Polymorphisms of the β-1,4 galactosyltransferase-I gene in Holsteins

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a b s t r a c t

The scope of this study was to identify sequence polymorphisms in the β-1,4-galactosyltransferase-I gene (B4GALT1), the gene which encodes the catalytic part of lactose synthase enzyme. Exons of the gene were scanned for the presence of allelic variation among a sample of 400 Iranian Holsteins, using the single strand conformation polymorphism technique followed by sequencing. Nine polymorphic nucleotide sites and 16 different genotypes of B4GALT1 were identified. We concluded therefore that the β-1,4-galactosyltransferase-I gene was polymorphic in Holsteins. Considering the biological roles of the B4GALT1 gene, we suggest that further studies are critical to investigate the biological effects of the SNPs reported in this study.

Keywords:
Galactosyltransferase
Lactose synthase
SNP

1. Introduction

The galactosyltransferases in the presence of a metal ion, transfers galactose from UDP-Gal to an acceptor sugar molecule (Shaper et al., 1998; Ramakrishnan et al., 2002). Three subfamilies, β1-4-, β1-3-, and α1-3-, have been well characterized and they generate β1-4-, β1-3-, and α1-3-linkages between galactose and the acceptor sugar, respectively. The β1-4-galactosyltransferase (Gal-T or B4GalT) subfamily consists of at least seven members, Gal-T1 to Gal-T7 (Ramakrishnan et al., 2002). These enzymes are expressed in different tissues and show differences in the oligosaccharide acceptor specificity (Brodbeck et al., 1967; Powell and Brew, 1974; Ramakrishnan et al., 2001). B4GalT-I (Gal-T1) is one of the best studied galactosyltransferase and is a widely distributed enzyme found in mammals, non-mammalian vertebrates and also in a subset of plants (Powell and Brew, 1974) which diverged from animals an estimated 1 billion years ago (Shaper et al., 1998). This enzyme is a trans-Golgi resident, membrane-bound glycoprotein that catalyzes the transfer of galactose to N-acetylglucosamine residues, forming the β4-N-acetyllactosamine (Gal β4-GlcNAc) or poly-β4-N-acetyllactosamine structures found in glycoconjugates (Shaper et al., 1998). Since the biosynthesis of glycans occurs in essentially all cell types, the participation of β4GalT-I in this process is considered to be a housekeeping or ubiquitous function (Shaper et al., 1998). Roles as a tumor marker, cell surface component, signal transducer, adhesion and recognition molecule (Berger and Rohrer, 2003) and involvement in angiogenesis, collagen deposition in the skin and wound healing (Shen et al., 2008) are suggested for B4GalT-I. In mammals, B4GalT-I has been recruited for a second biosynthetic function which is the tissue-specific production of lactose in the mammary gland (Brodbeck et al., 1967).In the mammary gland, B4GalT-I interacts with the calcium binding non-catalytic protein, α-lactalbumin, that is expressed only in the epithelial cells of the mammary gland beginning in late pregnancy and continuing throughout lactation (Brew et al., 1968), to form the lactose synthase complex (Ramakrishnan et al., 2002). This complex transfers galactose moiety to free glucose to produce lactose (Sasaki et al., 1978). Lactose is the major carbohydrate component and the most important osmole of milk which regulates the osmotic pressure and

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volume of the milk (Vilotte, 2002) and its synthesis potential affects milk volume and composition (Shahbazkia et al., 2009).

The bovine B4GALT1 gene is 53,285 bp in length and encompasses 6 exons and 5 introns. However, the total coding sequence of the gene is only 1209 bp responsible for encoding the protein with 402 amino acids. In spite of its involvement in various physiological and biochemical reactions, there isn't any information about naturally occurring mutations, polymorphisms and their involvement in physiological and biochemical aspects such as milk production traits in cattle. The scope of this study was to discover possible naturally occurring mutations and polymorphism in the B4GALT1 gene in Holsteins.

2. Materials and methods

The animal sample consisted of 400 Holstein cattle randomly selected from the first calved cows in the Ghiam Dairy Co. (Iran). Peripheral whole blood was collected from jugular veins into tubes containing citrate as an anticoagulant. High molecular weight DNA was extracted by a modified salting-out method (Garner, 2000).

Primer pairs (Table 1) targeting various coding regions of the B4GALT1 gene were designed based on the reference GenBank sequence NW_001495470 (bases 102,361 to 155,645) using Vector NTI software v10.1 (Invitrogen). A range of annealing temperatures (52–62 ℃) and conc entrations of MgCl2 (1–4 mM) was applied to optimize the PCR, which consisted of template DNA (50 ng), primers (16 pmol each), dNTPs (0.2 mM), 1× buffer and 1 U Taq polymerase in a 25 μl reaction. The DNA was denatured at 95 ℃ for 5 mi n, reactions were cycled 30 times through 95 ℃/30 s, annealing temperature (Table 1) /30 s and 72 ℃/30 s, and finally incubated at 72 ℃ for 5 min. All of the PCR produc ts were electrophoresed at 150 V for 40 min through a 2% agarose gel containing 1× TBE buffer and 0.14 mg/ml ethidium bromide to check that amplification had been successful.

For the SSCP procedure, 4–5 μl of each PCR was mixed with 3 vol denaturant solution (95% v/v formamide, 10 mM NaOH, 0.05% w/v xylene cyanol and 0.05% w/v bromophenol blue), heated to 95 ℃ for 5 min, chilled on ice, a nd loaded onto nondenaturing polyacrylamide gels (20 cm × 20 cm; T%: 8, 10 or 12%; C%: 2.5%), containing variable concentrations of TBE (0.5× and 1×) and glycerol (0, 0.05% and 0.1% v/v). Electrophoresis was carried out for between 4 and 9 h at either 25, 35, or 45 W constant power, with the temperature held at 4, 8, 15 or 20 ℃ on a DcodeTM Universal Mu tation Detection System (Bio-Rad, Hercules CA, USA) platform. Signal detection was by silver staining (PlusOneTM DNA Silver Staining method, Amersham Bioscience, Uppsala, Sweden). Templates displaying polymorphism were re-amplified, separated by agarose gel electrophoresis and the amplicons removed from the gel and purified using a PCR Purification Kit (Qiagen, Germany). The purified PCR products were sequenced (Macrogen, Korea) in both directions using the appropriate PCR primers.

3. Results

The optimized PCR conditions for each of the seven B4GALT1 fragments (Table 1) allowed each to be well amplified. The optimized SSCP conditions are also given in Table 1. The SSCP analysis led to the recognition of two patterns in both exon 1 and its upstream region, and exon 6 and its 3′ flanking sequence; and three patterns in exons 2, 3 and 5. Exon 4 was monomorphic. Sequencing of the polymorphic amplicons identified nine polymorphic nucleotide sites, sixteen different alleles and corresponding genotypes which are all summarized in Table 2.

4. Discussion

The most frequent genotype in this sample of Holstein cattle completely matches the Hereford breed B4GALT1 gene sequence deposited in the GenBank database. Thus, the sequence of this gene appears to be well conserved.

Two distinct isoforms of the B4GALT1 gene product have been reported in the mouse, cow (Russo et al., 1990) and human (Mengle-Gaw et al., 1991). The transcription start sites which are responsible for these allelic transcripts have been defined (Shaper et al., 1988, 1998). The amount of

Table 1
Primer pairs and optimized conditions for PCR amplification and SSCP analysis of specific regions of the bovine B4GALT1 gene.

Exon	Primers sequences from 5′ to 3′	Product size (bp)	T_m difference between sense and antisense primers (℃)	Optimized PCR conditions		Optimized conditions for SSCP analysis			
				$Mgcl_2$ concentration	Annealing temperature	Temperature (℃)	T%	Glycerol (%)	Volt-hours
Exon 1 part A	GCCTTCCTCGCGGTAGCCCAC GGAGGGCTGCCCGATAGCGG	269	0.8	1	57	15	10%	0	3500
Exon 1 part B	TTCAGGGAAGCTCTCACGGC TGTCTGTCGTGGAAGAGGGC	293	1.8	1	55	20	8%	0	3500
Exon 2	TCTCCTTTCCCCCACCTCTCTAG ATGCATGCCTTCCCTCCCTC	301	0.8	1.4	58	13	12%	0	4000
Exon 3	TTTGTTTCTGCACCAGGCTG GTGTTCCCTGGTTGTCATGG	269	1.4	2	61	8	12%	0	3500
Exon 4	GTCCTCTGTGTGTGTGCATG AGGGAAGCACGAGATCTAAG	196	1.0	1.1	53	20	10%	0.1	3000
Exon 5	ACCACTTGTCCCTTTGCTCC TTTCCCTTGAGGCACAGAGC	236	1.3	1.8	58	8	8%	0	3000
Exon 6	ACCCTTTGTCTGTTTGTTCC TTGTGAGAATGAGAGGGACC	252	0.6	2	57	18	10%	0.05	2500

Table 2
SNPs of B4GALT1 gene in the studied Holsteins, their positions, corresponding alleles and genotypes with respective frequencies.

Position [a]	Sequence	SNP allele name	Number of allele (SNP) observation	Allele (SNP) frequency %	SNP genotype [b]	SNP genotype frequency [c] %
224	WT [d]	14Met	795	99.375	14 Met/Met	98.75
	ATG → AAG	14Lys	5	0.625	14 Met/Lys	1.25
29554	WT	174Met	778	97.250	174 Met/Met	94.50
	ATG → ACG	174Thr	22	2.750	174 Met/Thr	5.50
29693	WT	220Gln	755	94.375	220 Gln/Gln	88.75
	CAG → CAC	220His	45	5.625	220 Gln/His	11.25
41860	WT	223Glu	796	99.500	223 Glu/Glu	99
	GAG → GAA	223Glu*	4	0.500	223 Glu/Glu*	1.00
42030	WT	280Phe	759	94.875	280 Phe/Phe	89.75
	TTT → TAT	280Tyr	41	5.125	280 Phe/Tyr	10.25
52294, 52295	WT	340Gly	779	97.375	340 Gly/Gly	94.75
	GGG → GAA	340Glu	21	2.625	340 Gly/Glu	5.25
52320	WT	349Arg	799	99.875	349 Arg/Arg	99.75
	AGA → CGA [e]	349Arg*	1	0.125	349 Arg/Arg*	0.25
52906	WT	389Pro	788	98.500	389 Pro/Pro	97.00
	CCG → CCA	389Pro*	12	1.500	389 Pro/Pro*	3.00

*Shows silent allele carrying different genetic code which encodes the same amino acid.
Bold data shows the site of the mutation in the related codon.
[a]Position is related to the location of the B4GALT1 gene in the reference GenBank sequence NW_001495470.
[b]SNP genotypes of each exon of the gene are given.
[c]Frequencies of the SNP genotypes are given.
[d]Wild type sequence which matches the Hereford breed B4GALT1 sequence deposited in the GenBank database; NW_001495470.
[e]The A → C mutation in exon 5 was present only in one productive cow. As a result, the related traits couldn't be included in the statistical analysis but are reported in the table.

B4GALT1 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 the level of B4GALT1 transcript (Shaper et al., 1998). Thus, allelic variation which affects the critical transcription start codon can be expected to disturb this mechanism and prevent the synthesis of higher levels of lactose. The exon 1 T → A transversion (allele 14Lys) reported in this study alters the second transcription start site therefore it may have impact on the expression of the gene.

The bovine B4GALT1 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). The exon 2 polymorphisms (alleles 174Thr and 220His) are located in the catalytic domain, and they may change some of the catalytic properties of the enzyme. The Phe280 residue, together with Tyr286, Gln288, Tyr289, Phe360 and Ile363, are involved in the interactions between B4GALT1 and α-lactalbumin to form the lactose synthase enzyme (Qasba et al., 2008). Therefore, Phe280Tyr substitution may alter this interaction and thereby the properties of the lactose synthase complex.

As the polymorphisms were identified by sequencing purified PCR fragments, it was not possible to define whether the SNPs in exon 5 were a GGG/GAA or a GAG/GGA heterozygote. Each involves a change at position 340 from Gly to Glu, (allele 340Glu) and this may alter the structural and catalytic properties of the enzyme.

The above discussion just explains the potential and molecular basis of uncovered SNPs to affect the structure, activity and expression of the enzyme and confirmation of the above hypothesis will clearly requires experimental biochemical evidence. If further studies reveal some advantageous mutations (leading to higher production traits) among identified mutations reported in this study, the mutations can be consider to be used as molecular markers to assist selection in dairy breeding. In this case considering the lower frequencies of the identified mutations, the beneficial mutation (if exists) must be newly occurring and marker assisted selection is able to fasten increasing its relative frequency.

5. Conclusions

We have reported here for the first time single nucleotide polymorphisms of the B4GALT1 gene in cattle. Some of the uncovered SNPs are located in the biologically important regions of the gene. We concluded that the identified SNPs lend themselves readily for further studies on their biochemical and/or physiological impacts such as milk and lactose production traits in livestock.

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References

Berger, E.G., Rohrer, J., 2003. Galactosyltransferase—still up and running. Biochimie 85, 261–274.
Brew, K., Vanaman, T.C., Hill, R.L., 1968. The role of alpha-lactalbumin and the A protein in lactose synthetase: a unique mechanism for the control of a biological reaction. Proceedings of the National Academy of Sciences 59, 491.
Brodbeck, U., Denton, W.L., Tanahashi, N., Ebner, K.E., 1967. The isolation and identification of the B protein of lactose synthetase alpha-lactalbumin. Journal of Biological Chemistry 242, 1391.

Garner, I., 2000. Isolation of high-molecular-weight DNA from animal cells. In: Rapley, R. (Ed.), The Nucleic Acid Protocols Handbook. Humana Press Inc, Totowa, NJ, pp. 3–6.
Mengle-Gaw, L., McCoy-Haman, M.F., Tiemeier, D.C., 1991. Genomic structure and expression of human beta-1, 4-galactosyltransferase. Biochemical and Biophysical Research Communications 176, 1269.
Powell, J.T., Brew, K., 1974. Glycosyltransferases in the Golgi membranes of onion stem. Biochemical Journal 142, 203.
Qasba, P.K., Ramakrishnan, B., Boeggeman, E., 2008. Structure and function of -1, 4-galactosyltransferase. Current Drug Targets 9, 292.
Ramakrishnan, B., Shah, P.S., Qasba, P.K., 2001. Alpha-lactalbumin (LA) stimulates milk beta-1, 4-galactosyltransferase I to transfer glucose from UDP-glucose to N-acetylglucosamine. Crystal structure of beta 4Gal-T1 LA complex with UDP-Glc. Journal of Biological Chemistry 276, 37665.
Ramakrishnan, B., Boeggeman, E., Qasba, P.K., 2002. 1, 4-galactosyltransferase and lactose synthase: molecular mechanical devices. Biochemical and Biophysical Research Communications 291, 1113–1118.
Russo, R.N., Shaper, N.L., Shaper, J.H., 1990. Bovine beta 1-4-galactosyltransferase: two sets of mRNA transcripts encode two forms of the protein with different amino-terminal domains. In vitro translation experiments demonstrate that both the short and the long forms of the enzyme are type II membrane-bound glycoproteins. Journal of Biological Chemistry 265, 3324.
Sasaki, M., Eigel, W.N., Keenan, T.W., 1978. Lactose and major milk proteins are present in secretory vesicle-rich fractions from lactating mammary gland. Proceedings of the National Academy of Sciences 75, 5020–5024.
Shahbazkia, H.R., Aminlari, M., Tavasoli, A., Mohamadnia, A.R., Cravador, A., 2009. Associations among milk production traits and glycosylated haemoglobin in dairy cattle; importance of lactose synthesis potential. Veterinary Research Communications.
Shaper, N.L., Hollis, G.F., Douglas, J.G., Kirsch, I.R., Shaper, J.H., 1988. Characterization of the full length cDNA for murine beta-1, 4-galactosyltransferase. Novel features at the 5′-end predict two translational start sites at two in-frame AUGs. Journal of Biological Chemistry 263, 10420.
Shaper, N.L., Charron, M., Lo, N.W., Shaper, J.H., 1998. 1, 4-Galactosyltransferase and lactose biosynthesis: recruitment of a housekeeping gene from the nonmammalian vertebrate gene pool for a mammary gland specific function. Journal of Mammary Gland Biology and Neoplasia 3, 315–324.
Shen, A., Qian, J., Liu, L., Liu, H., Chen, J., Niu, S., Yan, M., Chen, X., Shen, C., Gu, J., 2008. The role of [beta]-1, 4-galactosyltransferase-I in the skin wound-healing process. The American Journal of Dermatopathology 30, 10.
Vilotte, J.L., 2002. Lowering the milk lactose content in vivo: potential interests, strategies and physiological consequences. Reproduction Nutrition Development 42, 127–132.