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TEN-YEAR EXPERIENCE OF NEWBORN SCREENING FOR GLUTARIC ACIDURIA TYPE 1 IN NORTHEAST ITALY: SUCCESSES, CHALLENGES AND UNEXPECTED FINDINGS

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***ABSTRACT* – Objective:** Glutaric aciduria type 1 (GA1) is an autosomal recessive disorder caused by a deficiency of glutaryl-CoA dehydrogenase, leading to the accumulation of neurotoxic metabolites. Early diagnosis and treatment through newborn screening (NBS) can prevent severe neurological complications. However, the effectiveness of NBS for GA1, particularly in identifying low-excretor phenotypes, remains a subject of ongoing research. Our study aims to evaluate the effectiveness and limitations of NBS for GA1 over a 10-year period in northeast Italy.

Materials and Methods: We analyzed data from 301,360 newborns screened between 1 January 2014 and 31 December 2024. Screening was performed using tandem mass spectrometry to measure glutarylcarnitine (C5DC) levels in dried blood spots. Positive cases underwent confirmatory testing and long-term follow-up.

Results: Four patients were identified through NBS, with one false-positive and one false-negative case, resulting in a sensitivity of 80%. The mean age at diagnosis was 4.25 days. Despite early treatment, one patient (25%) experienced an acute encephalopathic crisis. Two out of three patients with available follow-up MRI showed white matter abnormalities. The false-negative case, homozygous for the p.Met405Val variant associated with a low-excretor phenotype, was diagnosed at nine months following an acute encephalopathic crisis.

Conclusions: NBS for GA1 allows early diagnosis and treatment, potentially improving neurological outcomes. However, challenges remain, including the occurrence of acute crises in treated patients and false-negative results in low-excretor phenotypes. Further research is needed to enhance screening sensitivity, particularly for low-excretor patients, and to optimize long-term management strategies.

Keywords: Newborn screening, Glutaric aciduria type 1, Glutarylcarnitine.

INTRODUCTION

Glutaric aciduria type 1 (GA1; OMIM #231670) is an autosomal recessive disorder caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH; EC 1.3.99.7), a mitochondrial enzyme that catalyzes the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA in the catabolic pathway of lysine, hydroxylysine, and tryptophan[1,2]. GCDH deficiency results in the accumulation of glutaryl-CoA and its neurotoxic dicarboxylic derivatives – glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA), glutaconic acid, and glutarylcarnitine (C5DC) – in body tissues, especially in the brain[2].

Infants are typically asymptomatic at birth, making clinical identification of affected individuals challenging. Macrocephaly, present at or shortly after birth, is a frequent (75%) but nonspecific finding[3,4]. Without treatment, 80-90% of infants develop irreversible striatal damage during a vulnerable period of brain development (mostly between the ages of 3 months and 36 months). This damage often follows an acute encephalopathic crisis precipitated by intercurrent febrile illness or surgical intervention[5-9]. These crises cause acute striatal damage, particularly affecting the putamen, and subsequently, a complex movement disorder (MD) with predominant dystonia[6,10,11]. Insidious-onset dystonia is less severe and usually manifests later in infancy[12]. Neuroimaging tests can reveal features, such as fronto-operculo-temporal hypoplasia, basal ganglia degeneration, cystic dilation of the Sylvian fissure, leukoencephalopathy, or subdural hematomas[5].

Two biochemical subgroups, low excretors (LE) and high excretors (HE), have been arbitrarily defined. High excretors are characterized by large quantities of GA (>100 mmol/mol creatinine) and 3-HGA in the urine. Conversely, low excretors have low/absent GA excretion (<100 mmol/mol creatinine), only slightly elevated 3-HGA excretion[10,13,14]. Importantly, the LE phenotype should not be mistaken for an attenuated disease variant, as both HE and LE individuals share the same high risk of developing acute MD[6,15].

GA1 is considered a treatable disorder. Maintenance treatment (MT) consists of a low-lysine diet with administration of a lysine-free, tryptophan-reduced, arginine-fortified amino acid mixture and oral carnitine supplementation. Intensified intermittent emergency treatment (ET) is recommended during intercurrent illness or surgery and consists of a carbohydrate-enriched, low-to-no-protein protocol to prevent catabolic episodes[16]. Over 90% of individuals remain asymptomatic if maintenance and emergency treatments are initiated before symptom onset and continuously maintained according to guideline recommendations, as recently confirmed in a meta-analysis[5] including 647 patients worldwide. Deviations from recommended treatment have different negative effects on neurological outcomes: deviations from MT increase the risk of insidious-onset MD, while deviations from ET are highly frequent in individuals with acute-onset MD. The effectiveness of treatment implemented after the manifestation of neurologic disease is strongly limited, as striatal damage cannot be reversed[5].

Since C5DC can be detected in dried blood spots (DBS) by MS/MS and early treatment is neuroprotective, GA1 has been included in many national newborn screening (NBS) panels[17], including the Recommended Uniform Screening Panel in the USA and several European countries[10,18]. In Italy, a nationwide newborn screening program for inborn errors of metabolism, including GA1, was introduced by law between 2016 and 2017 (Law 167/2016)[19]. Ruoppolo et al[20] reported the results on 806,770 newborns screened between January 2019 and December 2020, identifying five neonates affected by GA1 (incidence 1:161,354). No false negative results were reported at that time.

In northeast Italy, expanded newborn screening, including GA1, has been active by regional law since 2014. We report the results of 10 years of screening and follow-up, discussing the pros and cons, also in comparison with available data from other screening programs.

MATERIALS AND METHODS

Study Population

From 1 January 2014 to 31 December 2024, 301,360 neonates born in northeast Italy were screened at the Regional Center for Expanded Newborn Screening, Padua University Hospital. Dried blood spots (DBS) were collected between 36 and 48 hours after birth. A second sample was required for premature infants (<34 gestational weeks and/or weight <2,000 g) and for sick newborns (those receiving transfusions or parenteral nutrition).

Analysis of Acylcarnitines in DBS

Analysis of acylcarnitines in DBS was performed by electrospray ionization tandem mass spectrometry (MS/MS) using the Waters TQD tandem mass spectrometer and NeoBase Non-derivatized MSMS Kit (PerkinElmer, Waltham, MA, USA).

For GA1, C5DC was evaluated, and C5DC/acylcarnitine ratios (C5DC/C0, C5DC/C8, C5DC/C16) were used to improve diagnostic sensitivity and specificity. The cut-off for C5DC and its ratios was set to the 99th percentile.

If the screened value was equal to or greater than the positive cut-off value, the newborn was immediately referred to the clinic unit for confirmatory testing and management.

Confirmatory Testing

Confirmatory tests included clinical evaluation, plasma glutaric acid analysis, urinary organic acid analysis, and mutation analysis of the *GCDH* gene.

Quantitative determination of plasma glutaric acid was performed by liquid chromatography-mass spectrometry (LC-MS/MS). Urinary organic acid assay was performed by gas chromatography-mass spectrometry (GC-MS/MS), allowing quantitative determination of GA and qualitative determination of 3-OHGA. Biochemical phenotype (HE or LE) was classified according to previous definitions[10,13,14].

For molecular analysis, genomic DNA was extracted from peripheral leukocytes, and next-generation sequencing (NGS) was carried out using the Illumina MiSeq Sequencing System (Illumina Inc., San Diego, CA, USA) to determine specific exon regions and exon-intron boundaries.

Patient Management

Treatment was initiated immediately after diagnosis. Maintenance treatment (MT) consisted of a low-lysine diet supplemented with GA1 special formula and carnitine supplementation. Parents were educated on the importance of recognizing symptoms indicating impending catabolism and early initiation of emergency treatment.

Emergency treatment (ET) consisted of a carbohydrate-enriched, low-to-no-protein protocol used intermittently and increased carnitine supplementation during potentially catabolic episodes and initiated within 24 hours after the onset of alarming symptoms (e.g., fever, vomiting, feeding problems).

Follow-up

Patients were seen at our clinic every 3 months. Standard deviation scores for weight and height were calculated using the World Health Organization growth charts[21]. Neurological assessment was performed at every visit. The severity of movement disorder was assessed as previously described[7], classified as “mild” if children showed no significant disability in daily life, “moderate” if motor dysfunction caused disability but some motor functions were preserved, and “severe” if movement disorder caused significant disability with few motor skills remaining.

Biochemical monitoring included laboratory and nutritional parameters to detect insufficient intake of micronutrients or energy substrates, quantitative analysis of plasma amino acids to evaluate the supply of essential amino acids in patients on a low-lysine diet, and carnitine status to compensate for secondary depletion of free carnitine.

Non-contrast magnetic resonance imaging (MRI), including T1-weighted and T2-weighted images, diffusion-weighted imaging (DWI), and apparent diffusion coefficient (ADC) maps, was obtained at diagnosis and when new neurological symptoms presented.

Ethics Approval

The study was approved by the Ethics Committee of the Province of Padua (approval number 0032402, 7 May 2025).

The parents of all the patients signed an informed consent form to participate in the study and allowed publication of their data.

RESULTS

Screening Outcome

Of the 301,360 screened newborns, five positive cases for GA1 were referred to our Clinical Unit for confirmatory testing (Table 1). In four (three males and one female) patients, the diagnosis was confirmed by biochemical and molecular analysis. In the fifth case, biochemical analysis and molecular tests were negative (false positive).

Table 1. Baseline clinical and biochemical findings of positive newborns for GA1 by NBS.

Patient	Sex	Ethnicity	Newborn screening				Clinical presentation at birth	Metabolic examination at diagnosis			Biochemical phenotype	*GCDH* gene		Neuro-radiological findings
			C5DC µmol/L (NV <0.30)	C5DC/C0	C5DC/C8	C5DC/C16		Plasma free carnitine µmol/L (NV >11.9)	uGA mmol/mol creat (NV 0.1-5.3)/ u3-HGA	Plasma GA µmol/L (NV 0.4-0.9)		Variant 1	Variant 2	
#1	Male	European	3.04	0.32	101.33	1.35	Macrocrania, mild axial hypotonia	8.8	1,434.6/↑↑	62.8	High excretor	c.262C>T p.(Arg88Cys)	c.1204C>T p.(Arg402Trp)	Fronto-temporal hypoplasia, white matter changes
#2	Male	European	3.27	0.13	109	1.08	Asymptomatic	18.5	1,894.9/↑↑	13.9	High excretor	c.1218C>A p.(Asn406Lys)	c.1262C>T p.(Ala421Val)	Fronto-temporal hypoplasia, white matter changes
#3	Female	European	1.84	0.21	20.44	0.63	Asymptomatic	16.6	771.4/↑↑	1.2	High excretor	c.281G>T p.(Arg94Leu)	c.281G>T p.(Arg94Leu)	Fronto-temporal hypoplasia, white matter changes, striatal lesions
#4	Male	European	5.97	0.81	3.08	1.94	Mild axial hypotonia	6.3	12,093/↑↑	120.1	High excretor	c.1064G>A p.(Arg355His)	c.1204C>T p.(Arg402Trp)	Fronto-temporal hypoplasia, white matter changes
#5	Male	Arabic	0.55	0.02	6.11	0.61	End-stage kidney disease	22	NA	0.2		No variant	No variant	Not performed

↑↑: Elevated, NV: normal value.

During the study period, one patient whose NBS was negative was later diagnosed with GA1 due to clinical onset. Based on these data, the estimated incidence of GA1 in NE Italy is one in 60,272 newborns, and the overall sensitivity of NBS for GA1 was 80%.

GA-1 Patients Identified by NBS

Clinical, biochemical, and molecular results of the four GA1 patients identified by NBS are shown in Table 1.

Baseline Evaluation

The mean age at diagnosis was 4.25 days (range 4-5). Clinical evaluation revealed macrocephaly in 1/4 patients (25%) and mild axial hypotonia in 2/4 patients (50%). Biochemical tests showed increased C5DC values on DBS in all patients (mean 3.53 µmol/L, SD 1.74 µmol/L; range 1.84-5.96, normal value <0.3). Glutaric acid levels were also high in plasma in all patients (mean 82 µmol/L, SD 54 µmol/L, range 1.2-250.1, normal value 0.4-0.9), and 2/4 patients showed plasma free carnitine deficiency (mean 12.4 µmol/L, SD 5.97 µmol/L, range 6.3-18.5, normal value 33.8-49.6). Urinary organic acid assay revealed increased urinary glutaric acid (mean 4,048.48 mmol/mol creatinine, SD 382.8 mmol/mol creatinine, range 771.4-12,093, normal value 0.1-5.3) and 3-hydroxyglutaric acid levels in all patients. According to previous biochemical definitions[10,13,14], they were all classified as "high excretors".

The diagnosis was confirmed by molecular examination of the *GCDH* gene. One patient was homozygous for the c.281G>T p.(Arg94Leu) variant. The parents reported no known consanguinity but came from a small, geographically isolated city, suggesting a founder effect. Two patients had the c.1204C>T p.(Arg402Trp) variant in compound heterozygosity with c.262C>T p.(Arg88Cys) and c.1064G>A p.(Arg355His) variants, respectively. One patient was compound heterozygous for c.1218C>A p.(Asn406Lys) and c.1262C>T p.(Ala421Val) variants. All variants were known to be pathogenic (ACMG 5), except for p.(Asn406Lys), not yet reported.

Magnetic resonance imaging was performed within the first month of life (mean 14.75 days; range 9-24) (Figure 1). Significant brain MRI abnormalities were present in all patients, showing widening of the sylvian fissure, fronto-operculo-temporal hypoplasia, and hyperintensity of white matter in T2-weighted images. Interestingly, patient #3 (Table 1) already presented with striatal lesions (posterior putamen and pale nuclei). This finding is consistent with the previous concept that GA1 brain damage starts prenatally[22].

All patients started a low-lysine diet with administration of lysine-free, tryptophan-reduced, and arginine-fortified amino acid mixture (synthetic protein mean intake 1.66 mg/kg/day; range 1.48-2.5; lysine mean intake 114 mg/kg/day; range 98-142) and carnitine supplementation (100 mg/kg/day).

Follow-up

All patients participated in regular follow-up and had good compliance with maintenance and emergency treatment. Only one patient (patient #2) presented with an acute encephalopathic crisis at 13 months of age during an upper airway infection. The patient presented with acute bronchospasm and low-grade fever. Despite initiating home emergency therapy, he experienced progressive neurological deterioration. Upon arrival at the emergency department, the patient showed generalized hypotonia with loss of head control and seizures. Blood gas analysis revealed metabolic acidosis (pH 7.28, HCO_3^- 17.1 mmol/L, base excess -9.7, anion gap 24, lactate 5.8 mmol/L), and urine dipstick showed significant ketonuria (3+). Laboratory tests indicated elevated glutaric acid levels in plasma (18.4 µmol/L) and urine (1,028 mmol/mol creatinine), along with increased 3-hydroxyglutaric acid. MRI findings showed bilateral cytotoxic edema in the basal nuclei, affecting both the putamen and caudate nuclei.

Feeding was temporarily suspended, and IV glucose was immediately started. Carnitine 200 mg/kg was administered intravenously. Seizure episodes were managed initially with a bolus of midazolam, followed by maintenance therapy with levetiracetam. Nevertheless, the patient developed a serious movement disorder with dystonia and dyskinesia of the limbs, as well as marked axial hypotonia with poor trunk and head control.

At the last follow-up, all patients were alive with a mean age of 4.5 years (range 1-8) (Table 2). Three out of four were older than 3 years at the last visit, and one was older than 6 years. The three patients <6 years old followed a low-lysine diet with good compliance, supplemented with specific supplements (synthetic protein mean 1.12 mg/kg/day; range 0.6-2; lysine mean 68 mg/kg/day; range 60-84). The

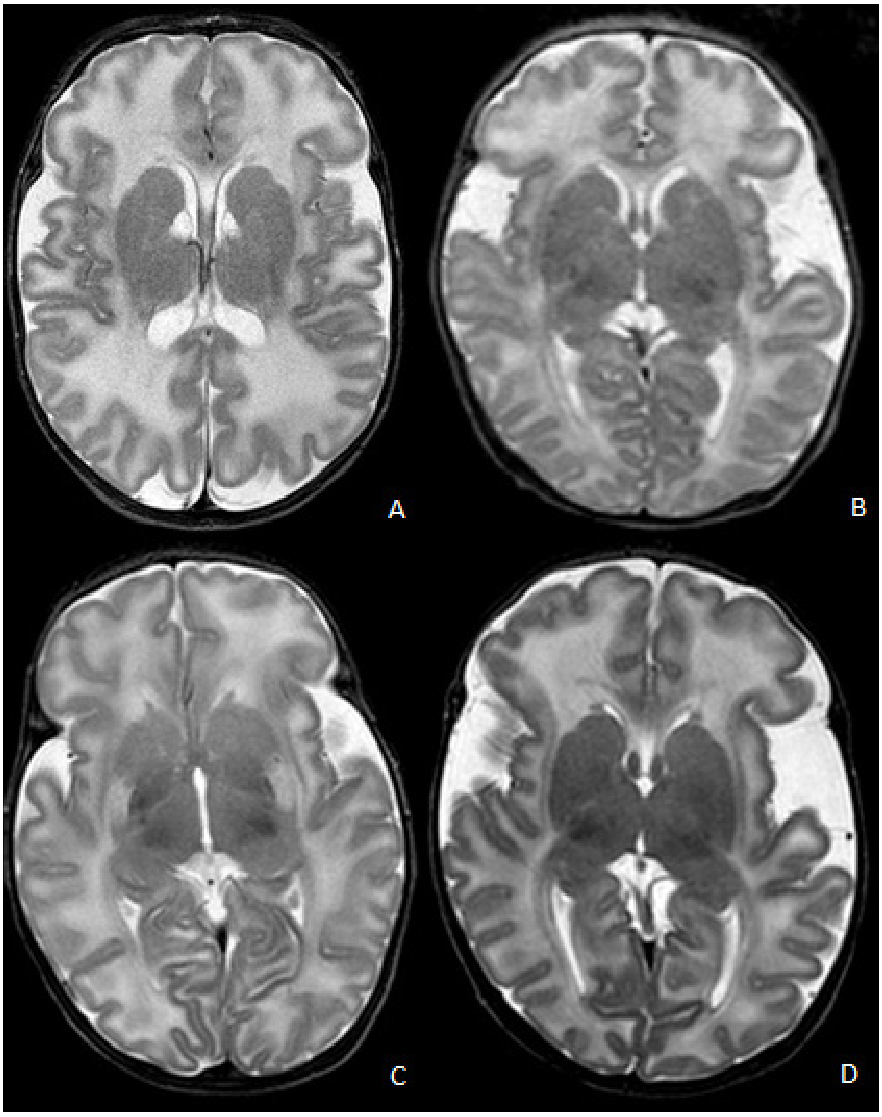


Figure 1. T2-weighted brain images at birth in GA1 patients identified by NBS. **A**, Widening of the Sylvian fissure and white matter changes in patient 1. **B**, Widely open Sylvian fissure with incomplete opercularization and white matter changes in patient 2. **C**, Moderate hypoplasia involving the subcentral gyrus and T2 hyperintense dorsolateral putamen; **D**, Severe frontotemporal hypoplasia with enlarged anterior temporal CSF spaces and diffuse white matter changes.

patient was >6 years old and followed a diet with controlled natural protein content. All supplemented with carnitine at a dosage of 100-150 mg/kg/day.

None presented with growth deficiency; patient #1 had macrocephaly, patient #2 presented with a severe dystonic-dyskinetic movement disorder, and patient #3 had a mild MD, with dystonic movements in the upper limbs while walking, and brief dyskinesias of the trunk and limbs when at rest. Cognitive function was within normal values in all subjects.

MRI images at the last follow-up visit in patients >3 years showed hyperintensity signal in T2-weighted images in 2/3 patients (patients #1 and #2) and basal ganglia involvement in 2/3 patients (patients #2 and #3) (Figure 2). Regarding basal ganglia involvement, patient #3, who had presented with an insidious onset, had an altered signal in the posterior portion of the putamen. Patient #2, who had experienced an acute encephalopathic crisis, had an atrophic appearance of the basal nuclei (especially the putamen).

Table 2. Clinical and biochemical findings of GA1 patients at the last follow-up visit.

Patient	Last follow-up visit		Biochemical findings				Neuroradiological findings	Management		Acute decompensation
	Age	Clinical presentation*	DBS C5DC DBS µmol/L (NV <0.30)	Plasma Free carnitine µmol/L (NV 33.8-49.6)	uGA mmol/mol creat (NV 0.1-5.3)/ u3-HGA	Plasma GA µmol/L (NV 0.4-0.9)		Drug therapy	Diet	
#1	8 years 7 months	Macrocrania	2.59	43.7	948.4/↑↑	18.3	White matter changes	Carnitine 100 mg/kg/day	NP 1.5 g/kg	No
#2	5 years	Severe movement disorder	1.44	77.4	1,708.6/↑↑	7.6	White matter changes, basal ganglia atrophy	Carnitine 150 mg/kg	SP 0.75 g/kg, Lys 60 mg/kg	Yes (13 months)
#3	3 years 3 months	Minor motor symptoms	0.57	53.5	5.4/↑↑	2.4	Abnormal signal of basal ganglia	Carnitine 150 mg/kg/day	SP 0.75 g/kg, Lys 60 mg/kg	No
#4	12 months	Asymptomatic	6.55	33.8	267/↑↑	3.3	NA	Carnitine 150 mg/kg	SP 2 g/kg, Lys 84 mg/kg	No

*Major motor symptoms (movement disorder) or minor motor symptoms (fine motor deficits and/or delayed achievement of motor milestones) or asymptomatic.
Lys: Lysine; NP: natural proteins; SP: synthetic proteins; NV: normal value; DBS: dried blood spots; ↑↑: elevated.

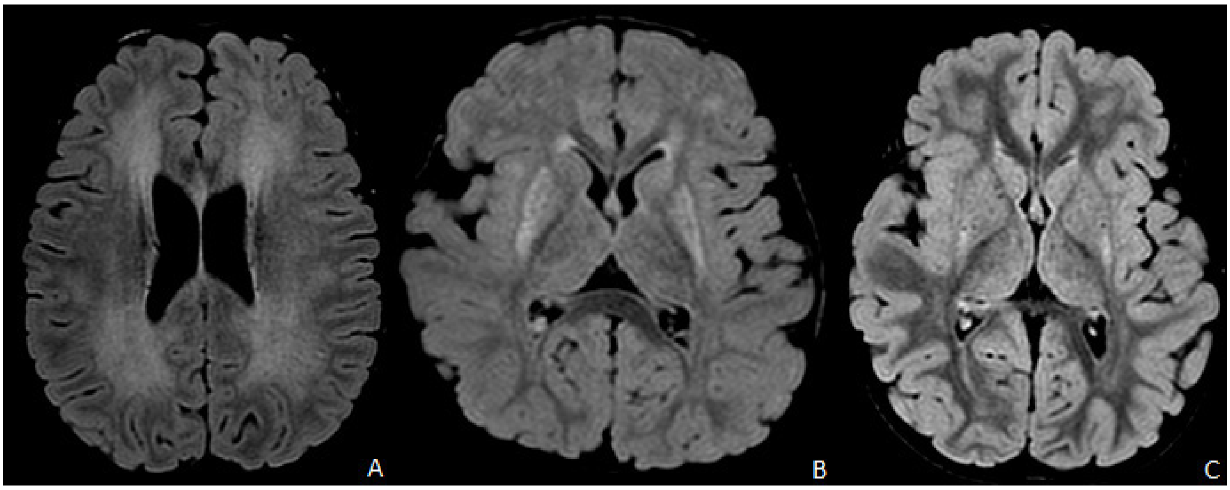


Figure 2. Brain MRI at the last follow-up. **A**, Extensive alteration of the white matter of the cerebral hemispheres and of the corpus callosum. **B**, Atrophic involution of the basal nuclei and diffuse white matter changes. **C**, Atrophic involution of dorsolateral putamen.

False-Positive

One patient in the NBS group had prenatal findings of bilateral renal dysplasia and anhydramnios at 20 weeks. He was born at 34+4 weeks. At birth, a diagnosis of end-stage kidney disease (creatinine 164 µmol/L, normal value 15-31) secondary to bilateral cystic renal dysplasia in autosomal recessive polycystic kidney disease was made. NBS showed increased values of C5DC (0.6 µmol/L). Due to anuric acute kidney injury, urinary organic acid analysis was not possible; however, glutaric acid in plasma was normal, and genetic testing was negative. The patient was treated with peritoneal dialysis and later underwent bilateral nephrectomy and is waiting for renal transplantation.

False-Negative

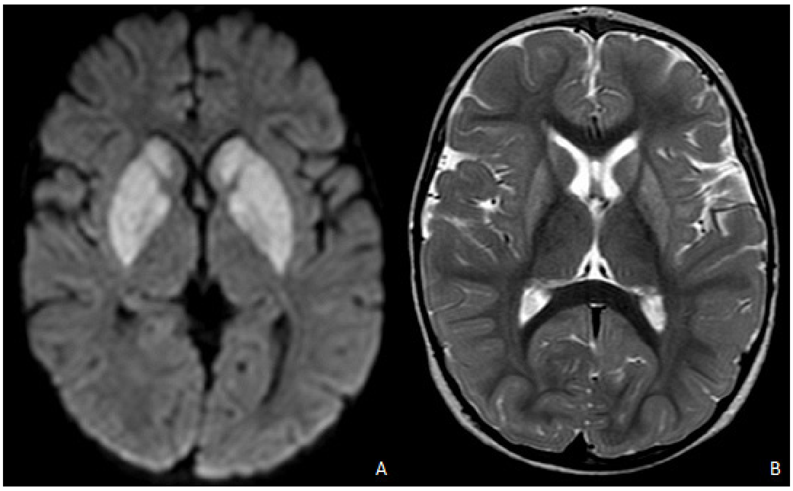
One patient whose NBS was negative was diagnosed with GA1 at the age of nine months following an acute encephalopathic crisis[23]. He was born in Italy to Moroccan parents, and the NBS was performed correctly, with unremarkable results. At 9 months of age, during a febrile upper airway infection, he presented with persistent tonic seizures and progressive poor responsiveness. His head circumference was 43.5 cm (7th percentile). Routine exams and blood gas analysis were normal. Brain MRI showed bilateral signal alterations in the basal ganglia, affecting the caudate, putamen, and pallidum (Figure 3). DBS acylcarnitine profile was normal (C5DC 0.067 µmol/L, normal value <0.3), with normal free carnitine levels (27.1 µmol/L, normal value 29-42). Urinary organic acid analysis showed only slightly elevated 3-HGA levels, with absent GA. Suspecting a GA1 diagnosis, feeding was suspended, and IV glucose was initiated along with carnitine 200 mg IV. Seizures were treated with midazolam (intravenous), followed by levetiracetam. The diagnosis of GA1 was genetically confirmed, showing a compound homozygous *GCDH* genotype with pathogenic variants c.1213A>G/p.Met405Val. The patient began treatment with a low-lysine diet with administration of lysine-free, tryptophan-reduced, and arginine-fortified amino acid mixture (synthetic protein 1 g/Kg; lysine 67 mg/kg) and carnitine supplementation (150 mg/kg/day).

However, the patient developed dystonic quadriplegia, and follow-up MRI showed atrophy of the basal ganglia.

DISCUSSION

We report the experience of 10 years of screening for GA1 on over 300,000 newborns. This allowed the identification of four affected newborns at an average age of 4.25 days and their timely management both chronically and during catabolic intercurrent episodes. The follow-up of these patients highlighted the benefits and limitations of this newborn screening.

Figure 3. Brain MRI during acute decompensation (**A**) and during follow-up (**B**) of the patient missed by NBS. **A**, Swelling and T2-hyperintensity of the putamen and caudate. **B**, Atrophic involution of lenticular nuclei.



Newborn screening has modified the natural history of the disease. In the absence of screening and early therapy, 90% of patients presented with acute encephalopathic crisis, resulting in major motor symptoms with severe dystonia. Our study suggests that the frequency of encephalopathic crises decreases notably after newborn screening, which is consistent with previous reports[5,10].

However, despite early treatment, in our experience, 25% of patients still suffered from an encephalopathic crisis, with outcomes comparable to those of the unscreened population. This rate is similar to that reported by a recent meta-analysis[5] including 261 presymptomatically diagnosed patients. The adherence to therapy in the screened population for GA1 is the major predictor of neurological outcome. More research is needed for the development of more effective therapies in GA1[10].

Moreover, there is evidence of a prenatal injury. At the time of diagnosis, all screened individuals were asymptomatic except for mild clinical signs, such as macrocephaly (one of four) and hypotonia (two of four). All patients showed signs of prenatal damage already at the first MRI. In particular, in patient #3, the dorsolateral putamen was already T2-hyperintense in initial scans acquired at 16 days of life. This typical pattern of circumscribed T2-hyperintensity of the dorsolateral putamen has been recently well described by Boy et al[10] in insidious-onset patients. The authors reported that all patients with T2-hyperintensity of the dorsolateral putamen on initial scans developed insidious onset movement disorder, with a latency of 3.5 months to 6.5 years. Accordingly, our patient developed a mild, insidious-onset movement disorder with dystonia after a latency period of 24 months.

Since newborn screening and early initiation of metabolic therapy reduce the risk of striatal damage and increase survival, long-term phenotypic changes, such as white matter abnormalities, could become more prominent in individuals who remain asymptomatic into adolescence or adulthood. In particular, in HE, an increased frequency of extrastriatal abnormalities, progressive neuroaxonal damage, and higher cerebral GA concentrations have been observed as they age, although their clinical significance remains unclear[2,24]. Neuroradiological abnormalities do not appear to correlate directly with genotype, but rather with the biochemical phenotype that results from it[2,24]. In our population, at the last clinical follow-up visit, MRI brain images were available for three patients older than three years. Two of these (patients #1 and #2) showed white matter abnormalities. This finding requires a thorough and critical reassessment of current long-term management approaches[5,10,24,25].

Regarding the newborn screening test, the diagnostic quality of glutarylcarnitine screening has been continuously improved using more precise cut-off adjustments or glutarylcarnitine ratios to other acylcarnitines. In our population, the method showed a sensitivity of 80%, with one false-positive and one false-negative result.

The false-positive patient was affected by renal insufficiency. The mechanism that led to the elevation of blood C5DC in the case of renal insufficiency is unclear. Renal damage might result in decreased excretion of C5DC or lead to a defect in the uptake or secretion of glutaric acid and its metabolites through specific active renal transporter systems[26]. In a previous study[26], eleven children with a positive newborn screening result indicating GA1 were suffering from renal insufficiency: 5/11 from congenital renal insufficiency, 6/11 from acquired renal insufficiency. C5DC normalized in all children with acquired renal disease within 13-39 days of life and in 3/5 children with congenital renal insufficiency. This decrease in C5DC was clearly correlated with normalization of serum creatinine, thus reflecting improvement of renal function. In one child, C5DC and its ratios remained elevated until renal transplantation. The combination of C5DC with the variables C5DC/(C8 + C10), C5DC/C0, and C5DC/C8 showed the highest correlation with renal affection, but in contrast to children with GA1, C5DC/C16 was not highly correlated with renal insufficiency. In our case, the ratios C5DC/C0 were significantly lower than the true positive, while the C5DC/C8 and C5DC/C16 ratios showed some overlap. C5DC/C0 ratio may help to differentiate between glutarylcarnitinemia due to GA I and glutarylcarnitinemia due to renal insufficiency[26].

Finally, newborn screening does not reliably identify all affected individuals, as some low excretor (LE) patients may show normal C5DC concentrations, resulting in false-negative NBS results[7,10,18,27-29].

Our patient, who was missed by newborn screening, was homozygous for the p.Met405Val variant, which has a high incidence in individuals of African origin. This variant is associated with a predicted LE phenotype[30]. Several other cases of GA1 have been reported as being missed by NBS and diagnosed after the onset of symptoms[27,29,31-33]. All had an LE phenotype and, when assessed, high residual enzyme activity (10-25%)[31]. The clinical onset, between five and 28 months, manifested as either an acute encephalopathic crisis[28,31-33] or a more insidious scenario[27,32,33]. In most cases, including our patient, the characteristic macrocephaly of GA-I was absent[31,32]. Moreover, all patients exhibited involvement of the basal ganglia, but no other typical neuroradiological findings of GA-I[28,31,32]. In addition to p.Met405Val[31-33], the most frequent variants were p.Arg227Pro[28] and p.Val400Met[31]. Patients missed by NBS face high morbidity and mortality, similar to the pre-NBS era[10]. Thus, a negative NBS result does not unequivocally exclude the diagnosis of GA1. In case of suggestive findings, a targeted diagnostic work-up should always be performed, even if NBS was normal[16].

The exact number of missed patients is unknown, as NBS programs often lack a tracking system. In the German NBS program, three patients have been missed in about 5 million newborns. The overall sensitivity of C5DC screening in a mixed population is about 95%[32], with a discrepancy between patients with the HE (100%) and LE (84%) phenotypes[10]. In our experience, after ten years of newborn screening, four patients were identified, and one patient was missed, demonstrating a sensitivity of 80%.

To further improve the diagnostic sensitivity for LE patients, a genetic NBS program was initiated for the Oji-Cree First Nation in Canada, a known high-risk population with LE phenotype[34]. However, this approach is not suitable for populations with mostly private *GCDH* gene variations, as is the case in most countries[5].

CONCLUSIONS

In conclusion, our study demonstrates that newborn screening for GA1 improves neurological outcomes in most patients. Some critical issues, such as acute encephalopathic decompensation in a subgroup of treated patients, require further studies to improve the therapeutic approach. The presence of false negatives in LE patients necessitates further research. In the meantime, a high level of awareness should be maintained, especially in populations where GA1 is more frequent.

Artificial Intelligence-Assisted Technologies:

The authors declare that no artificial intelligence (AI) tools were used in the writing, analysis and preparation of this paper.

Authors' Contributions:

Conceptualization: VG; methodology: VG, AP; validation: ABB; formal analysis; VG, AP; investigations: DG, CC, CL, EP, IDE, LS; data curation: VG, AP; writing-original draft: VG, AP; writing-review and editing: ABB; supervision: ABB; project administration ABB.

Availability of Data and Material:

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest:

The authors declare that they have no conflicts of interest to disclose.

Ethics Approval:

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