>Frequency</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bn1</td>
<td>61</td>
<td>a&gt;G</td>
<td>0.025</td>
<td>GQ415438</td>
</tr>
<tr>
<td>7</td>
<td>S3, S4 &amp; S7</td>
<td>423</td>
<td>t &gt; C</td>
<td>0.075</td>
<td>GQ478035, GQ478036 &amp; GQ478039</td>
</tr>
<tr>
<td>8</td>
<td>Jb3, Jb8</td>
<td>843</td>
<td>t &gt; C</td>
<td>0.05</td>
<td>GQ478025 and GQ478030</td>
</tr>
<tr>
<td>9</td>
<td>S3</td>
<td>866</td>
<td>t &gt; A</td>
<td>0.025</td>
<td>GQ478035</td>
</tr>
</tbody>
</table>

Jb : Jaffarabadi and S : Surti.

### Table 2. Effect of breed composition on single nucleotide polymorphism occurrence.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Sequence position</th>
<th>Frequency</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surti</td>
</tr>
<tr>
<td>1</td>
<td>423</td>
<td>0.075</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>843</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>846</td>
<td>0.025</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Number of the animals with the detected SNP by breed: Surti, Jaffarabadi, Mehsani and Banni.

2 Percentage of buffaloes with that SNP as observed in 40 buffaloes.
Effect of Breed composition on SNPs

Breed specific SNPs are not uncommon. In a similar study conducted in Mehasani and Banni breeds of buffalo involving the investigation of SNPs in intron I of OLR1 gene (Jawale, 2009), which is not in agreement with the present study and gives a clue that these SNPs could be breed specific.

In a study conducted on heat shock protein 70 in pure Brahman, pure Angus and their intercross breeds of cattle, breed specific SNPs were explored that tended to be associated with the particular breeds only, [9] which is in agreement with the present study. The frequency and breed composition of each of the three SNPs detected in the present study are summarized in Table 2. The presence of all the SNP was affected by breed. Surti ancestry tended to be related to the occurrence of SNP at positions 423 and 866 while the presence of SNP at position 843 tended to be associated with Jaffarabadi lineage. SNP 423 was found in three buffaloes, 7.5% of the total population, SNP 843 occurred in two Jaffarabadi buffalo. All the SNPs showed breed specific occurrence and might be correlated to milk fat percentage. However, as these SNPs are present in intronic region, their functional significance needs to be verified.

REFERENCES


