Molecular analysis of *GALT* gene in Argentinian population: Correlation with enzyme activity and characterization of a novel Duarte-like allele

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ABSTRACT

Background: Classical galactosemia is an autosomal recessive inherited metabolic disorder caused by mutations in the galactose-1-phosphate uridyltransferase (*GALT*) gene. GALT enzyme deficiency leads to the accumulation of galactose-1-phosphate in various organs, causing hepatic, renal and cerebral impairment. Over 300 mutations have been reported in the *GALT* gene. The aim of this study was to describe molecular characterization of *GALT* gene in Argentinian patients with decreased GALT activity, and to correlate molecular results with enzyme activity.
Methods: 37 patients with enzyme activity below 6.3 μmol/h/g Hb (35% of normal value) were included. GALT activity was measured on red blood cells. DNA was extracted from peripheral blood. p.Gln188Arg mutation was studied by PCR-RFLP and, on samples negative or heterozygous, *GALT* gene was sequenced. *In vivo* splicing analysis of the *GALT* gene was performed on RNA extracted from leukocytes of one patient.
Results: 14 different sequence variations were identified among 72 unrelated alleles. The two most common disease-causing mutations were p.Gln188Arg (24/72) and p.Lys285Asn (9/72). Three novel mutations were detected. One of them, c.688G>A, caused partial skipping of exon 9 of the *GALT* gene. Enzyme activity correlated with *GALT* genotype in 36 of the 37 patients.
Conclusion: This is the first report of sequence variations in the *GALT* gene in the Argentinian population. This study highlights the contribution of the molecular analysis to the diagnosis of Galactosemia and reveals c.688G>A as a novel Duarte-like variant, with a high prevalence in our population.

1. Introduction

Classical galactosemia (CG, OMIM 230400) is an autosomal recessive metabolic disorder due to deficient galactose-1-phosphate uridyl-transferase (GALT; EC 2.7.7.12), which catalyzes the reaction of galactose-1-phosphate with UDP-glucose to form glucose-1-phosphate and UDP-galactose [1,2]. CG is a potentially fatal disease that manifests within the first week of life with poor feeding, jaundice, vomiting, liver dysfunction, increased bleeding tendency, and septicemia, leading to death if untreated [2–4]. Early detection and management with dietary restriction of lactose containing foods can prevent infants from a deteriorating state and lead to rapid clinical improvement. However,

despite of early diagnosis by newborn screening and diet treatment, many patients develop long-term complications such as impairment of mental development, disorders of speech and motor function, and premature ovarian insufficiency [4–8]. GALT deficiency occurs with a prevalence of 1:40,000–60,000 in Europe and USA [4]. In Argentina, estimated from newborn screening (NBS) data, it is approximately 1:50,000 [9].

GALT enzyme is encoded by the *GALT* gene, which is located on chromosome 9 in the p13 region, with approximately 4 Kb of DNA sequence arranged into 11 exons [10,11]. The active enzyme is an 88 kDa homodimer, formed by 379 amino acids for each monomer [12]. More than 300 variations have been reported in the ARUP galactosemia

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database [13]. Most of them are classified as pathogenic, but there are also polymorphisms and variants of uncertain significance. The most common CG pathogenic variant among people with European ancestry is c.563A>G (p.Gln188Arg), which account for 64% of all galactosemic alleles, followed by c.855G>T (p.Lys285Asn). Both are associated with essentially null red blood cells (RCB) GALT activity and a poor outcome [13–16]. The presence of the c.404C>T (p.Ser135Leu) variant, frequent in individuals of African origen [14,17], determines an enzyme activity in RBC of 1–10% of normal, and is associated with a milder phenotype, probably due to a higher GALT activity in the liver and other tissues, like intestinal epithelial [3,4,18]. This variant, in homozygous state, is a common genotype of Clinical Variant Galactosemia. Upon dietary treatment, individuals with this form of galactosemia seem not to develop long-term complications [3,4]. Besides the deleterious mutations, the most common sequence variation is c.940A>G (p. Asn314Asp), which presents a frequency of approximately 8–11% in the European general population [14,19–21]. c.940A>G is associated with both Duarte-1 (D1 or Los Angeles) and Duarte-2 (D2) variant alleles, which are characterized by increased or decreased enzyme activity, respectively. In the D1 variant, c.940A>G is in linkage disequilibrium with the silent substitution c.652C>T, and in the D2, with three intronic variants, c.378-27G>C, c.507+62G>A, and c.508-24G>A, and the promoter deletion c.-119-116delGTCA [19,21]. The D2 allele in heterozygous with one CG allele causes Biochemical Variant galactosemia, also called Duarte galactosemia (DG). Individuals with this variant are usually asymptomatic, but are identified due to abnormal NBS, with moderate elevations in total blood galactose and 15–35% GALT activity [19,22].

The international clinical guidelines for the management of galactosemia [23] stated that diagnosis of CG should be confirmed by the measurement of GALT enzyme activity in RBC and/or *GALT* gene analysis, and recommend treating patients with RBC activity below 10% and/or pathogenic variants on both alleles of the *GALT* gene, including p.Ser135Leu. There is not enough evidence to conclude whether patients with 10–15% red blood cell residual GALT activity should or should not be treated. On the other hand, individuals with the Duarte variant should not be treated [24]. Therefore, it is important to differentiate those patients who will need treatment from those who will not, since both groups are detected by NBS [25].

The aim of this study was to describe molecular characterization of *GALT* gene in Argentinian patients with decreased GALT activity, and to correlate molecular results with enzyme activity.

2. Methods

Thirty six unrelated patients, and one sibling, were included in this study. Whole blood samples of the patients (21 males and 16 females) were collected. Twenty eight individuals were referred because of a positive newborn screening test (Total galactose >10 mg/dL on a dried blood spot sample) and enzyme activity in erythrocytes below 6.3 μmol/h/g Hb (35% of normal value). Nine patients had been diagnosed prior to the implementation of the NBS in 2007, with enzyme activity in erythrocytes below 1.8 μmol/h/g Hb (10% of normal value). Samples of the parents of 17 patients were also available.

2.1. Red blood cell GALT assay

The method is based on the uridinediphosphoglucose (UDPG) consumption test of Anderson *et al* [26] with suitable modifications in order to measure the transferase activity quantitatively. Hemolysate of RBD is incubated with galactose-1-phosphate and UDPG in suitable excess, the reaction is stopped and then the residual UDPG is determined spectrophotometrically by measuring the NADH formed after incubation with uridine diphosphoglucose dehydrogenase: the increase in absorbance at 340 nm is proportional to the residual UDPG. Patients with activity values between 0 and 0.5 μmol/h/g hemoglobin (Hb) are considered as

galactosemic, between 0.6 and 1.5 μmol/h/g Hb as possible galactosemic, between 1.6 and 18 μmol/h/g Hb as carriers of a pathogenic variant and activity values greater than 18 μmol/h/g Hb as normal.

2.2. Molecular analysis

Genomic DNA was obtained from peripheral blood leukocytes by the salting out method [27]. For *GALT* gene analysis, samples were first screened for the presence of the most common pathogenic variant c.563A>G (p.Gln188Arg) in exon 6, using polymerase chain reaction (PCR) based restriction fragment length polymorphism technique (RFLP) with *MspI* restriction enzyme. Samples negative or heterozygous for c.563A>G (p.Gln188Arg) were sequentially sequenced using five pairs of primers [28] to amplify the 11 exons and the exon-intron boundaries of the *GALT* gene. The PCR-amplified DNA fragments were subjected to direct sequencing in an automatic capillary sequencing system ABI 3130 Genetic Analyzer (Applied Biosystems), using the Big Dye® terminator v1.1 cycle sequencing kit (Applied Biosystems) and the same primers as for PCR amplification. The results were analyzed using the FinchTV version 1.4.0 (Geospiza), and the sequences obtained were compared with the reference sequence NG_009029.2 from GenBank database. A triplex assay [29] was used to assess the presence of the common deletion, with two primers flanking the *GALT* gene and a third one located within the deletion. This protocol detects both the deleted and the normal alleles. PCR products were resolved by electrophoresis in 2% agarose gel and visualized by ethidium bromide staining. Nucleotides are numbered from the first adenine of the ATG translation initiation codon at position 117 in the *GALT* cDNA reference sequence NM_000155.4. Amino acid numbering starts with the translation initiator methionine as +1 in the reference sequence NP_000146.2.

2.3. In silico analysis

The following databases were utilized for the establishment of the novelty of the variants: ClinVar [30], ARUP-GALT [31] and HGMD [32], last accessed in July 2020. In order to predict damage effects of missense novel variants, the following bioinformatics tools were used: Mutation Taster [33], SIFT [34], and PolyPhen2 [35]. Mutation Taster predicts the effect of the alteration and the probability value of the prediction: a value close to 1 indicates a high possibility. The server also displays a score for the amino acid change, took from the Grantham Matrix, which may range from 0 to 215. Higher values indicate a more deleterious alteration. SIFT score ranges from 0 to 1. The alleles with scores lower than 0.05 are more confidently predicted to be deleterious; those greater than or equal to 0.05 are tolerated. PolyPhen2 estimates the probability of the missense mutation being damaging with a score ranging from 0 to 1. If it is smaller than 0.15, the allele is predicted to be benign, if the probability is larger than or equal to 0.15, the allele is predicted to be probably or possibly damaging. Sequence variants thought to have impact on the splicing were analyzed with Human Splicing Finder [36], SROOGLE [37] and SplicePort [38]. Human Splicing Finder uses algorithms based on either Position Weight Matrices (HSF) or Maximum Entropy principle (MaxEntScan). A higher HSF score implies a more potential splice site. A higher MaxEntScan score implies a higher probability the sequence being a true splice site. SROOGLE algorithms for the acceptor site are based also on MaxEntScan and on the position-specific scoring matrix (PSSM) method, in which a higher score implies a more similar splice site sequence with the consensus sequence. Finally, SplicePort uses a machine learning approach to identify sequence features. Higher scores imply a more precise prediction of splice site.

2.4. In vivo splicing analysis

Total RNA was isolated from patient and control individual lymphocytes with TRIZOL. RNA was reverse transcribed and PCR analysis was performed using a pair of primers that amplifies a fragment from the

end of exon 7 to the beginning of exon 10 [39]. PCR products were resolved using the Qiagen QIAxcel® capillary electrophoresis system, and the results were analyzed with the QIAxcel® ScreenGel software 1.3.0. After separation by 1.2% agarose gel electrophoresis, PCR products were purified and analyzed by direct sequencing with the same primers as for PCR.

2.5. Statistical tests

Kruskal-Wallis test with *post-hoc* analysis was applied for comparison GALT enzyme activity among patients and carriers with different *GALT* genotypes using SPSS statistical package. A *p* value of 0.05 or less was considered significant.

3. Results

The biochemical characteristics of the 37 patients included in this study are summarized in Table 1. Sixteen patients had CG; median enzyme activity in this group was 0, with a maximum of 1.1 μmol/h/g Hb (6% of normal activity). Among the twenty one individuals with enzyme activity in the DG range, the mean was 3.3 μmol/h/g Hb (18% of normal activity), ranging from 0.5 to 5.7 (3 to 32% of normal activity).

Sequencing analysis of *GALT* gene identified at least one variant in each of the 72 alleles of the 36 non related patients. Fourteen different sequence variations were identified, being c.563A>G (p.Gln188Arg) (24/72) and c.855G>T (p.Lys285Asn) (9/72) the two most frequent pathogenic variants detected. D2 allele was found in 18/72 alleles,

always in *trans* with a known pathogenic variant. Two patients were heterozygous for c.512T>C (p.Phe171Ser) and also for c.876G>A (p. Thr292=) and c.945T>C (p.His315=), which has been described previously [31]. In patient 17, molecular analysis of its parents confirmed that the three sequence variations were located on the same chromosome.

In two patients, sequencing of the complete coding region of the *GALT* gene revealed D2 variant in homozygous state. These individuals would be predicted to have approximately half the normal GALT enzyme activity. However, their GALT activities were 3.3 and 5.5 μmol/h/g Hb, both values more consistent with C/D2 genotypes. Molecular analysis identified one of the parents of each patient as carrier of D2 allele, whereas the other resulted negative, thus the presence of a *GALT* deletion was suspected in both patients. This condition was confirmed with a duplex PCR and by sequencing of the deletion breakpoints. The rearrangement found in these two patients corresponded to the one already described [29], a 5458 bp deletion covering exons 1 to 11 with the inclusion of a 117 bp sequence from exon 8 to intron 8 region, and a 12 bp sequence of unknown origin. Afterwards, DNA sample of a patient with undetectable GALT activity failed to amplify. Then, a homozygous gene deletion was suspected, which was confirmed as described below.

Three novel variations were identified in this study: c.601C>A (p.Arg201Ser), c.666C>A (p.Ser222Arg) and c.688G>A (p.Glu230Lys). None of them were found neither in ClinVar nor HGMD, but have been submitted by the present authors to the ARUP-GALT mutation database. *In silico* results for each novel variant are shown in Table 2.

The c.601C>A (p.Arg201Ser) variant was detected in two female

Table 1
Genotype and GALT activity of the patients.

N°	Allele 1	Allele 2	Genotype	GALT activity		Type of Galactosemia
				μmol/h/g Hb	%	
1	c.820+13A>G	c.820+13A>G	C/C	0	0	Classical
2	p.Lys285Asn	p.Lys285Asn	C/C*	0	0	Classical
3	p.Lys285Asn	p.Lys285Asn	C/C	0	0	Classical
4	p.Gln188Arg	p.Leu195Pro	C/C*	0	0	Classical
5	p.Gln188Arg	c.820+13A>G	C/C*	0	0	Classical
6	p.Gln188Arg	p.Lys285Asn	C/C	0	0	Classical
7	p.Gln188Arg	p.Gln188Arg	C/C	0	0	Classical
8	p.Gln188Arg	p.Ser222Arg	C/C	0	0	Classical
9	p.Gln188Arg	p.Arg201Ser	C/C*	0	0	Classical
10	p.Arg148Trp	c.2T>C	C/C	0	0	Classical
11	p.Ser135Leu	p.Tyr209Cys	C/C	0	0	Classical
12	Del GALT	Del GALT	C/C	0	0	Classical
13	p.Gln188Arg	p.Gln188Arg	C/C	0.2	1	Classical
14	p.Gln188Arg	p.Lys285Asn	C/C	0.5	3	Classical
15	p.Gln188Arg	p.Lys285Asn	C/C	0.5	3	Classical
16	p.Gln188Arg	p.Arg201Ser	C/C*	1.1	6	Classical
17	p.Phe171Ser	D2 allele	C/D2*	0	0	Classical
18	p.Gln188Arg	D2 allele	C/D2	0.5	3	Duarte
19	p.Gln188Arg	D2 allele	C/D2*	1	6	Duarte
20	p.Gln188Arg	D2 allele	C/D2*	2.3	13	Duarte
21	p.Gln188Arg	D2 allele	C/D2	2.5	14	Duarte
22	p.Lys285Asn	D2 allele	C/D2	2.9	16	Duarte
23	Del GALT	D2 allele	C/D2*	3.3	18	Duarte
24	p.Phe171Ser	D2 allele	C/D2	3.3	18	Duarte
25	p.Gln188Arg	D2 allele	C/D2*	3.4	19	Duarte
26	p.Arg148Trp	D2 allele	C/D2	3.4	19	Duarte
27	p.Leu195Pro	D2 allele	C/D2*	3.5	19	Duarte
28	p.Gln188Arg	D2 allele	C/D2*	3.5	19	Duarte
29	p.Gln188Arg	D2 allele	C/D2*	3.5	19	Duarte
30	p.Lys285Asn	D2 allele	C/D2	3.7	21	Duarte
31	p.Gln188Arg	D2 allele	C/D2*	3.8	21	Duarte
32	p.Gln188Arg	D2 allele	C/D2	3.9	22	Duarte
33	Del GALT	D2 allele	C/D2*	5.5	31	Duarte
34	p.Gln188Arg	D2 allele	C/D2*	5.7	32	Duarte
35	p.Gln188Arg	c.688G>A	G/?	2.3	13	Duarte like
36	p.Gln188Arg	c.688G>A	G/?*	5.5	31	Duarte like
37	p.Gln188Arg	c.688G>A	G/?*	n.a.	–	Duarte like

n.a.: GALT activity value not available, referred as low, in DG range.
  * variant phase confirmed by parental analysis.

Table 2

In silico predictions for novel variants in the *GALT* gene.

Mutation		Mutation Taster			SIFT		PolyPhen2	
c.DNA	Protein	Prob.	Prediction	Grantham	Score	Prediction	Score	Prediction
c.601C>A	p.Arg201Ser	0.73	Disease causing	110	0.14	Tolerated	0.286	Benign
c.666C>A	p.Ser222Arg	0.99	Polymorphism	110	0	Damaging	0.063	Benign
c.688G>A	p.Glu230Lys	0.99	Disease causing	56	0.06	Tolerated	0.217	Benign

siblings with c.563A>G (p.Gln188Arg). The older sister acceded to the molecular analysis of the *GALT* gene because of a positive newborn screening test and undetectable GALT enzyme activity in red blood cells. The genotype was confirmed through molecular analysis of her parents. The second sister, a year younger, also presented with a positive newborn screening test but a GALT enzyme activity of 1.1 μmol/h/g Hb. A different change at the same DNA position and a variant that alters the same amino acid are described as pathogenic variants in ARUP-GALT database.

The c.666C>A (p.Ser222Arg) variant was found with c.563A>G (p. Gln188Arg) in a patient clinically diagnosed at the age of one month. GALT enzyme activity was null.

The c.688G>A variant was detected in three unrelated patients, always with c.563A>G (p.Gln188Arg), that were referred to the molecular analysis of *GALT* gene because of a positive newborn screening test. Enzyme activity was available in two of them, being 5.5 and 2.3 μmol/h/ g Hb respectively. In the former patient, and in the third one, DNA samples of both parents were available to confirm that the novel mutation and c.563A>G (p.Gln188Arg) were *in trans*. The nucleotide change could result in a missense mutation, causing an exchange of a glutamic acid for lysine at amino acid residue 230, but, as it is located in the first nucleotide position of exon 8, could also affect the splicing at the 3′ splice site. Two of three *in silico* tools predicted the missense change as benign, meanwhile through the splice site analysis, the nucleotide change was predicted to reduce the strength of the exon 8 splice donor site and likely cause decrease of the correctly spliced RNA (Table 3). RT-PCR was performed on RNA samples of patient 2 and a control. Control individual presented the expected fragment (378 bp) including the end of exon 7, complete exons 8 and 9 and the beginning of exon 10, and a very faint fragment (294 bp), resulting from an alternative splicing event with skipping of exon 9. Patient profile presented the 378 bp and the faint 294 bp fragments resulting from transcription of both alleles, the one yielding the c.688G>A and the wild type for that position, and another faint fragment (161 bp), resulting from the skipping of both exons 8 and 9. No fragment without exon 8 but including exon 9 was detected (Fig. 1). *In vivo*, c.688G>A might induce a frameshift in the open reading frame and introduce an premature termination codon 57 amino acids downstream position 229 of the protein.

GALT enzyme activities of patients and parents, expressed as percentage of normal value, 18 μmol/h/g Hb, were stratified according to the genotype (Fig. 2). Among the 16 patients with C/C genotype, 3 presented with outlier activity values above the median, and among the 18 patients with C/D genotype, 2 presented activity values above and 3 below the outlier limits. GALT activity of CG patients was significantly different from DG individuals ($p = 0.014$), and from C and D carriers ($p < 0.001$). No significant differences were found in GALT activity between C carriers and D carriers ($p > 0.05$), and between these two and DG individuals. In one patient (N°17 from Table 1) enzyme activity did

not correlate with *GALT* genotype, since a 15–35% of activity is expected for a C/D genotype. This could be due to an heterozygous deletion not detected by the PCR-based method used, or a deep intronic variation not covered by the molecular analysis.
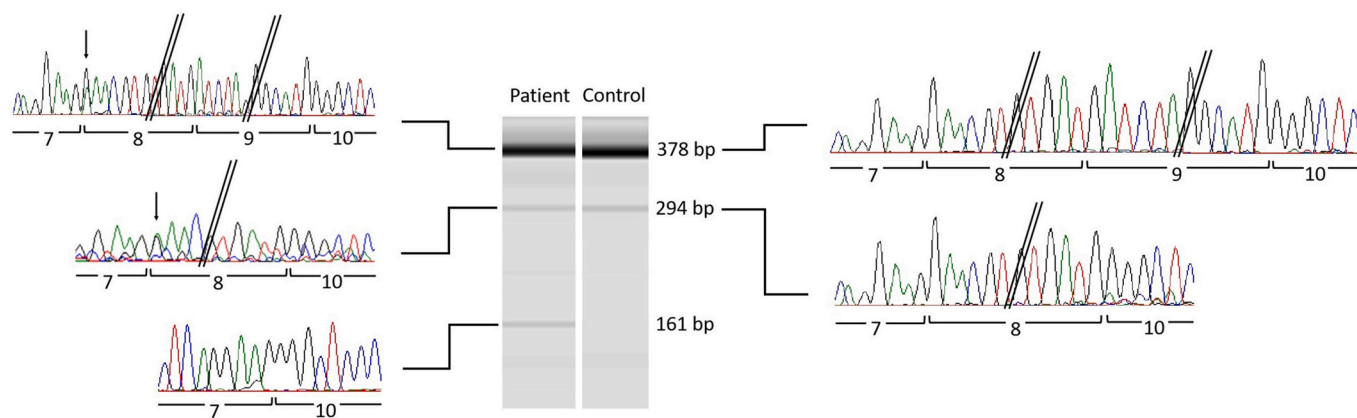
4. Discussion

Classical Galactosemia is an autosomal recessive disorder caused by a deficiency of GALT enzyme, which catalyzes the metabolism of galactose-1-phosphate to uridine diphosphate galactose. The resulting elevation of gal-1-phosphate leads to intellectual retardation, liver dysfunction and cataract formation [2,3]. Classic, Clinical Variant and Duarte galactosemia are caused by mutations in the *GALT* gene. More than 300 mutations have been described, and their frequencies vary among different populations [4]. In this study, we performed biochemical and molecular characterization of 37 Argentinian individuals with low GALT enzyme activity.

We identified in our cohort of patients a total of 14 different variations on *GALT* gene, 3 of which were novel variations. As expected, c.563A>G (p.Gln188Arg) was the most frequent pathogenic mutation detected in this cohort, followed by c.855G>T (p.Lys285Asn), accounting for 33% and 13% of the alleles, respectively. These prevalences are lower than the ones reported for Caucasian populations in general [13–16], although similar to those found in Italian patients [40]. The third most frequent pathogenic mutation was the 5.5 Kb deletion, which has shown a high carrier rate in Ashkenazi Jewish population [29,41]. In this study, two patients were heterozygous for this pathogenic variant, and one was homozygous. The second most frequent mutation in Portuguese patients, c.820+13A>G [42], was detected in three galactosemic alleles, and two prevalent mutations among African patients, c.404C>T (p.Ser135Leu) and c.512T>C (p.Phe171Ser) [12], were found in this cohort once and twice, respectively. Finally, as far as we know, c.2T>C has been only reported in a Brazilian cohort of galactosemic patients [43]. These data is consistent with the heterogeneous genetic background described for our population. To our knowledge, there are no additional reports available about molecular analysis of *GALT* gene in other South American countries.

Regarding the three novel mutations found in this study, two of them belonged each one to a single family, meanwhile the third one was identified in three unrelated patients. According to the ACMG/AMP 2015 guidelines [44], p.Arg201Ser is classified as pathogenic, since two variants affecting the same amino acid are also classified as pathogenic, and p.Ser222Arg as likely pathogenic, taking into account that the patient had no enzyme activity, which allows us to consider the PP4 ACMG/AMP rule [44].

Three patients were suspected of carrying the D2 allele because of their GALT enzyme activities, but resulted negative for that variant. Instead, the same novel variant was identified among them, c.688G>A, in heterozygous with c.563A>G (p.Gln188Arg). This variant affects the first G nucleotide of exon 8, thus probably disrupting the 3′ splicing site, one of the essential splicing *cis*-elements. Such type of mutations has been scrutinized in different genes [45,46]. In some cases, has been reported to cause aberrant splicing, while in others has been reported to have no effect on it. It has been hypothesized that in introns with a short polypyrimidine tract (PPT), *i.e.* less than 9 nucleotides long, changing the first G nucleotide of the following exon causes aberrant splicing, meanwhile a long PPT, *i.e.* from 10 to 15 nucleotides or more, makes a

Table 3

In silico splicing analysis of c.688G>A.

		Wild Type	Variant	Variation
HSF		85.25	82.12	−3.67%
SROOGLE	Max Ent	10.6	9.08	−14.34%
	PSSM	87.44	86.35	−1.25%
SplicePort		0.973	0.248	−74,47%

Fig. 1. Transcript analysis of lymphocytes of a control and a patient heterozygous for the c.688G>A variant.
The size of the transcripts detected in each sample are indicated, along with their sequence Arrows indicate the position of the c.688G>A variant.
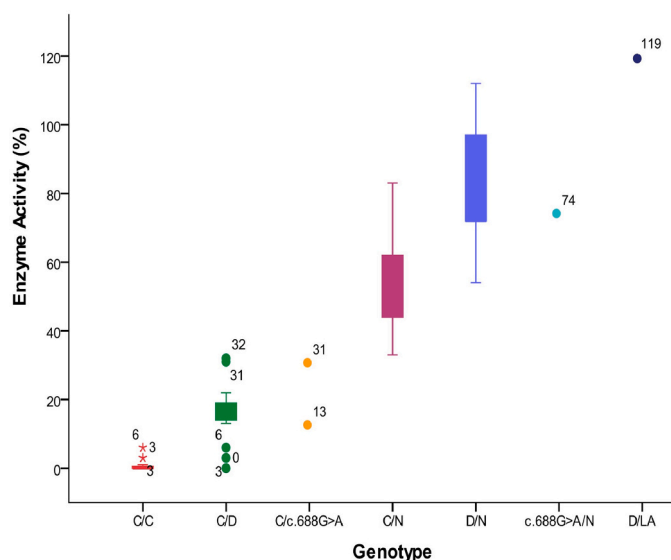
Fig. 2. Box plot showing the distribution of GALT activity among genotype groups.
GALT activity is expressed as percentage of 18 μmol/h/g Hb. Outliers values, those above or below more than 1.5 times the inter-quartile range on either side of the box, are indicated. GALT activity of 2 individuals carrying both p. Gln188Arg and c.688G>A are represented with circles, as well as of a c.688G>A carrier and a Duarte 2 and Los Angeles variants carrier. C: CG allele, D: DG allele, N: Wild Type allele, LA: LA allele.

variant at the first G of the exon immune to splicing errors. This rule would be applicable only when the last nucleotide of the exon is a G [47]. In this case, the longest uninterrupted stretch of pyrimidines at the PPT of exon 8 is 7 nucleotides, which would make the splicing of exon 8 susceptible to variants at the first G nucleotide. When studying RNA sample from one of the patients carrying the c.688G>A novel mutation, two mRNA species were found: one with the point mutation and one with the aberrant splicing of exons 8 and 9, along with the normally spliced RNA from the opposite allele, which carried the c.563A>G (p. Gln188Arg) mutation. Causing alternative splicing rather than aberrant splicing of the RNA has been described for other mutations at the first nucleotide of the exon [48]. This would be consistent with the GALT enzyme activity detected in the three patients of our study, of approximately 25%. The evidence suggests that c.688G>A should be considered as a Duarte2-like variant, causing a DG-like form in a different mechanism from the proposed for the D2 variant.

Duarte Galactosemia is a milder form, caused by the combinations of a CG allele and a D2 allele. DG individuals retain about 25% of normal GALT enzyme activity, and are usually asymptomatic [19–21]. In this study, D2 allele was identified in 18 children, always with a known pathogenic mutation, being c.563A>G (p.Gln188Arg) and c.855G>T (p. Lys285Asn) the most frequent ones. All individuals with DG were detected through newborn screening test, and showed no symptoms at the time of diagnosis.

Previous studies have reported opposite opinions on whether DG individuals could present or not long term developmental complications seen in CG [49,50]. Recently Carlock *et al* found no evidence of increased risks for developmental complications among a larger cohort of children with DG regardless of milk exposure in infancy [24]. In our country, once DG is confirmed by enzyme activity and/or molecular analysis after a NBS positive result, preventive dietary restriction of galactose is discontinued. Patients with Duarte-like variants, or those in which pathogenicity remain unclear, and enzyme activity in the DG range, are clinically evaluated: if symptomatic, dietary restriction of galactose is resumed, if not, dietary intake of galactose is gradually liberalized.

The present study reaffirms that, although the difference in GALT activity between CG patients and DG individuals was statistically significant, the measurement of enzyme activity was not enough to make a decision about treatment, as, especially for DG individuals, it was highly variable and with overlapping values between both groups. It is important to distinguish CG from DG, in order to avoid unnecessary treatments following newborn screening [23]. Furthermore, in light of the high incidence of Duarte and Duarte-like variants in our population, it would be recommended to review the present strategy of newborn screening, incorporating enzyme activity on dried blood spots. Eventually, current cut off values could be discussed as well. This could be beneficial for not only infants and their families, in order to avoid the stress of a positive NBS, but also for the public health system, minimizing unnecessary follow-up procedures.

5. Conclusions

This is the first report of sequence variations in the *GALT* gene in the Argentinian population. This study highlights the contribution of the molecular analysis to the diagnosis of Galactosemia, especially in those cases where enzyme activity is not enough to make a treatment decision. Moreover, it reveals a novel Duarte-like variant, with a high prevalence in our population.

Declaration of Competing Interest

None.

Acknowledgments

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