β(1,4)-GALACTOSYLTRANSFERASE-I GENE POLYMORPHISMS IN PAKISTANI NILI RAVI BUFFALO

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ABSTRACT

β4GalT-I interacts with α-lactalbumin to form the lactose synthase in mammary gland. Considering the biological function of the lactose synthase complex, β4GalT-I gene can be considered as a candidate gene for milk production in dairy animals. The bovine β4GalT-I gene, 3,283 base pairs in length, is mapped on chromosome 8. The present research work was planned to identify the polymorphism in β4GalT-I gene in Nili Ravi buffalo. Blood Samples were collected, DNA was extracted and primers were designed for PCR. After amplification, PCR products were sequenced for the identification of allelic variation. Thirteen polymorphic, six in coding and seven in non-coding region of gene, were identified. This is a first report toward genetic screening of β4GalT-I gene at molecular level in Nili Ravi buffalo. The present study will provide a better selection to develop association of identified polymorphisms with production traits in buffalo population.

Keywords: polymorphisms, β4GalT-I Gene, Nili Ravi, buffalo, Pakistan

INTRODUCTION

The β 1, 4-galactosyltransferase-I (β4GalT-I) belongs to a family of enzymes called galactosyl transferases. In the mammalian mammary gland, lactose is synthesized from blood glucose and galactose by a lactose synthase enzyme (Strucken *et al*., 2015). The βeta 1, 4-galactosyltransferase-I gene (β4GalT-*I*) interacts with α-lactalbumin, the calcium binding non-catalytic protein, in the golgi complex of mammary secretory cells to form the lactose synthase complex (Ramakrishnan *et al*., 2002; Shahbazkia *et al*., 2010; Strucken *et al*., 2015). This enzyme is a membrane-bound glycoprotein which is widely distributed among in mammals, non-mammalian vertebrates and also in some plants (Powell and Brew, 1974). Considering the biological function of the lactose synthase complex, β4GalT-I gene can be a potential candidate gene for milk production in dairy animals. It is considered a housekeeping gene in the biosynthesis of glycans which occurs in essentially all cell types (Shaper *et al*., 1998). β4GalT-I act as a cell surface component, adhesion and recognition molecule, signal transducer, tumor marker (Berger and Rohrer, 2003) and also involve in angiogenesis, wound healing and collagen deposition in the skin (Shen *et al*., 2008). However, in spite of its involvement in various physiological

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and biochemical reactions, little is known regarding the presence of polymorphisms in dairy animals and any consequent effects on production traits. It is a membrane-bound glycoprotein widely distributed in the mammals, non-mammalian vertebrates and also in some plants. Two distinct isoforms of the β4GalT-I gene product have been reported in the human, mouse and in cattle.

The bovine β4GalT-I gene is 53,283 base pairs in length, comprises of six exons and five introns and is located on chromosome 8. The bovine β4GalT-I protein consists of four domains- the cytoplasmic domain (residues 8-24), the transmembrane domain (residues 25-44), the stem region (residues 45-145) and the catalytic domain (residues 146-402) (Qasba *et al*., 2008). Thus, it was accepted that the polymorphisms which are located in the catalytic domain of the protein may affect some of the catalytic properties of the enzyme. This study was aimed to identify the probable polymorphism in the β4GalT-I gene of Nili Ravi buffalo breed of Pakistan.

MATERIALS AND METHODS

Blood samples (n=50) of Nili Ravi buffaloes were collected from Livestock Production Research Institute (LPRI) Bahadarnagar, Okara and preserved in EDTA (0.5 M) coated falcon tubes. Genomic DNA was extracted from blood samples using inorganic method (Sambrook and Russel 2001). DNA quantification was carried out using 0.8% Agarose gel. 50 ng/uL of genomic DNA was used for the amplification of coding regions of the β4GalT-I gene. PCR primers were designed from GenBank accession no. AC_000165.1 by web based software "Primer3" (http://bioinfo.ut.ee/primer3-0.4.0/) (Untergasser *et al*., 2012). All primers were amplified by touchdown PCR protocol with annealing temperature (62-52°C) on Bio-Rad and peQ Lab thermocycler. After the precipitation with 70% ethanol in dark, PCR products were sequenced through ABI prism 3100 genetic analyzer (Applied Biosystems Inc., Foster City, CA). Sequencing results were analyzed with BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Pair wise alignment of sequence was done with the help of blast2 sequence. Allelic and genotypic frequencies for the identified polymorphisms were calculated using bioinformatics software POPGENE (Yeh *et al*., 1999).

RESULT

The overall sequence variation across the bovine β4GalT-I locus is high. Thirteen polymorphic sites were identified by using BLAST in local Nili Ravi buffalo breed. Aligned sequence represent that six identified substitutions were in coding region of the gene while seven were positioned in intronic (near to exonic) region of the gene. All these identified polymorphism can be used as strong association markers with economic traits in Nili Ravi buffalo population. The reported exonic polymorphisms do not bring amino acid change and appeared as synonymous substitution. However, these sites may be related to detect causative mutation or adjacent QTL. The distribution pattern of alleles and genomic frequencies against each identified polymorphisms are presented in Table 1. However the identified polymorphic sites were considered breed specific and might be correlated to milk production and other economic traits.

DISCUSSION

The β4GalT-I encodes the catalytic part of lactose synthase enzyme which is responsible for lactose synthesis in the mammary gland. The whole coding region of the gene was screened for the presence of allelic variation among a sample of fifty buffaloes, using PCR technique followed by Sanger sequencing. Altogethersixteen polymorphic sites were identified across the whole bovine β4GalT-I locus.

Two distinct isoforms of the β4GalT-I gene product have been reported in the mouse (Shaper *et al*., 1998) and similar variants have been

Table 1. Change in nucleotide, genotypic and allelic frequency of all identified polymorphisms in bovine β4GalT-I gene.

SNP ID	Chromosomal Position	Change in Nucleotide	Genotype Frequency			Allele Frequency	
GALT1	76203115	G>A	GG	AG	AA	G	A
			0.5806	0.0646	0.3548	0.5968	0.4032
GALT2	76183169	C>T	CC	CT	TT	C	T
			0.5484	0.3187	0.1329	0.6613	0.3387
GALT3	76183187	C>T	CC	CT	TT	C	T
			0.4516	0.2113	0.3371	0.4861	0.5139
GALT4	76183243	T>G	TT	TG	GG	T	G
			0.5881	0.2791	0.1328	0.8226	0.1774
GALT5	76183252	T>C	TT	TC	CC	T	C
			0.5137	0.3319	0.1544	0.4677	0.5323
GALT6	76183296	T>A	TT	TA	AA	T	A
			0.5741	0.2897	0.1362	0.3548	0.6452
GALT7	76183328	T>C	TT	TC	CC	T	C
			0.6134	0.1844	0.2022	0.4918	0.5082
GALT8	76183346	A>G	AA	AG	GG	A	G
			0.3147	0.2741	0.4112	0.3953	0.6047
GALT9	76183447	T>C	TT	CT	CC	T	C
			0.4918	0.3147	0.1935	0.5173	0.4827
GALT10	76180158	C>G	CC	CG	GG	C	G
			0.5178	0.1928	0.2894	0.5712	0.4288
GALT11	76180262	G>A	GG	AG	AA	G	A
			0.4617	0.2349	0.3034	0.4911	0.5089
GALT12	76179615	C>T	CC	CT	TT	C	T
			0.4143	0.2316	0.3541	0.6197	0.3803
GALT13	76179669	C>T	CC	CT	TT	C	T
			0.5349	0.2199	0.2452	0.5763	0.437

identified in the bovine (Russo *et al*., 1990) and human (Mengle-Gaw *et al*., 1991) homologues. Both of these isoforms, ‘‘short’’ and ‘‘long’’ are differ only by an N-terminal sequence of 13 residues (Shahbazkia *et al*., 2012). The amount of β4GalT-I enzyme in the lactating mammary gland increases during the lactation period to meet the demand for lactose synthesis.

The most important mechanism by which this increase is ensured is the switch from the long to the short variant, which has the effect of raising β4GalT-I transcript level (Shaper *et al*., 1998). Thus, allelic variation which affects the transcription start codon probablydisturbs this mechanismand prevents the synthesis of higher levels of lactose.

Shahbazkia *et al*., (2012) reported the exon 1 T→A (14Lys) transversion alters the second transcription start site of the codon and may have impact on the gene expression. However, no such type of transversion was seen in this study. Other polymorphisms (174 Thr and 220 His) in exon 2 were identified in the catalytic domain that may alter some of the catalytic properties of the enzyme (Shahbazkia *et al*., 2012). In another study, Qasba *et al*., 2008 reported that the Phe280 residue, together with Tyr286, Gln288, Tyr289, Phe360 and Ile363, were concerned in the relations between β4GalT-I and α-lactalbumin and may alter this interaction and the properties of the lactose synthase complex.

In our study all the identified substitutions GT**G**> GT**A** (236Val), AA**T**> AA**C** (296Asp), GG**A**> GG**G** (302Gly), AT**C**> AT**T** (326 Ile), GT**G**> GT**A** (369Val), and AC**G**> AC**A** (387Thr) were synonymous in nature and located in the catalytic domain of enzyme. However, this Phe280 Tyr substitution was not identified in Nili Ravi buffaloes.

To conclude, it was the first screening of β4GalT-I gene single nucleotide polymorphisms in Nili Ravi buffalo breed of Pakistan. The above discussion just explains the potential and molecular basis of identified polymorphisms. These SNPs may serve as a potent genetic reserve for the development of molecular markers to assist selection in dairy breeding.

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