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CASE REPORT

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Clinical and biochemical evolution after partial dietary liberalization of two cases of galactosemia due to galactose mutarotase deficiency

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Abstract

Background The recommended diet attitude in the recently described galactose mutarotase (GALM) deficiency is not yet established. We describe two 9-years twins who remain asymptomatic despite prolonged partial dietary liberalization from 18 months of age, after two periods of galactose-free diet. It represents the second report in Europe of GALM deficiency.

Case presentation Two male monochorionic diamniotic twins were detected through newborn screening by galactosuria and increased total blood galactose. They started galactose dietary restriction with biochemical normalization. After exclusion of the three previously described types of galactosemia, a progressively galactose reintroduction was initiated. The clinical follow-up developed include neurological assessment and intelligence quotient, annual ophthalmological evaluation and biannual abdominal ultrasound; whereas the biochemical assessment comprises quarterly determinations of galactose 1-phosphate and galactosuria and annual determination of liver and renal function, 25-OH-vitamin D and calcium levels. Sanger sequencing of GALM gene was complemented by the study of gene dose using SNPs array and a protein modeling to study the conformational changes induced in GALM protein. In both siblings a novel and complete deletion of exon 4 in GALM gene was detected. Both remained asymptomatic, with normal growth and intellectual development, despite dietary liberalization. Evolutionarily, the biochemical profile in blood remained normal with intermittent galactosuria.

Conclusions The absence of clinical involvement after 7 years of dietary liberalization is interesting to expand the knowledge about the recommended dietary management in this pathology.

Keywords Type IV galactosemia, GALM deficiency, Galactose mutarotase deficiency

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Background

Galactosemia is an inborn error of metabolism due to disturbances in different stages of galactose metabolism related to a deficiency in one of the four enzymes of the Leloir pathway [1].

We present the second description in Europe [2] of galactosemia due to galactose mutarotase (GALM) deficiency, also known as aldose 1-epimerase deficiency or type IV galactosemia (OMIM # 618881). This entity, described in 2019 [3], is due to the defect of the enzyme that catalyzes the first step of the referred pathway consisting of epimerization between β- and α-D-galactose [4, 5]. The clinical spectrum of the few reported patients suggests that it is similar to galactosemia due to galactokinase (GALK) deficiency, with mild symptoms and risk of early cataracts [3, 6].

The recommended treatment for classic galactosemia due to galactose-1-phosphate uridyltransferase (GALT) deficiency, for GALK deficiency and for UDP-galactose 4-epimerase (GALE) deficiency is dietary restriction of lactose. Nevertheless, the diet attitude in this apparently benign form of type IV galactosemia, recently described, is not yet established. We present two 9-year-old twins diagnosed with galactosemia by newborn screening (NBS) with subsequent confirmation of this type IV galactosemia. They remain asymptomatic despite partial progressively dietary relaxation after 18 months of life, in line with other patients reported with early diagnosis who follow a diet without galactose restriction [2].

Case presentation

Patients

Two monochorionic diamniotic male siblings, currently 9-years old, born at term (38 weeks) from non-consanguineous parents. One of the siblings, referred as Patient 2, was small for gestational age (birth weight: 1920 g) and needed respiratory support with continuous positive airway pressure during 24 h after birth due to transient tachypnea. In both, urinary excretion of galactose was detected by NBS in the second sample developed at 15 days of life, with normal blood galactose-1-phosphate (Gal 1-P) value in both NBS samples, and increased blood total galactose (TG) determination in the second sample. NBS in our community (Galicia, Spain) comprises generally a single dried-blood and urine samples collected between 48 and 72 h of life, after the start of sufficient oral intake. In some conditions, as in premature newborns < 34 weeks of gestational age; birth weight < 2000 g; and monochorionic twins, a second sample is required between 2nd and 4th week of life in order to minimize the risk of false negatives. Hexoses monophosphate determination is carried out by mass spectrometry (MS/MS)(Applied Biosystems Sciex API 2000) [7] and urine galactose is first by determining reducing substances and, in case of a positive result, thin layer chromatography for galactose is performed as confirming technique [8]. The quantification of blood TG is employed as a second-level test.

With the initial suspected diagnosis of galactosemia due to GALK deficiency, dietary treatment was established with galactose-restricted soy-based diet with consequent normalization of galactosuria and blood TG levels. Given the negative result of the genetic study by massive sequencing of the *GALT*, *GALK1* and *GALE* genes; the normal enzymatic activity of GALT, GALK1 and GALE; and the absence of data suggestive of secondary galactosemia, a free diet was started at 6 months of age with reappearance of galactosuria. Once again, a galactose-free diet was indicated for one year. They maintained during this period a normal ophthalmological examination and normal liver function tests.

Additionally, Patient 2 presented two episodes of febrile status epilepticus, the first one at 12 months of age during a urinary infection by Proteus mirabilis with normal cerebrospinal fluid study and cerebral magnetic resonance imaging, and the second one at 14 months related with a respiratory infection by Influenza A. Treatment with valproic acid was established until 3 years of age with subsequent withdrawal, without presenting new convulsive episodes.

After the description of galactosemia due to GALM deficiency the genetic study was extended by Sanger sequencing of the 7 exons of *GALM* gene and complemented by the study of gene dose using SNPs arrays by CytoScan XON of Affymetrix. Sanger sequency enabled the identification that both patients are compound heterozygous for two different deletions in *GALM* gene: a heterozygous deletion comprising exons 1–4 arr[hg19] 2p22.1(38,893,070 – 38,925,887) in the maternal allele, and a heterozygous deletion comprising exon 4 arr[hg19] 2p22.1(38,916,650 – 38,925,887) in the paternal allele, entailing, as illustrated in Fig. 1, an homozygous deletion of complete exon 4 of *GALM* gene in both siblings. Segregation studies performed by array confirm that both parents are heterozygous carriers of one of the deletions identified in their offspring.

Clinical and biochemical evolution after diet liberalization

From 18 months of age a progressive dietary liberalization was established with gradual incorporation of milk and dairy products (cheese and yoghurt) until daily intake. They currently receive an estimated contribution of 10.6 g / day of galactose (0.38 and 0.41 g/kg/day respectively). The clinical follow up of the described patients include annual ophthalmological evaluation, neurological assessment and intelligence quotient (IQ), and biannual abdominal ultrasound. In the follow-up period quarterly quantitative determinations of Gal 1-P,

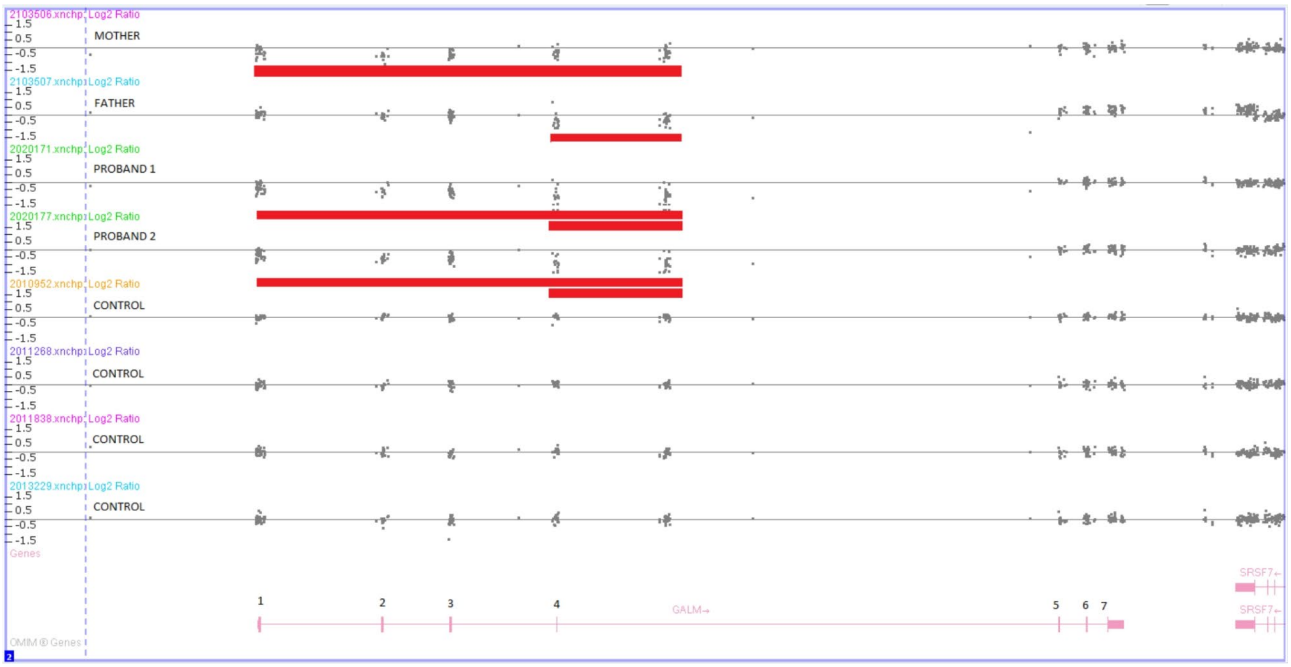

Fig. 1 Deletions of *GALM* gene in both siblings and their parents compared with normal controls. To facilitate the understanding of the image, the extension of the deletion is outlined in red

TG and qualitative galactosuria were performed, complemented with annual liver and renal function, plasma 25-OH-vitamin D levels and calcium assessment.

Both siblings remained asymptomatic, with normal blood Gal 1-P and TG levels and intermittent galactosuria (Table 1). They show a normal growth and cognitive function, with psychomotor development according to age. No ophthalmological, liver or kidney involvement was observed during follow-up and both maintain evolutionary normal abdominal ultrasounds and normal plasmatic 25-OH vitamin D and calcium levels. Currently, IQ of both cases is >110.

Discussion and conclusions

GALM gene encodes an enzyme composed of 342-amino acid that plays a key role by catalyzing the interconversion of beta-D-galactose and alpha-D-galactose during galactose metabolism [4]. Beta-D-galactose is metabolized in the liver into glucose 1-phosphate, the primary metabolic fuel, by the action of four enzymes that constitute the Leloir pathway: GALM, GALK1, GALT and GALE. Human GALM is monomeric and its catalytic mechanism requires a histidine residue acting as an acid, and a glutamate acting as a base. Together, these residues open the pyranose ring of d-galactose enabling free rotation of the bond between the first two carbon atoms in the monosaccharide. This can cause reversal of the configuration of the hydroxyl group attached to carbon [5]. The encoded protein is expressed in the cytoplasm and has a preference for galactose and is involved in the maintenance of the equilibrium between the beta- and alpha-anomers of galactose [6].

Three-dimensional structure study of human galactose mutarotase [4] shown that the sugar ligand sits in a shallow cleft and is surrounded by Asn-81, Arg-82, His-107, His-176, Asp-243, Gln-279, and Glu-307. Both the side chains of Glu-307 and His-176 act as a catalytic base and a catalytic acid, respectively. The deletion in exon 4 in our patients, causes the loss of three amino acids (Asp-243, Gln-279 and Glu-307), being the last part of the catalytic center of the enzyme.

GALM deficiency was initially described in 2019 [3]. The current carrier frequency estimated in European non-Finnish population is 1:655, and the estimated incidence of this entity in all populations is 1:228,411, higher in African population (1:10,388). Until date 42 cases were described, mainly in Japanese population [3, 9] with only two previous cases in Europe [2]. Table 2 summarizes the clinical and biochemical features of the reported patients with GALM deficiency, except for the data from the Mikami-Saito et al. report [9]. So far, in three patients (7%) [2, 3, 9, 10] the development of cataracts was prior to the start of galactose-restricted diet. An improvement in cataracts has been described in some patients after starting lactose-free diet. Transient cholestasis (2/43) and mild transaminitis (10 /43) are also documented findings in GALM patients, but globally, none developed liver or growth failure, or neurodevelopment involvement [11].

The biochemical profile in NBS in our patients, in line with previous NBS studies in Japan [9, 12], show

Table 1 Clinical and biochemical patient characteristics before and after galactose diet liberalization

	Patient 1				**Patient 2**			
Epidemiological characteristics								
Sex	Male				Male			
Gestational age, weeks	38				38			
Birth weight, g	2570				1920			
Current age, years	**9**				**9**			
	TG (mg/dL)	**Gal 1–P (mmol/L)**	**Galactose in urine**	**HF**	**TG (mg/dL)**	**Gal 1–P (mmol/L)**	**Galactose in urine**	**HF**
At newborn screening								
First sample	11	0.38	-	N	18.9	0.33	-	N
Second sample	21.6	0.12	+	N	23.7	0.15	+	N
First period with galactose-free diet								
3 m	9	0.04	-	N	7.7	0.06	-	N
6m	17.4	0.03	-	N	15.2	0.06	-	N
First period of dietary galactose introduction								
7m	12.1	0.04	+	N	13.4	0.03	+	N
Second period with galactose-free diet								
12m	-	0.03	-	N	-	0.03	-	N
18m	14.2	0.02	-	N	10	0.03	-	
Second period with dietary galactose intake								
21m	9.8	0.03			8.7	0.02		
24m	15.7	0.09	-	N	12.3	0.09	+	N
27m	9.7	0.03	-		9.6	0.02	+	
2.5y	15.7	0.05	-	N	14.6	0.06	+	N
3 y	7	0.07	-		4.9	0.08	-	
3.5y	9.7	0.06	+	N	8.2	0.07	-	N
4.5y	11	0.09	+		8.6	0.07	+	N
5.5y	8.5	0.03	-	N	6.8	0.11	+	N
6.5y	8.8	0.03	-		5.1	0,04	-	N
7.5y	10.9	0,03	-	N	8.9	0,03	-	N
8 y	3.4	0,05		N	2.6	0,04	-	N

Gal 1-P: galactose 1-phosphate; HF, hepatic function; M: months; N: normal; TG, total galactose; y: years. Normal value for Gal 1-P: <0.7mmol/L and for TG < 18 mg/dL. The determination of galactose in urine is qualitative

mild elevation of TG and detection of Gal 1-P without exceed cut-off value (0.12–0.38, cut-off level: 0.7mmol/L), with a declination of Gal 1-P after neonatal period. This trend does not change in our patients despite the slight dietary liberalization. The initial high TG levels in neonatal period normalize with diet restriction in both twins and persisted within normal levels after galactose diet reintroduction.

GALM deficiency is considered as a relatively mild entity with a symptomatology most like as type II galactosemia [6]. The main clinical manifestation observed is the development of cataracts with an estimated risk of 11.9% (5/42), according the current casuistry, including our patients. Four Japanese patients [9] and one of the two Turkish siblings) [2] developed cataracts involvement.

Although, given that all the patients described are children so the risk of long-term complications cannot be well established, the indication for dietary treatment in asymptomatic patients is not clear. In our series, after 7.5 years of galactose dietary reintroduction, both patients remain asymptomatic with normal ophthalmological explorations. Recently ß-galactosidase was suggested as a potential novel treatment agent to reduce blood galactose increase caused in lactose loading, that could contribute to mitigate the galactose dietary restrictions despite unknown efficacy in preventing or treating complications [13].

Agreed dietary-therapeutic recommendations are necessary for GALM deficiency management. The evolutionary absence of clinical manifestations after prolonged partial dietary reintroduction of galactose in our patients provides valuable information to delve into the dietary treatment of this pathology.

Table 2 Characteristics of the reported patients with GALM deficiency

		Gender	Age at GALM deficiency diagnoses	Type of galactosemia diagnosis	Follow-up period	*GALM*variants	Clinical manifestations						Biochemical profile			Free diet	Dietary restriction period
							Asymp	Cataracts	HI	Cholestasis	GI	NI	TG	Gal 1-P	Galactose		
Wada *et al.* (2018) [3, 13]	1	M	NR	NBS	2y	c.244C > T/c.294delC		+	-	-	-	-	21.3	8.9	NR		1m- UR*
	2	F	NR	NBS	4y	c.294delC/c.799C > G	+	-	-	-	-	-	17.6	8.9	NR		4m -18m
	3	M	NR	NBS	1y	c.294delC/c.294delC		-	-	Transient	-	-	18.5	6.6	NR		1m - UR
	4	F	NR	NBS	2y	c.932G > A/c.932G > A	+	-	-	-	-	-	12.1	2.4	NR		1m-18m
	5	M	NR	NBS	2y	c.424G > A/c.424G > A	+	-	-	-	-	-	20.8	10.8	NR		2m- UR*
	6	F	NR	NBS	4y	c.424G > A/c.424G > A	+	-	-	-	-	-	13.1	0.3	NR		2m-UR
	7	M	NR	NBS	12y	c.424G > A/c.799C > G		-	-	Transient	-	-	26.1	10.4	NR		1m-11y*
	8	M	NR	NBS	1y	c.424G > A/c.424G > A		+	-	-	-	-	18.1	6.7	NR		3w-UR*
Yacizi *et al.*(2021) [2]	9	F	3 m	Clinical	3 y	c.829G > A/c.829G > A		+	Mild	-	-	-	High	High	NR	3m	33m
	10	M	6y	FI	NR	c.829G > A/c.829G > A	+	-	-	-	-	-	N	N	NR	6y	0
Sánchez-Pintos *et al.* (2024)	11	M	5y	NBS	9y	arr[hg19] 2p22.1(38,893,070 – 38,916,650) X1;2p22.1 (38,916,650 – 38,925,887)x0	+	-	-	-	-	-	11/21.6	10.1/3.1	Normal/ Elevated	7y	18m
	12	M	5y	NBS	9y	arr[hg19] 2p22.1(38,893,070 – 38,916,650) X1;2p22.1 (38,916,650 – 38,925,887)x0	+	-	-	-	-	-	18.9/23.7	8.6/3.9	Normal/ Elevated	7y	18m

Asymp, asymptomatic; d, days; F,female, FI, familial study; Gal 1-P, galactose 1-Phosphate; GF, growth failure; HI, hepatic involvement; M, male; m: months; NBS, newborn screening; NI, neurological involvement; N, normal; NP, neonatal period; NR, not reported; TG, total galactose; UR, until report data; w: weeks. To facilitate comparation Gal 1-P levels of our patients are expressed in mg/dL. TG values from Wada et al., 2018[3] were calculated as galactose + Gal 1-P

The asterisk (*) in the section Dietary restriction period represent a failed attempt to relax the dietary restriction

Abbreviations

Gal 1-P	Galactose-1-phosphate
GALK	Galactokinase
GALE	UDP-galactose 4-epimerase
GALM	Galactose mutarotase
GALT	Galactose-1-phosphate uridyltransferase
NBS	Newborn screening
TG	Total galactose

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Author contributions
P.S-P and M.L.C contributed to conceptualization, design, analysis and writing of the original draft. M.J.C.G and B.M.L-P contributed to methodology and data curation. J.A.C.J, M.D.B., M.E.V-M, S.G., R.F.P. and F.B-A contributed to formal analysis and to the presentation of the results and helped draft the manuscript. All authors discussed, revised, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets analyzed during the current study are avaliable from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
In our autonomous community (Galicia, Spain), clinical cases are not subject to a request for approval by the Research Ethics Committee.

Consent for publication
Both parents gave written informed consent for publication.

Competing interests
The authors declare no competing interests.

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