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RESEARCH REPORT

Galactose Epimerase Deficiency: Expanding the Phenotype

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Abstract Galactose epimerase deficiency is an inborn error of metabolism due to uridine diphosphate-galactose-4′-epimerase (GALE) deficiency. We report the clinical presentation, genetic and biochemical studies in two siblings with generalized GALE deficiency.

Patient 1: The first child was born with a dysmorphic syndrome. Failure to thrive was noticed during the first year. Episodes of heart failure due to dilated cardiomyopathy, followed by liver failure, occurred between 12 and 42 months. The finding of a serum transferrin isoelectrofocusing (IEF) type 1 pattern led to the suspicion of a congenital disorder of glycosylation (CDG). Follow-up disclosed psychomotor disability, deafness, and nuclear cataracts.

Patient 2: The sibling of patient 1 was born with short limbs and hip dysplasia. She is deceased in the neonatal period due to intraventricular hemorrhage in the context of liver failure. Investigation disclosed galactosuria and normal transferrin glycosylation.

Next-generation sequence panel analysis for CDG syndrome revealed the previously reported c.280G>A (p.[V94M]) homozygous mutation in the *GALE* gene. Enzymatic studies in erythrocytes (patient 1) and fibroblasts (patients 1 and 2) revealed markedly reduced GALE activity confirming generalized GALE deficiency. This report describes the fourth family with generalized GALE deficiency, expanding the clinical spectrum of this disorder, since major cardiac involvement has not been reported before.

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Introduction

Uridine diphosphate-galactose-4′-epimerase (GALE) deficiency is a very rare disease of galactose metabolism and the Leloir pathway. This enzyme catalyzes the conversion of uridine diphosphate galactose (UDP-Gal) to uridine diphosphate glucose (UDP-Glc) (Segal and Berry 1995), as well as the reverse reaction when other sources of UDP-

Gal are scarce. GALE also plays a role in the interconversion of uridine diphosphate-*N*-acetylgalactosamine and uridine diphosphate-*N*-acetylglucosamine (Wohlers et al. 1999). These uridine diphosphate sugars are essential for the glycosylation of proteins and lipids, and their restriction can lead to the production and accumulation of hypogalactosylated glycans, which are suggested to contribute to the long-term complications in galactosemia (Charlwood et al. 1998). Thus, galactosemia is a cause of secondary hypoglycosylation (Sturiale et al. 2005).

The clinical severity of GALE deficiency ranges from benign to potentially lethal, depending on the degree of reduction of GALE activity and the tissues affected. The first reported case (Gitzelmann 1972) was described as a benign condition, a "peripheral" form of the disease, in which the GALE impairment was restricted to the circulating blood cells. In 1981, (Holton et al. 1981) described the first case of "generalized" GALE deficiency, a patient with a severe clinical presentation, similar to classic galactosemia, with reduced GALE activity in both circulating red blood cells and fibroblasts. As pointed out by Openo, GALE deficiency is not a binary condition but a continuum disorder (Openo et al. 2006). There are three forms currently recognized: (1) a peripheral form, with decreased enzyme activity in red blood cells and leucocytes, with normal or near normal levels in all other tested tissues; (2) an intermediate form, with decreased enzyme activity in circulating blood cells, and less than 50% in all other tissues tested; and (3) a generalized form, with a profound generalized decrease of enzyme activity (Fridovich-Keil 2013). The peripheral form is reported to occur with a frequency of 1:6,700 to 1:60,000 depending on the ethnic background (Alano et al. 1998), while the severe generalized form is extremely rare (Holton et al. 2000) with only three families (six patients) described (Holton et al. 1981; Sardharwalla et al. 1988; Walter et al. 1999; Sarkar et al. 2010).

The present report is on two sisters with different phenotypes, expanding the clinical spectrum of generalized GALE deficiency, and reviews the literature on this GALE deficiency form.

Patient Reports

Patient 1 (Fig. 1)

This first female child of non-consanguineous Caucasian parents was born after a full-term pregnancy. The mother was treated for hypothyroidism. The biochemical and ultrasound screening showed a high risk for trisomy 21, but there was a normal karyotype on amniocentesis.

The newborn was admitted to the NICU during the first week due to a dysmorphic syndrome: relative macrocephaly, hypertelorism, micrognathia, finger contractures, short limbs, hip dislocation, positional talipes, and ligament laxity. During the first year of life, failure to thrive, hypotonia, and psychomotor disability were noticed. Cerebral ultrasound and electromyography were normal.

At 12 and 16 months old, she was hospitalized in the PICU with heart failure due to dilated cardiomyopathy, followed by hepatomegaly and acute liver failure. She recovered under supportive therapy and was discharged on diuretic medication. Infectious etiologies were excluded, and pituitary hormones and brain MRI were normal.

At the ages of 18, 41, 42, and 43 months, she was hospitalized with heart decompensation that responded well to anticongestive heart therapy.

Results of other investigations included persistently raised serum aminotransferases (3–4 times the upper limit of normal) until 4 years old, negative urinary-reducing sugars, and unspecific alterations in urinary organic acids, plasma amino acids, and acylcarnitine profile. A diagnosis of congenital disorder of glycosylation (CDG) was hypothesized based on a high serum level of carbohydrate-deficient transferrin (CDT) on several occasions (3–10.2%; reference range <2.6%). Serum transferrin isoelectrofocusing (IEF) showed a type 1 pattern, and the fibroblast LLO (lipid-linked oligosaccharides) profile was normal (small mannose 2 glycan accumulation). Phosphomannomutase and phosphomannose isomerase activities in fibroblasts were normal, and there were no mutations in *ALG2, DPM3, DK1*, and *SRD5A3* genes.

During follow-up (Fig. 1a–c), on regular diet and till the present age of 12 years, the dysmorphic features attenuated. She showed a short stature (height, −2.9 SD; weight, −0.17 SD), global developmental disability with slow acquisitions (15–19 months at 10 years old on Vineland Adaptive Behavior Scales) (Sparrow et al. 2005), a behavior disorder controlled with risperidone, sensorineural hearing loss detected at 3 years old, and bilateral nuclear cataracts diagnosed and surgically treated at 8 years old. Cardiac function normalized under furosemide and spironolactone and dilated cardiomyopathy has progressively improved. The medication is being gradually withdrawn.

Patient 2 (Fig. 2)

After the birth of a healthy male sibling, the third child of the same couple, a female, was born following an uneventful term pregnancy. She presented relative macrocephaly, short limbs, and bilateral hip dislocation and was discharged under breastfeeding. At the 8th day of life, she was admitted to the local hospital for poor feeding and lethargy. Hypotonia, hypothermia, and hypoglycemia were noticed. After initial stabilization and because of persistent depressed consciousness, she was transferred to the PICU.

Fig. 1 (**a**–**c**) Case 1, older sibling with generalized GALE deficiency

On admission, encephalopathy, jaundice, and hepatomegaly were observed. Acute liver failure was confirmed, with severe coagulopathy, thrombocytopenia, and anemia, requiring multiple transfusions. Death occurred on the 17th day, with severe intraventricular hemorrhage, despite a lactose-free regimen.

Investigations revealed positive urine-reducing sugars (20 g/L) without glycosuria; generalized hyperaminoaciduria; high plasma glycine, glutamine, alanine, tyrosine, and phenylalanine, and unspecific abnormalities in urinary organic acids. Acylcarnitine profile on Guthrie card, pituitary hormones, and serum transferrin IEF were normal. Viral infections, neonatal hemochromatosis, and hemophagocytic lymphohistiocytosis were excluded. Autopsy showed a small liver with massive hepatic necrosis, cholestasis, large kidneys with intratubular renal calcifications, hepatopancreatic siderosis, and subarachnoid and intraventricular hemorrhages. *Postmortem* mitochondrial respiratory chain functional studies showed deficits of complexes I, II, IV, and V in the liver and heart and normal activities in the muscle, with normal results for mtDNA copy number, mtDNA genome, and DGUOK gene analysis.

Gene panel using massive parallel sequencing (target capture CDGv1, NimbleGen) revealed the previously reported homozygous c.[280G>A] p.[V94M] mutation in *GALE* in both sisters, leading to the diagnosis of GALE deficiency (MIM 230350). The parents are heterozygous for the same mutation. GALE activity was markedly reduced in erythrocytes of patient 1 [0.6 μmol/(h.gHb); reference range 5.7–22.1] and also in fibroblasts of both patients 1 and 2 confirming generalized GALE deficiency. In patient 1, erythrocyte galactose 1-phosphate and urinary polyols chromatography were normal on an unrestricted diet.

Table 1 shows clinical, biochemical, and genetic characteristics of the present patients and the reported ones.

Discussion

Generalized GALE deficiency has only been reported in six patients from three families (Table 1). In one of the patients previously described (case 8), GALE was only assayed in red cells, and no genetic studies were undertaken therefore making it difficult to confirm the diagnosis of generalized GALE deficiency, besides the severe clinical presentation described (Sarkar et al. 2010). The other five patients belong to two highly consanguineous families. This constitutes a difficulty in attributing some features to generalized GALE deficiency with certainty. Although the parents of patients 1 and 2 affirmed to be non-consanguineous, a farthermost possibility of a shared ancestry couldn't be ruled out. Nevertheless, excluding cardiac involvement, all of the other features described can be attributed to GALE deficiency, according to the previous published descriptions of this disease. A majority of patients showed hepatic symptoms (7/8), hypotonia (5/8), and, during follow-up, short stature (7/7), developmental disability (7/7), and sensorineural hearing loss (4/7). Symptoms present in a minority of patients were micrognathia, flexion deformities of the fingers, dislocatable hip/hip dysplasia, and positional talipes equinovarus. Galactosemia was not considered in patient 1, since liver involvement happened outside the neonatal period, reducing sugars were negative, recovery was almost complete under a normal diet, and cataracts appeared only at 8 years. Thus, the lack of the classic newborn severe liver presentation, which emerged in the younger sister, contributed to the diagnostic delay in this family. This

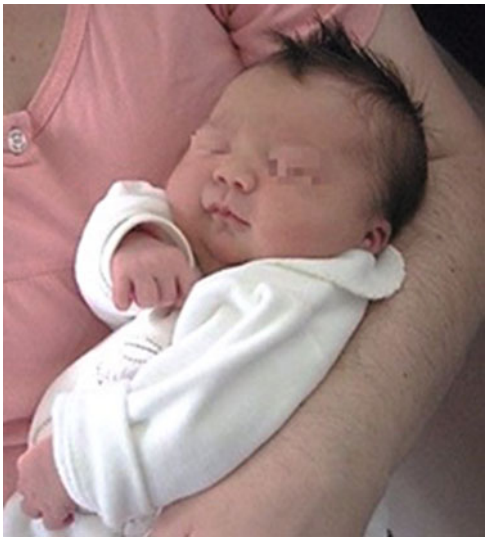

Fig. 2 Case 2, younger sibling with generalized GALE deficiency

different severity in clinical presentation overlaps the description made by Walter et al. (1999), in which the index cases for each family presented earlier with severe clinical illness similar to that seen in classic galactosemia, with poor feeding, weight loss, liver disease, and tubulopathy, while the other members of those families (cases 4, 6, and 7 of Table 1), who had the same homozygous mutation, were diagnosed before noticeable disease has developed. Another curious fact is that the older sibling (case 1) didn't show any sign of milk intolerance until date. The available knowledge about GALE deficiency is still insufficient to explain these differences in clinical presentation. In patient 1, the multisystem involvement with dysmorphism, cardiomyopathy, and developmental disability associated with high levels of serum CDT, and a type 1 pattern on IEF led to the suspicion of a CDG, which remained "type X," since no enzyme deficiency or mutation was found. Galactosemia type 1 and 3 are known causes of secondary hypoglycosylation of proteins and lipids (Charlwood et al. 1998, Sturiale et al. 2005). Hypertrophic and dilated cardiomyopathy is a known feature of some CDG, most commonly in a multisystem presentation, early in life, although late-onset and non-syndromic cases have also been reported (Lefeber et al. 2011). Dilated cardiomyopathy was the main clinical feature in patient 1 for some years, but it was absent in her younger sister. To our knowledge, this is the first generalized GALE-deficient patient with dilated cardiomyopathy.

The c.[280G>A] (p.[V94M]) mutation in the *GALE* gene has been found in a homozygous form in all of the patients tested with the severe phenotype (Table 1). Other mutations are associated with the intermediate or asymptomatic phenotype (Openo et al. 2006; Wasilenko et al. 2005).

A peculiar feature in patient 1 was the absence of accumulation of galactose metabolism products [total galactose and galactose-1-phosphate (Gal-1-P)]. A similar situation was already described in patients with intermediate and peripheral GALE deficiency (Openo et al. 2006).

Galactose is an essential constituent of the glycosphingolipids, required for brain growth and development. The galactose metabolism includes the Leloir and the pyrophosphorylase pathways, and epimerase is a key enzyme in both. In the Leloir pathway, the conversion of Gal-1-P to glucose-1-phosphate (Glc-1-P) is made by the enzyme galactose-1-phosphate uridyltransferase. In this reaction, UDP-GIc is converted to UDP-Gal. Epimerase regenerates UDP-Glc from UDP-Gal, allowing the maintenance of this cycle. The pyrophosphorylase constitutes an alternative pathway, which allows the synthesis of UDP-Gal in its direct route in patients with transferase deficiency or, in its reversal route, when galactose intake is restricted (Holton et al. 1981). In epimerase deficiency, the patients are unable to synthetize UDP-Gal by this way and depend on exogenous galactose for its synthesis (Holton et al. 1981).

Henderson et al. (1983) suggested that, in galactose epimerase deficient patients, a small amount of administered galactose will be metabolized via the Leloir pathway to produce UDP-Gal. UDP-Glc cannot be regenerated from UDP-Gal but could be formed from UTP and Glc-1-P and used as a cofactor in the maintenance of transferase activity. Thus, under these circumstances, galactose and Gal-1-P will not accumulate but UDP-Gal will. When dietary galactose is increased further, it was suggested that the synthesis of UDP-Glc might become limiting for the

Table 1 Clinical and laboratory features of the present patients and the previously reported ones

	Patient 1	Patient 2 (sibling of 1)	3 Holton et al. (1981)	4 Walter et al. (1999) (sibling of 3)[a]	5 Sardharwalla et al. (1988)	6 Walter et al. (1999) (sibling of 5)[a]	7 Walter et al. (1999) (cousin of 5,6)[a]	8 Sarkar et al. (2010)
Year of birth; gender	2004; Female	2012; Female	1980; Female	1991; Female	1984; Female	1985; Male	1994; Female	?; Male
Clinical presentation	Hypotonia Failure to thrive Dilated cardiomyopathy Cardiac failure Acute liver failure	Hypotonia Poor feeding Jaundice Hepatomegaly Acute liver failure	Hypotonia Weight loss Jaundice Vomiting	No clinical illness	Poor feeding Irritability Jaundice Hepatomegaly Cataracts	Hypotonia Poor feeding	Hypotonia	Poor feeding Jaundice Lethargy Vomiting Hepatomegaly
Dysmorphic features	Micrognathia Relative macrocephaly Hypertelorism Short limbs Flexion deformity of fingers Positional talipes Bilateral hip dysplasia Ligament laxity Short stature	Relative macrocephaly Bilateral hip dysplasia Short limbs	Hypotelorism Increased palpebral fissure length Posteriorly rotated ears Short philtrum Short stature	Short stature	Micrognathia Posteriorly rotated ears Ligament laxity Persistent femoral anteversion Internal tibial torsion Short stature	Micrognathia High palate Pigeon chest Flexion deformity of fingers Dislocatable hip Positional talipes Ligament laxity Short stature	Micrognathia Flexion deformity of fingers and toes Chest deformity Thick gums Short stature	?
Evolution	Severe SN hearing loss Global developmental disability Cataracts Cardiac function normalized under medication Normal pubertal development	Death at 17 days	Developmental disability Mod. learning difficulties Severe SN hearing loss Short stature Normal ovarian function	Mod. learning difficulties No SN deafness	Severe SN hearing loss Mod. learning difficulties Normal pubertal development	Tracheostomy (upper airway obstruction) Poor feeding Mod. developmental disability No SN deafness	Severe SN hearing loss Global developmental disability	No visual or hearing impairment Walking with support at 15 months
Laboratory investigations	↑ CDT Abn. transferrin IEF Urinary-reducing substances Ø Gal-1-P normal	Urinary-reducing substances +	Urinary-reducing substances + Galactosuria Gal-1-P and UDP-Gal increased Mod. generalized aminoaciduria		Gal-1-P increased	Gal-1-P increased Abn. transferrin IEF	Gal-1-P increased Abn. transferrin IEF	Urinary-reducing substances +
GALE activity	RBC: ↓↓↓ Fibroblasts: ↓↓↓	Fibroblasts: ↓↓↓	RBC: ↓↓↓ Fibroblasts: Ø Liver tissue:10%	Amniocytes: 5%	RBC: Ø Fibroblasts: Ø	RBC: Ø	RBC: ↓↓↓	RBC: ↓↓↓
GALE mutation	Homozygous c.280G>A (p.[V94M])	Homozygous c.280G>A (p.[V94M])	Homozygous c.280G>A (p.[V94M])	–	–	Homozygous c.280G>A (p.[V94M])	Homozygous c.280G>A (p.[V94M])	–

[a] Presymptomatic diagnosis; ↓↓↓, markedly reduced; Ø, absent; *Abn* abnormal, *CDT* carbohydrate-deficient transferrin, *Gal-1-P* galactose-1-phosphate, *Mod.* moderated, *RBC* red blood cells, *SN* sensorineural, *UDP-Gal* uridine diphosphate galactose

transferase reaction, possibly because of the limited availability of UTP, and at this point, Gal-1-P and galactose will begin to accumulate also. The responsibility of the different accumulating metabolites in the genesis of clinical manifestations and long-term complications remain unclear.

On the basis of present knowledge, the main objective in the therapeutic approach of generalized epimerase-deficient galactosemia seems to be the achievement of a balance between dietary restriction of galactose to prevent galactose toxicity and supplying enough galactose for essential requirements (Henderson et al. 1983, Walter et al. 1999, Openo et al. 2006, Sarkar et al. 2010, Fridovich-Keil 2013). However, the galactose intake for optimum outcome remains unknown, and, as in classical galactosemia, there is no guarantee that long-term complications will be completely prevented by this approach (Fridovich-Keil 2013).

In conclusion, the present report expands the clinical spectrum of GALE deficiency.

Take Home Message

Generalized GALE deficiency (galactosemia type 3) is a very rare inherited metabolic disease, associated with a secondary CDG and with a variable clinical spectrum that can include cardiomyopathy.

Compliance with Ethics Guidelines

Conflict of Interest

Filipa Dias Costa, Sacha Ferdinandusse, Carla Pinto, Andrea Dias, Liesbeth Keldermans, Dulce Quelhas, Gert Matthijs, Petra A. Mooijer, Luísa Diogo, Jaak Jaeken, and Paula Garcia declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from patients' legal guardians for being included in the study.

Additional informed consent was obtained from them for which identifying information is included in this article.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

Details of the Contributions of Individual Authors

FDC gathered the data and drafted the manuscript to report the work. PG and LD were responsible for planning and conducting the diagnostic investigation and patients' follow-up and for the process of critical review of the manuscript. AD and CP assisted both patients during their hospitalizations in PICU and made significant contributions to the content of the manuscript. DQ collaborated in diagnostic investigation and reviewed the manuscript. SF and PAM performed GALE enzymatic studies and critically reviewed the data. JJ, LK, and GM were responsible for the biochemical, enzymatic, and genetic CDG studies. JJ gave significant contributions in the process of manuscript's revision. All authors gave their final approval of the version to be published.

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