Investigation of the galactosyltransferase (GALT) gene polymorphism in Romanian Black and White and Romanian Grey Steppe cattle breeds

Teodora C. Carșai

University o Agricultural Sciences and Veterinary Medicine, Faculty of Animal Husbandry and Biotechnology, Cluj-Napoca, Romania. Corresponding author: T. C. Carșai, teodora_carsai@yahoo.com

Abstract. The 1,4-β-D-galactosyltransferase (1,4-β-D-GALT) is a Golgi apparatus membrane bound enzyme that participates in the biosynthesis of the oligosaccharide structures of glycoproteins and glycolipids. The GALT encoding gene in cattle is located on chromosome 8 and is expressed in two different length isoforms. The investigation of GALT polymorphisms was carried out on a sample of individuals of the Romanian Black and White cattle breed (individuals with a high milk production of 10,000 L/lactation and individuals with low milk production of 6,000 L of milk/lactation) and the ancestral breed, Romanian Grey Steppe, with a low milk production. Frequency 1 of the T allele found in this study in the Romanian Black and White breed suggests that the allele has been fixed in the course of breed evolution and specialisation.
Key Words: galactosyltransferase (GALT), polymorphisms, markers, cattle, milk.

Introduction. Understanding the lactogen secretion mechanisms of the mammary gland has been the goal of numerous studies. Variations in milk production parameters and milk secretion are controlled by numerous hormones and enzymes, including galactosyltransferase (GALT). The 1,4-β-D-GALT enzyme is unique given its double role in glycoconjugate and lactose biosynthesis (D'Agostaro et al 1989; Li et al 2017). For the first activity, the enzyme adds galactose to the N-acetylglucosamine residues. The second activity is limited to the mammary gland tissue, where the enzyme forms an alpha-lactalbumin (LALBA) heterodimer catalysing UDP-galactose + D-glucose and UDP + lactose formation. The two enzymatic variants result from the differentiation of initiation of the transcription site and from the posttranslational process. The two transcripts encode the same protein, being different only at the 5' end, with an approximate length of 4.0 and 3.9 kb, respectively (Russo et al 1990). The longer transcript encodes the type II Golgi receptor, membrane binding protein, involved in glycoconjugate biosynthesis. The shorter transcript encodes a protein which is cleaved to form the soluble lactose-synthase (Russo et al 1990).

It is also demonstrated that defficiency in activity of GALT may produce disorders in galactose metabolism (Coelho et al 2017), galactosemia being recognized as an inborn disease with severe consequences (Thakur et al 2017). Catera et al (2016) also identified a novel plasma glycosylation-associated markers of aging.

The secretory activity of the pituitary gland is over-coordinated by the brain through factors of pituitary hormone synthesis and release. One of these is the pituitary growth factor (PIT1) (Coșier et al 2008), a key protein synthesized in the hypothalamic nuclei and which causes adenohypophysis to activate the expression of genes encoding the growth or somatotropic hormone (GH) (Coșier et al 2012), and prolactin (PRL), essential hormones for the development of mammary tissue (mammogenesis), lactation and milk synthesis (lactogenesis), maintenance of dairy secretion (galactopoiesis) and resting of the mammary gland. The growth hormone (GH) acts on the mammary gland by way of two mechanisms: indirectly by stimulating in the liver the synthesis of a key

growth factor protein called insulin like 1 growth factor (IGF1), and directly by binding to its specifically located receptor and in the epithelial mammary secretion cell membrane.

Released into the blood, lactogen hormones bind to their specific protein receptors in the target secretory mammary epithelial cell membrane, that are encoded by two genes: the prolactin receptor gene (PRLR) and the growth hormone receptor (GHR) gene. This binding induces receptor dimerization and phosphorylation activation of a protein transcription factor, called signal transduction and activation transcription factor 5 (STAT5), linked to the intracellular domain of these two receptors. By activation, it reaches the nucleus where it binds to specific regions of several genes' promoters, including genes encoding milk proteins, causing their transcriptional activation and the synthesis of specific milk proteins (Coșier & Croitoriu 2012). They thus influence the synthesis of fat in the mammary gland, a process catalysed mainly by the acylCoA gene expression product: diacylglycerol acetyltransferase (DGAT), encoding the enzyme bearing the same name (Carșai & Bălteanu 2010; Coșier et al 2015). LALBA is a major lactose-rich protein with high lysozyme homology, encoded by a gene located on bovine chromosome 5. LALBA is only active in the secretory cells of the mammary gland. As a major constituent of the lactose-synthase enzyme, LALBA transits the membrane of the Golgi apparatus, where together with GALT, it forms the lactose synthase enzyme involved in the synthesis of milk lactose (Yadav & Brew 1991). In milk, fats are present in the form of small spherical or elliptical globules, consisting of triglycerides representing 98-99% of total fat. Our research was developed in an effort to identify a GALT polymorphism in low and high dairy production of Romanian Black and White (RBW) cattle breed and in ancestral cattle breed, Romanian Grey Steppe (RGS), respectively.

Material and Method

Animals and blood sampling For the study of polymorphisms at GALT gene locus, intron 1, exon 2 and part of intron 2, where a cytosine to thymine (C/T) substitution is located, which characterizes two alleles at this locus T and C, were amplified by PCR. Sixty individuals were studied, from farms located in Alba, Satu Mare and Iasi counties, of which 40 RBW, and 20 RGS. The study was developed in May 2012, and February 2013. Twenty (20) individuals of the total 40 RBW examined individuals had high milk production (RBW-H), while the other 20 had low milk production (RBW-L). Two (2) mL of jugular vein blood was taken from each individual in vacutainers with K3-EDTA for DNA extraction. Samples were stored at 4°C until DNA extraction.

Direct amplification of target DNA from blood

DNA extraction and quality assessment. For DNA extraction, 100 µL of whole blood from each sample was transferred to one 1.5 mL sterile Eppendorf tube. For each sample, 600 µL of sterile PBS solution was added, and after a quick vortexing the tubes were centrifuged. The supernatant was removed each time, and washing was repeated twice. After the final washing and the removal of the supernatant, the pellet containing white figurative elements was subjected to extraction by alkaline lysis (NaOH) and neutralization (200 mM HCl + 100 mM Tris HCl- pH = 8.5). After centrifugation and a quick vortexing, the amount, purity and concentration of DNA in the samples was determined by agarose gel electrophoresis (1%) and by spectrophotometry using the Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). The samples were diluted to a final concentration of 150 ng μL^{-1} and then stored at 4°C for the genetic analysis of the studied loci.

PCR amplification. A 301 bp product, where a cytosine to thymine (C/T) substitution is supposed to be located, which differentiates the two alleles from the GALT locus, was amplified by PCR. The PCR mix and amplification conditions are shown in Tables 1 and 2.

Table 1

PCR reaction setup

Component	*Volume (µL)*
H_2O miliQ	14
PCR buffer	1
Forward primer	1
Reverse primer	1
Taq polymerase	0,5
$MgCl_2$	0.5
DNA	2
Reaction volume	20

Table 2

PCR cycling conditions

Cycle (repetitions)	*Temperature (°C)*	*Time*
1x	98	5 min
35x	98	1 sec
	58	5 sec
	72	30 sec
1x	72	60 sec
1x	4	60 sec

PCR product digestion. To highlight the two alleles, the restriction of the 301 bp amplification product was carried out. Restriction was performed in a final volume of 30 µL, containing 12 µL of PCR product, 1 µL of N*coI* Fast digest enzyme, 3 µL of enzyme buffer and 14 µL of miliQ sterile water (Thermo Fisher Scientific, MA, USA). The samples thus prepared were incubated at 37°C for 30 minutes.

The amplification and digestion products were migrated and analysed in a 2% agarose gel, containing 1X Sybr Safe (Invitrogen, Eugene, OR, USA) in TBE 1X buffer at 65 V for 2 hours. The gel was then analysed with the Molecular Imager Gel Doc XR System (BioRadLaboratories, Hercules, CA, and SUA).

PCR fragment sequencing. Amplicons specific to the 301 bp product were purified using the ZR-96 DNA Clean & Concentrator™-5 (Zymo Research Corporation, Orange, CA, SUA). The sequencing reaction was performed by capillary electrophoresis chemistry using BigDye® Terminator v1.1 Kit (Applied Biosystems, Foster City, CA, USA) on Applied Biosystem 3730 sequencer (Applied Biosystems, Foster City, CA). Amplification for sequencing was performed using the dideoxynucleotide sequencing chain termination method and the BigDye® Terminator v1.1 Cycle Sequencing Kit. Each strand of PCR product from heterozygous individuals was sequenced with forward and reverse GALT primers, also used to obtain PCR products. Analysis of the sequencing chromatograms was performed using the BioEdit program (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

Results and Discussion

Genotyping at the GALT locus. Genotyping at the GALT locus of the cattle studied considered the presence/absence of cytosine/thymine (C/T) substitution from the 301 bp fragment amplified by PCR to distinguish the two alleles identified with the N*coI* restriction enzyme. Following the digestion of the amplified 301 bp fragment, three corresponding restriction profiles were revealed in three genotypes (CC, CT and TT).

The C allele of the PCR amplified 301 bp fragment does not contain any restriction site for the *NcoI* enzyme and therefore is not digested by the enzyme. The C/T type substitution creates a restriction site in the T allele, resulting in 2 fragments of 184 bp and 117 bp, respectively.

The analysis of electrophoretic profiles revealed in RGS individuals with low milk production the presence of the three genotypes: type TT homozygous genotypes corresponding to digested 184 bp and 117bp fragments, homozygous CC genotypes corresponding to undigested fragments of 301 bp and heterozygous CT genotypes showing all three fragments. In RBW-H and RBW-L batches only TT type genotypes were shown (Figure 1).

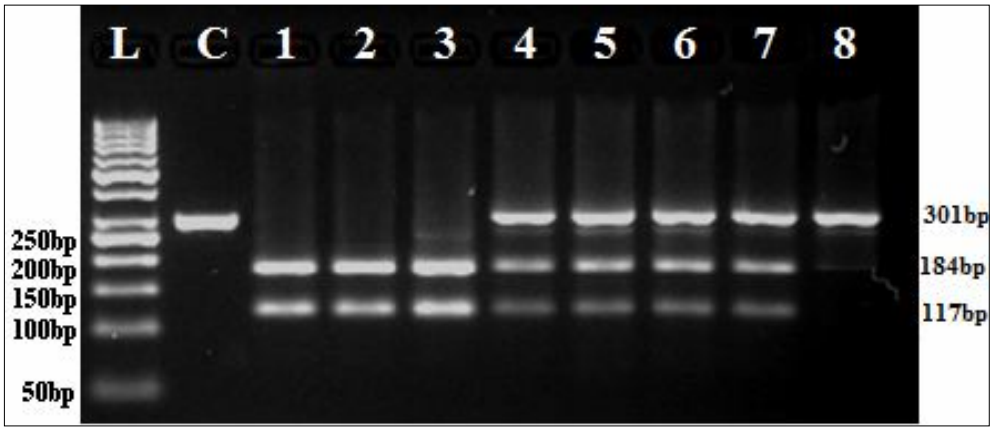

Figure 1. Electrophoretic profile highlighting the *Nco*I type polymorphism, located in 301 bp fragment of GALT gene; L: 50 bp DNA ladder (Fermentas, Vilnius, Lithuania); C – control PCR product of undigested 301 bp; fields: 1, 2 and 3 - TT genotypes; fields 4, 5, 6 and 7 - CT genotypes; field 8 - CC genotypes.

Confirmation of C/T substitutions by sequencing. The sequencing of the 301 bp GALT gene revealed the C/T substitution mutation characterizing the two alleles (C and T) at this locus (Figure 2).

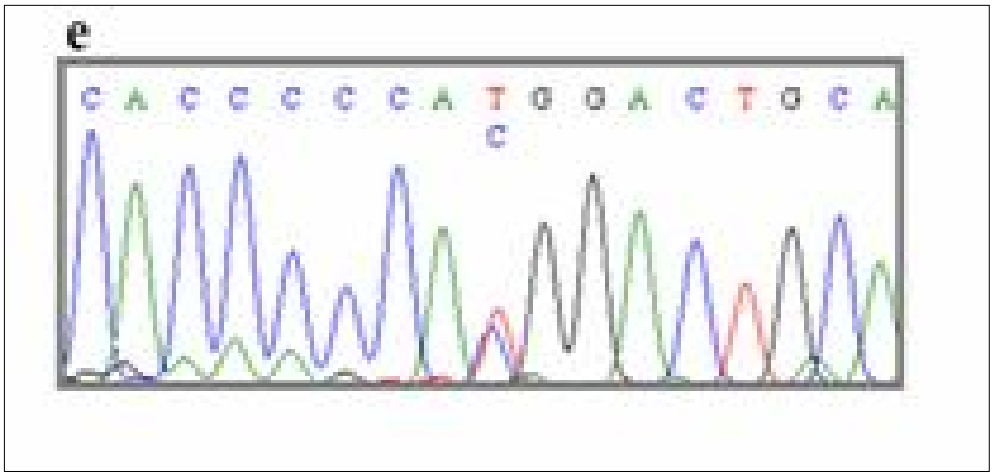

Figure 2. Part of sequencing chromatograms evidencing the C and T allele respectively in cattle (CT genotype).

Genetic structure at GALT locus. Following the compilation of the experimental data, allele and genotype frequencies were calculated in the two studied breeds (Table 3).

Table 3

Genetic structure at the GALT locus in the three examined groups of two studied breeds

Breed/group	*Number of genotyped individuals*	*Genotypic and allelic frequency*				
		TT	*CT*	*CC*	*T*	*C*
RBW-H	N = 20	1	0	0	1	0
RBW-L	N = 20	1	0	0	1	0
RGS	N = 20	0.375	0.5	0.125	0.625	0.375

RBW-H: Romanian Black and White cattle breed - individuals with high dairy production; RBW-L: Romanian Black and White cattle breed - individuals with low dairy production; RGS - Romanian Grey Steppe cattle breed.

A single genotype (TT genotype) with a frequency equal to 1 was identified in the individuals of the RBW-H and RBW-L batches. In individuals of the RGS batch the highest observed frequency was found for CT genotype (0.5). The lowest frequency was recorded for the CC genotype (0.125), while the homozygous TT genotype, with a frequency of 0.375, significantly differentiated the two breeds. The maximum frequency of the T allele (equal to 1) determined in the RBW breed suggests that the allele is fixed in the population. Additional studies on larger batches may confirm or invalidate the partial results of the study. However, the low frequency of the T allele in the rustic RGS, may explain the lower influence of the gene in the lactogen process.

Conclusions. The study shows the identification of a C/T substitution mutation and the genetic structure at this locus in three experimental groups from two native breeds characterized by differentiated milk production: the Romanian Black and White and the Romanian Grey Steppe. The PCR-RFLP technique was used to identify the mutation, and to confirm it the amplicons of heterozygous individuals were sequenced. The amplified fragment corresponds to a portion of the GALT gene that covers intron 1, exon 2 and part of intron 2, where cytosine/thymine (C/T) substitution is located, which differentiates the two alleles. The study was conducted on two groups of cattle expressing different phenotypes of mammary gland development and milk production in order to identify differences in genetic structure and to develop additional studies to establish associations with the attributes of dairy production. However, the results of the study have brought some clarification as regards the possible role of the T allele in relation to breed specialization.

Based on the results obtained, it was concluded that the possible fixation of the T allele in the Black and White Romanian breed could be related to the breed specialization for milk production and that the presence of the other allele only in the Romanian Grey Steppe breed confirm its existence in non-selected populations. However, a single mutation in a single gene from among the genes that interfere with the lactogenic process cannot explain the variability of the productive characteristics of the two breeds, which indicates a polygenic interaction with a multitude of factors involved and not just the GALT polymorphism. Confirmation of the absence of the C allele in the Romanian Black and White breed requires further studies on a larger sample.

Acknowledgements. This work was supported by UEFISCDI, Project PN-II-RU-TE No. 113/20140 and code 224.

References

Carşai T. C., Bălteanu V. A., 2010 The polymorphism of some key genes involved in some lactation traits. The 39th International Session of Scientific Communications of the Faculty of Animal Science, Bucharest, Scientific Papers 53:45-49.

Catera M., Borelli V., Malagolini N., Chiricolo M., Venturi G., Reis C. A., Osorio H., Abruzzo P. M., Capri M., Monti D., Ostan R., Franceschi C., Dall'Ollio F., 2016 Identification of novel plasma glycosylation-associated markers of aging. Oncotarget 7(7):7455-7468.

Coelho A. I., Rubio-Gozalbo M. E., Vicente J. B., Rivera I., 2017 Sweet and sour: an update on classic galactosemia. Journal of Inherited Metabolic Disease 40(3):325-342.
Coşier V., Croitoriu V., 2012 Research concerning the polymorphic expression of Pit-1 and STAT5A genes in cattle. Bulletin UASVM - Animal Science and Biotechnologies 69(1-2):70-79.
Coşier V., Vlaic A., Carşai C., Socol C., Constantinescu R., 2008 Associations between polymorphism at pit1 locus and milk yield in Romanian Simmental and Maramures brown breed cattle. Bulletin UASVM - Animal Science and Biotechnologies 65(1-2):471.
Coşier V., Păcurar A., Carşai C., Bălteanu V., 2012 Identification of C127G point mutation in growth hormone (GH1) in indigenous breeds' cattle. Bulletin UASVM - Animal Science and Biotechnologies 69(1-2):306-307.
Coșier V., Comșa E., Constantinescu R., 2015 The use of direct genetic markers K-casein and DGAT1, involved in milk production characteristics. Bulletin UASVM - Animal Science and Biotechnologies 72(2):257-258.
D'Agostaro G., Bendiak B., Tropak M., 1989 Cloning of cDNA encoding the membrane-bound form of bovine β1,4-galactosyltransferase. The FEBS Journal 183(1):211-217.
Li W., Zhu Z., Chen W., Feng Y., Dimitrov D. S., 2017 Crystallizable fragment glycoengineering for therapeutic antibodies development. Frontiers in Immunology 8:1554. doi: 10.3389/fimmu.2017.01554.
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 265:3324-3331.
Thakur M., Feldman G., Puscheck E. E., 2017 Primary ovarian insufficiency in classic galactosemia: current understanding and future research opportunities. Journal of Assisted Reproduction and Genetics, doi: 10.1007/s10815-017-1039-7.
Yadav S. P., Brew K., 1991 Structure and function in galactosyltransferase. Sequence locations of alpha-lactalbumin binding site, thiol groups, and disulfide bond. Journal of Biological Chemistry 266:698-703.
*** http://www.mbio.ncsu.edu/bioedit/bioedit.html.

Received: 29 October 2017. Accepted: 28 November 2017. Published online: 28 December 2017.
Author:
Teodora Crina Carșai, University o Agricultural Sciences and Veterinary Medicine, Faculty of Animal Husbandry and Biotechnology, Biotechnology Department, Calea Manastur 3-5, 400372 Cluj-Napoca, Romania, e-mail: teodora_carsai@yahoo.com

How to cite this article:
Carșai T. C., 2017 Investigation of the galactosyltransferase (GALT) gene polymorphism in Romanian Black and White and Romanian Grey Steppe cattle breeds. ABAH Bioflux 9(2):59-64.