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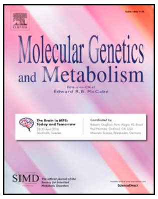

Glutaric acidemia type 1: Treatment and outcome of 168 patients over three decades

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ABSTRACT

Glutaric acidemia type 1 (GA1) is a disorder of cerebral organic acid metabolism resulting from biallelic mutations of *GCDH*. Without treatment, GA1 causes striatal degeneration in > 80% of affected children before two years of age. We analyzed clinical, biochemical, and developmental outcomes for 168 genotypically diverse GA1 patients managed at a single center over 31 years, here separated into three treatment cohorts: children in Cohort I ($n = 60$; DOB 2006–2019) were identified by newborn screening (NBS) and treated prospectively using a standardized protocol that included a lysine-free, arginine-enriched metabolic formula, enteral L-carnitine (100 mg/kg•day), and emergency intravenous (IV) infusions of dextrose, saline, and L-carnitine during illnesses; children in Cohort II ($n = 57$; DOB 1989–2018) were identified by NBS and treated with natural protein restriction (1.0–1.3 g/kg•day) and emergency IV infusions; children in Cohort III ($n = 51$; DOB 1973–2016) did not receive NBS or special diet. The incidence of striatal degeneration in Cohorts I, II, and III was 7%, 47%, and 90%, respectively ($p < .0001$). No neurologic injuries occurred after 19 months of age. Among uninjured children followed prospectively from birth (Cohort I), measures of growth, nutritional sufficiency, motor development, and cognitive function were normal. Adherence to metabolic formula and L-carnitine supplementation in Cohort I declined to 12% and 32%, respectively, by age 7 years. Cessation of strict dietary therapy altered plasma amino acid and carnitine concentrations but resulted in no serious adverse outcomes. In conclusion, neonatal diagnosis of GA1 coupled to management with lysine-free, arginine-enriched metabolic formula and emergency IV infusions during the first two years of life is safe and effective, preventing more than 90% of striatal injuries while supporting normal growth and psychomotor development. The need for dietary interventions and emergency IV therapies beyond early childhood is uncertain.

1. Introduction

Glutaric acidemia type 1 (GA1; OMIM #231670) is a disorder of cerebral organic acid metabolism caused by biallelic variants of *GCDH*, which encodes a mitochondrial flavin-dependent glutaryl-CoA dehydrogenase (GCDH) that mediates degradation of lysine and tryptophan (Fig. 1). Neuronal GCDH deficiency results in proximal accumulation of glutaryl-coenzyme A (CoA) and its dicarboxylic acid derivatives, glutaric acid (GA) and 3-hydroxyglutaric acid (3HGA). These metabolites become concentrated in the brain due to its limited capacity to form 5-carbon carnitine and glycine conjugates from glutaryl-CoA [1,2] or export medium-chain dicarboxylates [3,4].

GCDH deficiency causes sudden degeneration of striatal neurons in at least 80% of untreated patients [5–8]. More than 90% of these

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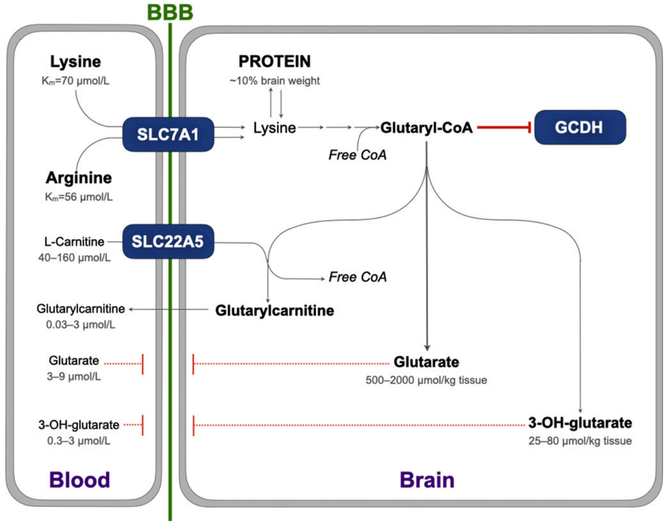

Fig. 1. Pathophysiology of Glutaric Acidemia Type 1. The brain imports lysine and arginine via a common facilitative transporter (SLC7A1) at the blood-brain barrier (BBB). Lysine and arginine have similar K_m values for SLC7A1 and compete for cerebral uptake within physiological concentrations ranges. The central nervous system (CNS) has a minimum lysine requirement for normal protein accretion and growth; lysine in excess of this requirement is normally oxidized via a GCDH-dependent degradation pathway. In GA1, the CNS produces glutaryl-CoA and its derivative dicarboxylic acids (glutarate and 3-hydroxyglutarate) in proportion to its rate of lysine uptake, which can be altered by competition with arginine at the BBB. The brain imports L-carnitine via one or more organic cation transporters (e.g., SLC22A5 and SLC22A4). In the mitochondrial matrix, acyltransferases theoretically drive the transfer of glutaryl moieties from glutaryl-CoA to L-carnitine to liberate free coenzyme A (CoA) and form glutarylcarnitine, which can traverse multiple cellular barriers and be excreted. The quantitative importance of this 'detoxification' reaction in vivo is uncertain. The BBB is relatively impermeable to 5-carbon dicarboxylic acids and therefore the brain accumulates glutarate and 3-hydroxyglutarate to concentrations two to three orders of magnitude higher than those measured in plasma. These compounds exhibit a range of neurotoxic actions in cell culture and *Gcdh−/−* mice.

encephalopathic crises strike during an infectious illness within the first 24 months of life but rarely occur without apparent provocation and may even happen in utero [9,10]. Regardless of their timing or mechanism, striatal lesions result in a complex extrapyramidal movement disorder that is the principal determinant of outcome. The predominant motor pattern observed is severe, generalized dystonia, which often abrogates speech, ambulation, and self-efficacy and results in comorbidities such as dysphagia, gastroesophageal reflux, joint dislocation, scoliosis, pulmonary aspiration, and chronic pain. Intellect typically remains intact despite profound functional impairment.

The birth incidence of GA1 is ~1 per 90,000 worldwide [11] but much higher among certain endogamous groups such as the Old Order Amish [12], a modern religious sect descended from a few hundred Swiss Anabaptists who immigrated to North America during the eighteenth century [13]. A population bottleneck followed by generations of genetic drift allowed a pathogenic *GCDH* c.1262C > T (p.Ala421Val) founder allele to reach carrier frequencies as high as 10% (birth incidence ~1 per 400) within certain extant Amish demes, and this apparently heritable form of 'cerebral palsy' was recognized within such communities long before it was linked to *GCDH* [14].

The Clinic for Special Children (CSC) is sited in rural Pennsylvania amid a large Amish population, and since 1989 has drawn an ethnically and genetically diverse group of 168 GA1 patients from 26 U.S. states and 6 countries. Here, we reflect upon this rich clinical experience to summarize historical trends in GA1 treatment, with special emphasis on the evolution of pathophysiological models and treatment strategies. Our data indicate that newborn screening (NBS) for GA1, when coupled to an appropriate metabolic formula and timely intravenous (IV) infusion therapy during the first two years of life, prevents more than 90% of striatal injuries while supporting normal growth and psychomotor development.

2. Patients and methods

2.1. Cohorts

The Institutional Review Board of Penn Medicine-Lancaster General Hospital approved the research protocol (2008–095-CSC) and parents consented in writing on behalf of their children. We studied a group of 168 individuals born with GA1 between August 1973 and October 2019 (current median age 11.8 years, range 0.2–43.6 years; 52% female), comprising 2276 patient-years of follow up and representing at least 41 different pathogenic *GCDH* allele combinations (Table S1). Ninety-one (54%) patients were homozygous for the c.1262C > T (p.Ala421Val) variant, 46 (27%) were homozygous or compound heterozygous for other *GCDH* alleles, and 31 (18%) had no molecular testing but a compelling clinical and biochemical GA1 phenotype, including elevated C5DC concentrations in plasma, elevations of both C5DC and 3HGA in urine, congenital or infantile-onset macrocephaly, and characteristic middle cranial fossa fluid collections detected by magnetic resonance imaging [6,15].

For comparative analyses, we established three separate cohorts based on timing of diagnosis and method of treatment (Table 1):

Cohort I 60 individuals (DOB 2006–2019) with GA1 were asymptomatic when identified between 0 and 14 days of age by one of two NBS methods (quantification of glutarylcarnitine [C5DC] from dried filter blood spots or detection of *GCDH* c.1262C > T from umbilical cord blood). After confirmatory biochemical and molecular testing, each child in Cohort I was treated consistently from birth to present (for patients < 2 years) or until ≥2 years of age using a standardized protocol that included a lysine-free, arginine-enriched ($Lys^{-}Arg^{+}$) metabolic formula (Glutarade, Nutricia North America), enteral L-carnitine (100 mg/kg•day), and emergency IV infusion of dextrose, saline, and L-carnitine (400 mg/kg•day) during illnesses (Table 2).

Cohort II: 57 individuals (DOB 1989–2018) were identified by NBS and subsequently treated with emergency IV infusions during illness and a diet restricted to 1.0–1.3 g/kg•day of intact (i.e., 'natural') protein, but did *not* receive a lysine-free metabolic formula.

Cohort III: 51 individuals (DOB 1973–2016) were diagnosed with GA1 either during a workup for motor disability (90%) or incidentally through cascade testing; they did not have NBS or receive preventative therapies.

Data for Cohort I were collected prospectively to extend findings of a study initiated in 2006 to evaluate the safety and efficacy of a novel $Lys^{-}Arg^{+}$ metabolic formula [16]. For Cohorts II and III, we conducted retrospective chart reviews to gather data about the method and timing of diagnosis, treatment strategy, neurologic outcome, and medical comorbidities.

2.2. Prospective treatment and monitoring protocol, cohort I (DOB 2006–2019)

2.2.1. Dietary therapy

Children in Cohort I ($n = 60$) were prescribed approximately equal quantities of intact protein (1.0–1.3 g/kg•day) and modified $Lys^{-}Arg^{+}$ protein equivalent (1.0–1.3 g/kg•day) from birth to their present age (for those < 2 years) or at least two years of age (Table 2). The protein equivalent of $Lys^{-}Arg^{+}$ metabolic formula was devoid of L-lysine, reduced in L-tryptophan (0.6%), and enriched with L-arginine (10.8%) as compared to protein in human milk (6.9% lysine, 1.8% tryptophan, 3.0% arginine) or commercial milk-based infant formulas (~9.1% lysine, 1.8% tryptophan, 3.0% arginine). The $Lys^{-}Arg^{+}$ metabolic formula otherwise contained fat, carbohydrate, micronutrients, and all

Table 1
Glutaric Acidemia Type 1 Cohorts: Clinic for Special Children, 1989–2019.

	Birth year	Age in years (Mean ± SD)	Newborn screening	L-Carnitine supplement[a]	Intact protein restriction[b]	Lys^{-}Arg^{+} Formula[c]	Striatal degeneration (%)			Relative Risk (95% CI)	P value[d]
							Total	*Acute*	*Insidious*		
Cohort I (n = 60)	2006–2019	5.4 ± 3.7	+	+	+	+	**7%**	*50%*	*50%*	–	–
Cohort II (n = 57)	1989–2018	14.8 ± 7.5	+	+/–	+	–	**47%**	*60%*	*40%*	7.1 (2.8–18.7)	*< 0.0001*
Cohort III (n = 51)	1973–2016	21.8 ± 10.4	–	–	–	–	**90%**	*84%*	*16%*	13.5 (5.6–34.5)	*< 0.0001*

[a] For subjects in Cohort II, supplemental L-carnitine dosing was variable and inconsistent; all subjects in Cohort I took ~100 mg/kg•day until at least two years of age.
[b] Restriction of total intact (i.e., 'natural') protein to ~1 g/kg•day was used for both Cohort I and Cohort II.
[c] The Lys-Arg + metabolic formula used to treat subjects in Cohort I was devoid of lysine and had 10.8% L-arginine and 0.6% L-tryptophan per gram of protein equivalent.
[d] Two-sided Fisher's exact test of risk for striatal degeneration relative to Cohort I.

other amino acids. Lys^{-}Arg^{+} metabolic formula powder was available in two forms containing either 12% (Glutarade Junior) or 30% (Glutarade Essential) amino acids per dry powder weight, intended for use in younger versus older children, respectively. Both formulations had an energy content of 4 kcal per gram of dry powder.

Infants in Cohort I ingested 70–90 mg/kg•day of lysine, which is close to that of nursing babies (107 mg/kg•day), matches recommended safe intake (62–89 mg/kg•day), and exceeds the factorial estimate for normal growth (45–50 mg/kg•day) [16,17]. Blending intact protein and Lys^{-}Arg^{+} formula protein in a 1:1 ratio decreases the proportion of ingested lysine to arginine approximately four-fold, from 2.3–3.0 to 0.54–0.73 mg:mg (Table S2). L-arginine was not added to the diet as a separate supplement. L-carnitine (100 mg/mL) was dosed consistently at 100 mg/kg•day, rounded up to the nearest 0.5 mL increment.

After age two years, we recommended children with GA1 continue an enteral L-carnitine supplement (~1000 mg daily), observe a modest natural protein restriction (1.0–1.3 g/kg•day), and supplement their diet with lysine-free, arginine-enriched amino acids from Glutarade Essential (30% Lys^{-}Arg^{+} amino acids per dry powder weight). However, the large majority of parents ultimately chose to disregard these recommendations, such that most individuals with GA1 had a fully unrestricted diet by seven years of age (see below).

2.2.2. *Feeding strategy*

Once daily, formula was prepared to a concentration and volume appropriate for age using components measured in milliliters, ounces, or grams (on a digital kitchen scale) (Table 3). In contrast to alternating nutritional sources throughout the day, we combined all components, including L-carnitine, into the same 24-h mixture. To allow for normal variation in both energy requirement and feeding behavior, infants were allowed to feed ad libitum according to their natural schedule within ± 20% of the prescribed daily volume. When human milk was used as the source of intact protein, it was expressed, precisely measured, and added along with Lys^{-}Arg^{+} powder and L-carnitine into the daily formula. Each child began a flexible transition to table foods between 6 and 14 months of age based on developmental cues. Table food was prescribed as average daily protein in grams (rather than milligrams of lysine), allowing for ± 15–25% variation day to day. We subtracted intact table food protein from the quantity in formula to maintain a total intact protein allocation of ~1 g/kg•day (Table 3). No children in Cohort I were fed via nasogastric or gastrostomy tube.

2.2.3. *Emergency intravenous infusion therapy*

We considered inpatient IV infusion therapy for any child who exhibited signs of an infectious illness or other serious medical condition. The decision to hospitalize was based on the perceived threat of encephalopathic crisis as influenced by age, feeding behavior, and the severity and duration of illness. Based on historical observations, we considered children to be at particularly high risk for brain injury if they were < 24 months of age and had anorexia, gastroenteritis, or signs of any infectious illness lasting more than 1–2 days, even in the absence of fever. The standard inpatient protocol consisted of 10% dextrose in normal saline infused at 1.5-times the maintenance rate (glucose infusion 10–12 mg/kg•min), IV L-carnitine (100 mg/kg•dose) every 6–8 h (300–400 mg/kg•day), and supportive antiemetic, antipyretic, and antimicrobial agents as indicated (Table 2). Because dextrose, saline, and L-carnitine were consistently administered in parallel, we could not isolate the independent therapeutic role of each, and therefore refer to this combination simply as emergency IV infusion therapy.

2.2.4. *Biochemical monitoring*

Children on protocol were seen monthly in clinic during the first year of life and every two months during the second year of life (Table 2). Plasma amino acids were collected at each visit and the dietary proportion of intact to Lys^{-}Arg^{+} protein equivalent was

Table 2
Cohort I (n = 60): Prospective Treatment and Monitoring Protocol.

Clinical Context	Treatment Goals	Interventions	Monitoring Principles
Outpatient ‘Well Day’	Normal growth trajectories Age-appropriate motor milestones Normal cognition and functional independence Prevention of nutritional deficiencies	Calories and micronutrients within age-appropriate DRIs Total protein: 2.0–2.6 g/kg•day • Intact (i.e., ‘natural’) protein: 1.0–1.3 g/kg•day • Lysine-free, arginine-fortified protein: 1.0–1.3 g/kg•day • Dietary ratio of lysine to arginine (mg:mg): 0.5 • Lysine: 70–90 mg/kg•day • Tryptophan: 20–25 mg/kg•day L-carnitine (100 mg/mL): 100 mg/kg•day Formula concentration: 0.7–0.9 kcal/mL (20–27 kcal/oz) Feeding schedule: *ad libitum* Standard immunizations	Outpatient visit schedule: • Birth to 1 year: once monthly • 1 to 2 years: every 2 months Plasma amino acid analysis each visit: • Lysine: 60–100 µmol/L • Lysine to arginine ratio (mol:mol): ≤1 • Tryptophan: within normal reference range Plasma acylcarnitines for known or suspected non-adherence
Outpatient ‘Sick Day’[a]	Reduce intact protein intake by ~50% Increase lysine-free, arginine-fortified protein by ~50% Increase feeding frequency Control fever, nausea, and vomiting Treat identified infections Maintain frequent clinical contact ***Triage to inpatient setting as indicated***	Calories and micronutrients within age-appropriate DRIs Total protein equivalent: 2.0–2.4 g/kg•day • Intact (i.e., ‘natural’) protein: 0.5–0.6 g/kg•day • Lysine-free protein: 1.5–1.8 g/kg•day • Dietary ratio of lysine to arginine (mg:mg): 0.2 • Lysine: 35–45 mg/kg–day L-carnitine (100 mg/mL): 100 mg/kg•day Formula concentration: 0.7–0.9 kcal/mL (20–27 kcal/oz) Feeding schedule: q3 hours around the clock Antimicrobials, antipyretics, and antiemetics as indicated	Physical examination: assess status, identify source of illness Phone and/or outpatient follow-up at least every 12 hours ***Triage to inpatient setting if:*** • Decreased formula intake • Vomiting and/or diarrhea • Unremitting fever • Persistent or worsening illness • Treatment non-adherence
Inpatient ‘Emergency’ Therapy	Rapidly initiate IV therapy Continuous IV saline and dextrose infusion High-dose IV L-carnitine Reduce intact protein intake by ~50% Increase lysine-free, arginine-fortified protein by ~50% Control fever, nausea, and vomiting Treat underlying infections as indicated Ensure smooth transition to enteral ‘Well Day’ diet	Replace fluid deficit by (repeated) IV saline bolus as indicated Dextrose 10% in normal saline: 1.5x maintenance requirement L-carnitine IV: 100 mg/kg•dose every 6 hours ‘Sick Day’ formula: *ad libitum* as tolerated Antimicrobials, antipyretics, and antiemetics as indicated	Laboratory studies at admission: • Glucose, general chemistries, and complete blood count Repeat POC glucose and laboratory studies as indicated Temperature and vital signs every 6–8 hours Neurologic assessment every 6–8 hours Strict quantification of total fluid intake and output Daily weight tracking

[a] For routine vaccinations, we administer ‘Sick Day’ diet plan in the outpatient setting for 24–48 h.

Abbreviations: DRI, dietary reference intake, National Institutes of Health (https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes); IV, intravenous; POC, point-of-care.

Table 3
Sample 24-h Diets for Infants and Children in Cohort I[a]

Age, months	2	6	12	15	20
Approximate Weight, kg	4.5	8.0	10.0	10.5	11.5
Dietary variables					
Intact protein, g/kg•day	1.2	1.1	1.0	1.0	1.0
Protein equivalent from $Lys^{-}Arg^{+}$ metabolic formula, g/kg•day	1.2	1.1	1.0	1.0	1.0
L-carnitine, mg/kg•day	100	100	100	100	100
Total calories, kcal/kg•day[b]	110	95	75	35	15
Total formula volume, mL/kg•day[c]	140	120	95	45	20
Sample dietary components					
Human milk, ounces[d]	16	–	–	–	–
Commercial milk-based formula powder, grams	–	90	85	–	–
Cow or soy milk, ounces[d]	–	–	–	6	–
$Lys^{-}Arg^{+}$ (12% AA content) formula powder, grams[e]	45	75	85	45	–
$Lys^{-}Arg^{+}$ (30% AA content) formula powder, grams[e]	–	–	–	20	40
Intact protein from table food, grams	–	–	1–2	3–5	10–13
L-carnitine (100 mg/mL), mL	4.5	8.0	10.0	11.0	11.5
Add potable water to make total 24-h volume, ounces	21	32	32	16	8
Formula concentration, kcal/oz	24	24	23	23	20

[a] Diets listed above are intended to serve as *examples only;* individual diets and formula components will vary based on patient/family preference and natural feeding behaviors.

[b] Total calories include those from human milk, cow/soy milk, or Lys-Arg + metabolic formula, *but not those from table food.*

[c] Total volume includes human milk, cow/soy milk, or Lys-Arg + metabolic formula, but not other fluid sources (e.g. juice, almond milk, rice milk, etc.), which we did not attempt to quantify.

[d] Composition per ounce: Human milk, 20 kcal, 0.33 g protein; Cow milk, 20 kcal, 1 g protein; Soy milk, 20 kcal, 1 g protein.

[e] There are two formulations of $Lys^{-}Arg^{+}$ formula powder which contain either 12% (Glutarade Junior) or 30% (Glutarade Essential) amino acids per dry powder weight, intended for younger versus older children, respectively. Both formulations have an energy content of 4 kcal per gram of dry powder.

adjusted to achieve a plasma lysine concentration of 60–100 μmol/L and a plasma Lys/Arg ratio ≤ 1.0 mol:mol. Following the initial diagnosis of GA1, we did not use blood C5DC or urine organic acid levels to guide management. Plasma acylcarnitines were monitored only for research documentation or suspected/known dietary non-adherence. To evaluate the long-term safety of $Lys^{-}Arg^{+}$ metabolic formula, we collected general laboratory measures in each subject at a median age of 2.5 (range 0.4–11.0) years.

2.2.5. Data collection

During routine outpatient visits, we collected data about growth, psychomotor development, metabolic formula intake, and plasma amino acid concentrations. For the purpose of this investigation, we restrict detailed diet and amino acid information to the first two years of life, which roughly defines the window of neurologic vulnerability observed in Cohorts I–III and allows for reliable dietary accounting. The timing of motor milestone acquisition was compared to that of healthy siblings without GA1. A subgroup of 10 children from Cohort I (median 7.0, range 5–12 years) and their age-matched (p = .086) unaffected siblings (median 8.9, range 6–17 years) completed Stanford-Binet Intelligence Scales, 5th Edition (SB-5), comprised of eight subscales: full-scale intelligence quotient (FSIQ), verbal IQ, non-verbal IQ, working memory, and knowledge, as well as visual-spatial, quantitative, and fluid reasoning [18].

2.3. Estimation of Cerebral Amino Acid Influx

For patients in Cohort I treated with $Lys^{-}Arg^{+}$ metabolic formula, we designed a custom Excel spreadsheet (Microsoft Corporation) to estimate cerebral uptake of circulating amino acids during the first two years of life [16]. Briefly, a group of three cationic amino acids (lysine, arginine, ornithine) compete for entry into the brain via a common facilitative transporter (SLC7A1; a.k.a. CAT1, y+) [19,20] which is saturated under physiological conditions, such that cerebral influx of each SLC7A1 substrate is influenced by ambient plasma concentrations of its competitors. Competition is expressed by an *apparent* K_m, called K_{app} (μmol/L), calculated for each amino acid according to the equation:

$$K_{app} = K_m \left[1 + \sum\nolimits_{1->n} (C_i/K_i)_n \right] \tag{1}$$

where K_m is the classical Michaelis-Menten affinity constant for the single amino acid of interest, C_i is the plasma concentration (μmol/L) for each of n competitors, and K_i is the classical affinity constant of that competitor (μmol/L). For a given plasma amino acid profile, K_{app} values were determined for each SLC7A1 substrate using empirically-derived Michaelis-Menten constants [21]. The K_{app} value was then used to estimate the brain influx (nmol per minute per gram of brain tissue) of each amino acid in the competing group, according to the equation:

$$\text{Influx} = (V_{max})(C)/(K_{app} + C) \tag{2}$$

where V_{max} and C are the maximal transport velocity (nmol/min•g) and plasma concentration (μmol/L), respectively, of each amino acid [22]. Estimated brain influx values were compared to those calculated from a pediatric control population (N = 52) and depicted as standard scores (i.e., z-scores), where z = [(study subject value – control mean)/control standard deviation]. All cerebral uptake values represent calculated heuristics and should not be interpreted as *direct* measurements of amino acid flux. An analogous method was used to estimate brain uptake of zwitterionic amino acids (glutamine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine) that compete for entry into the brain via the SLC7A5 transporter [21].

2.4. Statistics

Statistical calculations were performed using Prism 8 software (https://www.graphpad.com). Kaplan-Meier analyses were applied to outcomes of striatal degeneration, death, and time to motor milestone acquisition. The two-sided Fisher's exact test was used to calculate relative risk of brain injury in Cohorts II and III as compared to Cohort I. Normal reference plasma amino acid concentrations were derived from 52 non-fasted healthy children without disorders of amino or organic acid metabolism and converted to z-scores for graphical representation [16]. Developmental trajectories of GA1 patients were compared with those of healthy siblings using Mann-Whitney and logrank Mantel-Cox tests. The two-tailed Student's t-test was used to compare SB-5 scales between GA1 subjects and their age-matched sibling controls. Paired vital signs before and 18–36 h into IV infusion therapy were compared using the Wilcoxon signed-rank test. To investigate differences among three or more groups, we used one-way analysis of variance (ANOVA) followed by the Tukey post-test for pairwise comparisons. Most continuous data sets (e.g. plasma amino acid concentrations and concentration ratios) were not normally distributed, and thus their central tendency is reported as the median and 25th to 75th interquartile range (IQR) except where otherwise noted.

3. Results

3.1. Cohort I (DOB 2006–2019): prospective treatment and monitoring

3.1.1. Diagnosis

Forty-one (68%) children in Cohort I were homozygous for *GCDH* c.1262C > T (p.Ala421Val). For 17 of them, this risk for GA1 was

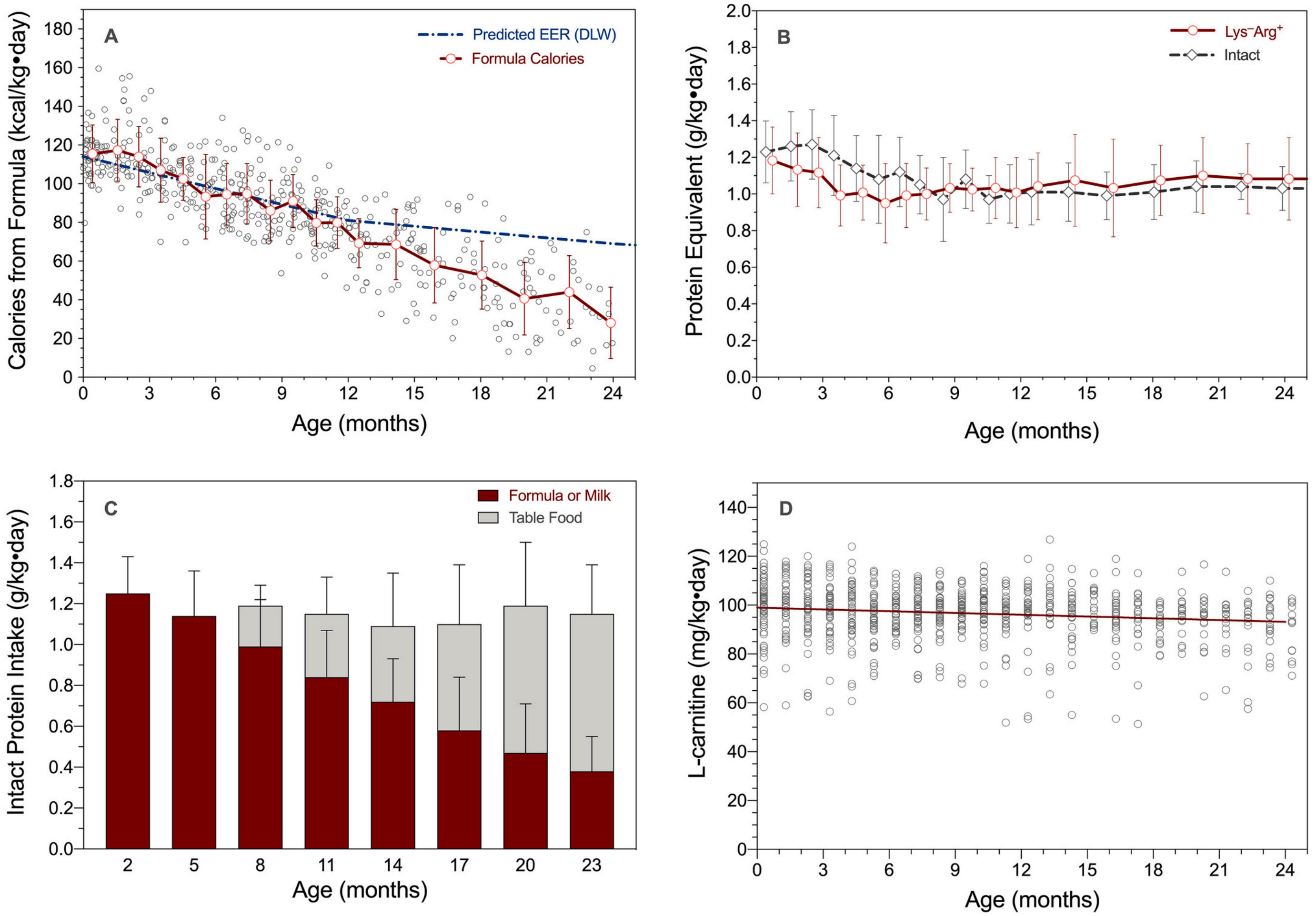

Fig. 2. Cohort I: Dietary variables from Birth to 24 Months. (A) For the first 12 months of life, average prescribed calories from formula (red circles, ± 1SD) closely matched estimated energy requirement (EER) as determined by the doubly-labeled water method (DLW; https://www.nap.edu/), shown as a blue dotted line. Unmeasured calories from table foods increase during the second year of life. (B) Intact protein (gray diamonds) and lysine-free, arginine-enriched amino acids from metabolic formula (Lys$^-$Arg$^+$; red circles) were prescribed in approximately equal quantities throughout the first two years of life. (C) After ~8 months of age, the proportion of intact protein from table food (gray) as compared to milk sources (red) increased. (D) The average enteral L-carnitine supplement was ~100 mg/kg•day (red line) and at no time decreased to < 50 mg/kg•day (gray circles).

known prenatally and molecular testing of umbilical cord blood in our CLIA-certified molecular laboratory yielded a definitive diagnosis at a median of one (range 0–4) postnatal day(s). Among 43 remaining patients in Cohort I, dried filter paper blood spots were collected at a median of 2 (range 0–5) postnatal days and results were reported through state NBS at a later median age of 8 (range 5–14) days ($p < .0001$). The median diagnostic filter paper C5DC concentration was 1.15 (IQR 0.88–1.78) μmol/L and the minimum was 0.42 μmol/L (normal reference value < 0.27 μmol/L). In Pennsylvania, NBS included reflex tier two testing for the *GCDH* c.1262C > T variant. We encountered no false negative or false positive screening results at our center between 1994 and present.

3.1.2. *Dietary therapy*

For each child in Cohort I, we analyzed an average of 18 diets prescribed between birth and two years of life (1061 dietary formulations in total). Calories represented in milk sources and medical food closely matched estimated energy expenditure until ~12 months of age, after which unmeasured calories from table foods increased (Fig. 2a). We routinely prescribed equal quantities of intact and modified protein but infants tolerated higher proportional intact protein (1.1–1.4 g/kg•day) during the first six months of rapid growth (Fig. 2b). After age two months, the most common source of intact protein shifted from breastmilk to commercial milk-based infant formula, and during the second year of life we introduced natural milk sources along with an increasing quantity and variety of table foods (Table 3, Fig. 2c). As children transitioned to natural foods, 42% switched to a Lys$^-$Arg$^+$ medical formula containing 30% (Glutarade Essential) as compared to 12% (Glutarade Junior) amino acids per dry powder weight, which decreased their prescribed formula volume by 50–75% (Table 3). The L-carnitine supplement for each child in Cohort I was consistently ~100 mg/kg•day during the first two years of life and at no time was < 50 mg/kg•day (Fig. 2d).

3.1.3. *Outpatient biochemical monitoring, birth to two years*

We collected and analyzed a total of 1054 plasma amino acid samples in the outpatient setting from children in Cohort I; 855 of these were obtained during the first two years of life under conditions of strict dietary adherence (average of 14 per subject and one per visit). Here, we define a 'strict' diet as one in which intakes of both intact and lysine-free protein were reliably known and the prescribed and ingested volume of Lys$^-$Arg$^+$ metabolic formula closely aligned. The 'relaxed' diet applies to older children on a recommended but unquantified (typically higher) range of intact protein, whose intake of metabolic formula often fell short of the recommended volume.

A strict Lys$^-$Arg$^+$ diet was associated with median plasma lysine and arginine concentrations of 80 (IQR 60–100) μmol/L and 98 (IQR 80–115) μmol/L, respectively (Fig. 3a), and a median plasma Lys/Arg

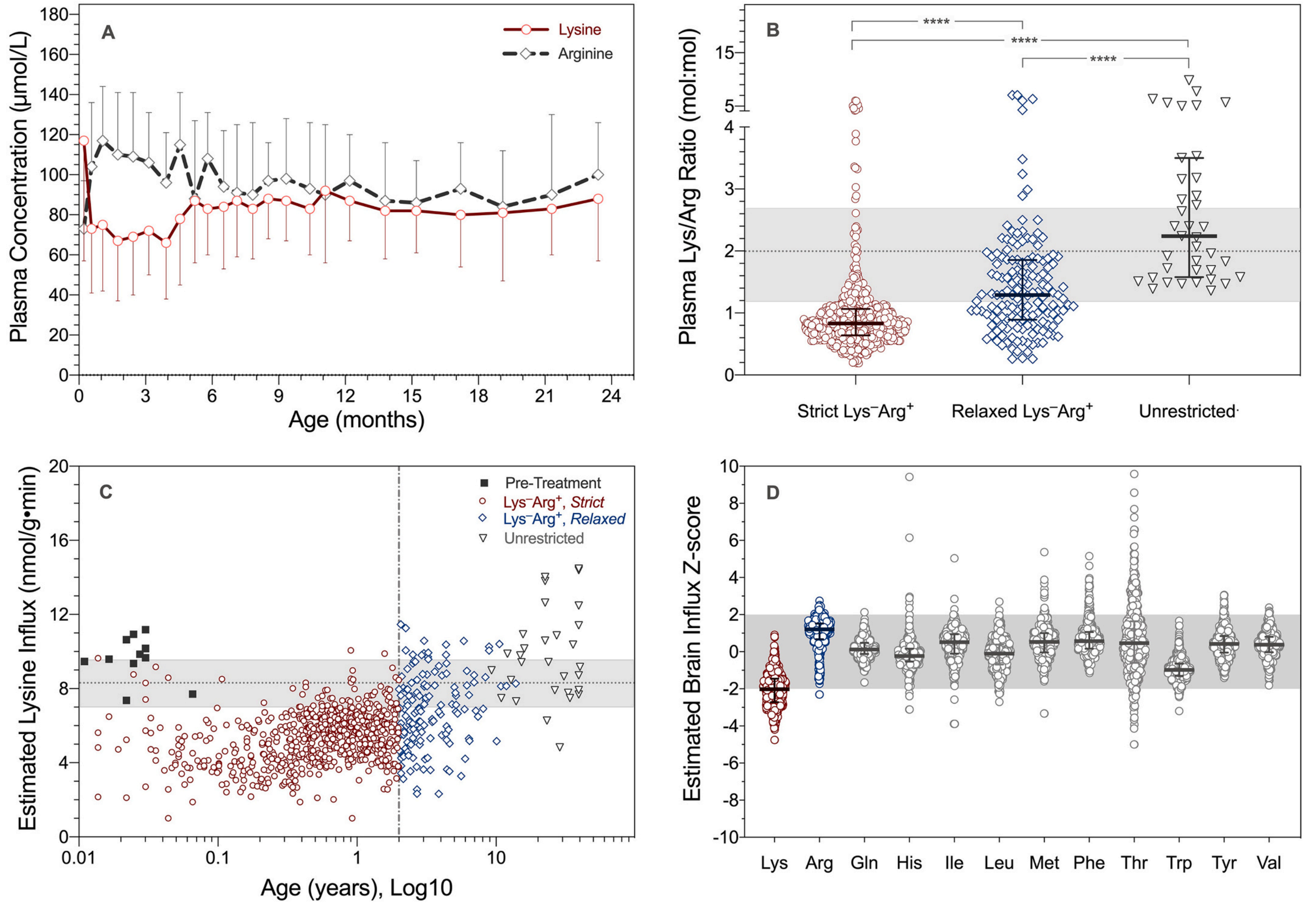

Fig. 3. Cohort I: Outpatient Biochemical Monitoring ($n = 1054$ amino acid samples). (A) The mixture of intact protein with Lys$^-$Arg$^+$ metabolic formula achieved a dietary lysine to arginine ratio of ~0.5 mg:mg, which maintained plasma lysine (red circles, ± 1SD) and arginine (gray diamonds) at nearly equal concentrations. (B) The average ratio of lysine to arginine in plasma (Lys/Arg) was ≤1.0 on strict diet (red circles, mean, ± 1SD) but increased as adherence to both intact protein recommendations and metabolic formula intake became more relaxed after age two years (blue diamonds). Most GA1 subjects older than 7 years (gray triangles) no longer restricted their natural protein intake or used Lys$^-$Arg$^+$ metabolic formula and their plasma Lys/Arg ratios increased to the normal control range (gray shading, median and IQR; ****$p < .0001$). (C) Plasma lysine, arginine, and ornithine concentrations were used to estimate cerebral lysine influx in relation to dietary exposure in four groups: pre-treated newborns (dark gray squares), young children on a strict diet using Lys$^-$Arg$^+$ metabolic formula (red circles), children on a relaxed intake of metabolic formula and natural protein (blued diamonds), and older children on an completely unrestricted natural diet (open gray triangles). The gray shaded area represents estimated cerebral lysine uptake (median and IQR) calculated from plasma amino acid profiles of 52 healthy children without GA1. Age on the abscissa is depicted as a log10 scale with a vertical dashed line at age two years. (D) For children ≤2 years of age on a strict Lys$^-$Arg$^+$ diet, lysine (red circles) and arginine (blue circles) had the lowest and highest adjusted cerebral uptake values, respectively, depicted here as z-scores (gray shading represents the normal z-score range of −2 to +2). Calculated brain uptake values for all other SLC7A1 and SLC7A5 substrates (gray circles), including tryptophan, were within the normal reference range.

ratio of 0.8 (IQR 0.6–1.1) mol:mol (Table 4, Fig. 3b). Children ingesting Lys$^-$Arg$^+$ formula had normal circulating concentrations of all SLC7A1 and SLC7A5 amino acid substrates (z scores +2 to −2) with the notable exception of lysine, which was 38% below the normal reference median and had the lowest reference-adjusted plasma concentration. Based on plasma concentrations of lysine, arginine, and ornithine in children on strict dietary therapy, we estimated median cerebral lysine uptake to be approximately 40% lower than values derived from either a control population or older GA1 subjects on unrestricted diet (Table 4, Fig. 3c). Calculated brain uptake values for all other SLC7A1 and SLC7A5 substrates, including tryptophan, were within the normal reference range (Fig. 3d).

Serum folate concentration exceeded the limit of quantitation (> 20 ng/mL) in children taking Lys$^-$Arg$^+$ metabolic formula; those on supplemental L-carnitine had elevated total and free plasma carnitine levels ($p < .0001$). Other laboratory indices of nutritional sufficiency and general health were within normal reference intervals (Table S3). Plasma tryptophan values were within the normal range (Table 4) and no child in Cohort I developed signs of tryptophan or niacin deficiency (i.e. pellagra) [23].

3.1.4. Dietary adherence

All Cohort I subjects remained on a strict diet using Lys$^-$Arg$^+$ metabolic formula until two years of age. Dietary adherence decreased steadily thereafter, so that the probability of a child remaining on Lys$^-$Arg$^+$ metabolic formula after age seven years was only 12% (Fig. 4a). Cessation of diet was associated with a higher median plasma lysine concentration and Lys/Arg ratio (Table 4, Fig. 3b). Similarly, only 32% of GA1 subjects remained on an L-carnitine supplement after age six years. Plasma free and total carnitine decreased markedly as a consequence but we observed no overt clinical signs of carnitine deficiency (Fig. 4b).

3.1.5. Hospitalizations

There were 153 recorded hospitalizations for children in Cohort I. During the first two years of life, each child was hospitalized a median

Table 4
Plasma Amino Acids and Estimated Cerebral Lysine Influx Relative to Dietary Exposure[a].

	Age Range (years)	Plasma concentration, μmol/L			Plasma Lys/Arg Ratio, mol:mol	Cerebral Lys Influx, nmol/min g[c]
		Lysine	Arginine	Tryptophan[b]		
Normal reference control (n = 52)		*130 (97–180)*	*73 (42–94)*	*52 (35–62)*	*2.0 (1.4–2.7)*	*8.6 (7.0–9.6)*
Strict Lys^-Arg^+ Metabolic Formula Diet (n = 855)[d]	Birth–2.0	80 (60–100)	98 (80–115)	38 (31 – 101)	0.8 (0.6–1.1)	5.4 (4.3–6.3)
Relaxed Lys^-Arg^+ Metabolic Formula Diet (n = 150)[d]	> 2.0–13.8	97 (73–129)	82 (62–102)	36 (30–44)	1.3 (0.9–1.9)	6.8 (5.3–8.2)
Unrestricted Natural Diet (n = 49)	9.3–30.8	167 (129–213)	74 (41–91)	na	2.3 (1.6–4.2)	9.7 (8.7–10.9)
ANOVA P value		*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*
Pairwise comparisons (Tukey)						
Control vs. Strict Lys^-Arg^+		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Control vs. Relaxed Lys^-Arg^+		< 0.0001	0.0651	< 0.0001	< 0.0001	< 0.0001
Control vs. Unrestricted		< 0.0001	0.9993	na	0.0448	0.0083
Strict Lys^-Arg^+ vs. Relaxed Lys^-Arg^+		< 0.0001	0.0006	0.8386	0.2626	< 0.0001
Strict Lys^-Arg^+ vs. Unrestricted		< 0.0001	< 0.0001	na	< 0.0001	< 0.0001
Relaxed Lys^-Arg^+ vs. Unrestricted		< 0.0001	0.1065	na	0.0038	< 0.0001

Abbreviations: IQR, interquartile (25th–75th) range; na, not applicable due to insufficient plasma tryptophan data.

[a] All descriptive statistics are expressed as median (IQR), where IQR is the 25%–75% interquartile range.

[b] Most amino acid profiles from the "Unrestricted Natural Diet" group were acquired before the CSC laboratory accurately quantified tryptophan in plasma.

[c] Cerebral lysine (Lys) influx is not a measured value; rather, it is calculated using empirically derived Michaelis-Menten constants for lysine, arginine, and ornithine.

[d] We define a 'strict' diet as one in which intact protein is precisely quantified; the 'relaxed' diet applies to older children on a recommended but unquantified range of intact protein.

of three (range 0–14) times and 85% of children were hospitalized at least once for emergency IV therapy. The median age of hospitalization was 12.6 months for the main indications of gastroenteritis (44%), respiratory tract infection (32%), fever without an identified source (16%), and acute otitis media (5%). Each hospitalization lasted a median of two (1–8) days.

For children in Cohort I, Table 5 lists vital signs and select laboratory values recorded at the time of admission and again an average of 24 (18–36) hours into the course of emergency IV therapy. Two-thirds of patients admitted to the hospital before age two years had objective signs of inflammation indicated by elevated body temperature (> 38 °C, 100.4 °F) and/or a serum C-reactive protein concentration > 8.0 mg/dL. These two variables were modestly correlated ($r_s = 0.42$, $p < .0001$). Average body temperature did not change during the first 18–36 h of hospitalization ($p = .620$). Heart rate and blood pressure were generally within the normal range at the time of admission and decreased 2.4–7.0% during IV therapy ($p < .0001$).

Laboratory studies collected at the time of admission did not show metabolic acidosis or hypoglycemia; this was true even in two patients who presented with acute striatal necrosis. The mean presenting serum total carbon dioxide and anion gap were 22 ± 3 (range 13–29) mmol/L and 11 ± 3 (range 5–21) mmol/L, respectively, and only two children (1.3%) presented with a serum total carbon dioxide concentration < 16 mmol/L. The average presenting serum glucose concentration was 95 ± 22 (range 47–202) mg/dL; only four values (2.6%) were < 60 mg/dL and these were not associated with signs of neuroglycopenia or metabolic stroke. For more than 90% of hospitalizations, blood was collected only at the time of admission. For the few subjects who had at least one repeat set of laboratory studies ($n = 14$), average serum glucose increased in response to IV dextrose, but this result was not significant ($p = .143$) due to broad variation (−17% to +174%) among a small number of samples. No child developed hyperglycemia sufficient to warrant insulin therapy.

There were no serious adverse events associated with dextrose

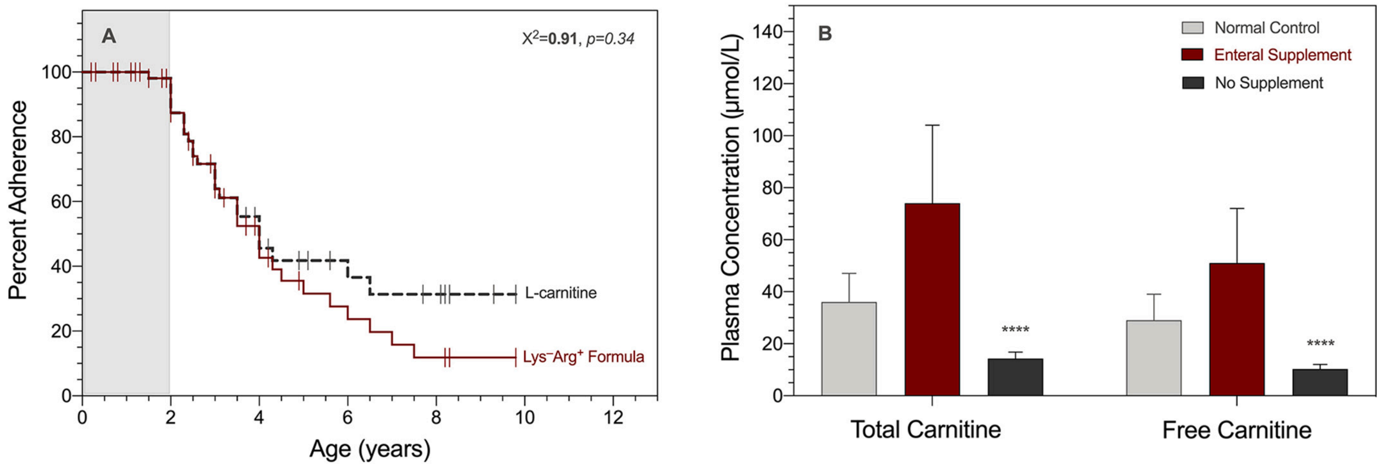

Fig. 4. Cohort I: Dietary Adherence. (A) All Cohort I subjects remained on Lys^-Arg^+ metabolic formula during the first two years of life (gray shaded area). Thereafter, the majority of families stopped dietary therapy of their own accord. By age seven years, the probability that a child from Cohort I remained on metabolic formula (red solid line) or enteral L-carnitine (gray dashed line) decreased to 12% and 32%, respectively. (B) Relative to healthy non-GA1 controls (light gray bars, mean ± 1SD), GA1 subjects on an L-carnitine supplement (red bars) had higher total and free carnitine concentrations in plasma. Cessation of L-carnitine therapy (dark gray bars) was associated with five-fold decreases of plasma total and free carnitine but no overt clinical signs of carnitine deficiency (****$p < .0001$).

Table 5

Cohort I: Measurements at the Time of Hospital Admission and 18–36 Hours Into IV Infusion Therapy (n = 153 Hospitalizations).

	Hospital admission	Infusion therapy 18–36 Hours	Relative change	Wilcoxon P
	Mean ± SD (Range)	Mean ± SD (Range)		
Vital signs				
Body temperature, C°	37.4 ± 0.8 (34.9–40.6)	37.5 ± 0.9 (36.1–39.9)	0.10%	0.620
Heart Rate, bpm	134 ± 16 (100–188)	130 ± 13 (86–170)	–2.4%	0.018
Systolic blood pressure, mmHg	110 ± 13 (81–145)	104 ± 11 (80–134)	−4.6%	< 0.0001
Diastolic blood pressure, mmHg	65 ± 12 (41–90)	58 ± 9 (42–88)	−7.0%	< 0.0001
Serum biomarkers				
Glucose, mg/dL	95 ± 22 (47–202)	110 ± 28 (79–182)	28%	0.143
Bicarbonate, mmol/L	22 ± 3 (13–29)	na		
Anion Gap, mmol/L	11 ± 3 (5–21)	na		
Blood Urea Nitrogen, mg/dL	12 ± 4 (5–30)	na		
Creatinine (mg/dL)	0.27 ± 0.06 (0.20–0.40)	na		
Alanine Aminotransferase (U/L)	27 ± 14 (11–89)	na		
C-reactive Protein (mg/L)	18.9 ± 7.7 (< 0.02–100)	na		

Abbreviations*:* C, Celsius; na, not available; SD, standard deviation.

infusions of 9–12 mg/kg•min or L-carnitine doses ≤400 mg/kg•day, which were administered exclusively by peripheral (as opposed to central) vein. No child developed motor regression while receiving emergency IV therapy.

3.2. *Clinical outcomes in cohorts I-III*

3.2.1. *Psychomotor outcomes*

The incidence of striatal degeneration in Cohorts I, II, and III was 7%, 47%, and 90%, respectively ($p < .0001$) (Fig. 5a). Newborn screening decreased the relative risk of striatal degeneration 7-fold ($p < .0001$; 95%CI 3- to 19-fold, $p < .0001$) and, when coupled to the use of Lys$^-$Arg$^+$ metabolic formula, 14-fold (95%CI 6- to 35-fold, $p < .0001$) (Table 1). No neurologic injuries occurred after 19 months of age.

The proportion of striatal lesions that presented as acute encephalopathic crisis versus insidious motor delay was 84% before the advent of NBS (Cohort III) and 50% in the modern NBS era (Cohort I). Children with insidious motor delay tended to have better functional outcomes as compared to those who presented with acute motor regression. In Cohort I, two children were rendered mute, non-ambulatory, and fully disabled after suffering acute striatal necrosis; two additional children from this cohort had infantile-onset hypotonia and dystonia but no witnessed encephalopathic crisis. They learned to crawl (12.5 and 13 months) and walk independently (24 months) and now enjoy a high degree of self-efficacy.

All 91 subjects with GA1 who were spared neurologic injury are alive and well at a mean follow up of 9.6 ± 8.3 (range 0.2–43.4) years. Uninjured children from Cohort I (n = 56) who took Lys$^-$Arg$^+$ metabolic formula grew normally during the first two years of life (Fig. 6). They achieved independent sitting (5–9 months) and crawling (8–11 months) as expected relative to their unaffected siblings (Fig. 7a-d), but walked about two months later, at a median of 14 as compared to 12 months (p = .045). First articulate words were similarly delayed about two months (p = .036). Ten subjects from Cohort I who were old enough for cognitive testing (5–12 years) scored similar to their age-matched control siblings on FSIQ and SB-5 subscales (Fig. 7e).

3.2.2. *Mortality and comorbidity*

Across cohorts, a total of 77 patients suffered brain injury (Cohort I, n = 4; Cohort II, n = 27; Cohort III, n = 46). Post-mortem and neuroimaging studies consistently showed bilateral neuronal loss and gliosis of the lentiform nuclei in a dorsolateral to ventromedial gradient ranging from mild to severe (Fig. 8a). Eighteen patients with striatal lesions were lost to clinical follow up. Of the 59 remaining, 24% (n = 14) died from complications of dystonia at a median age of 14.5 (range 2.5–39.1) years (Fig. 5b). Medical and surgical comorbidities were common among survivors; most became wheelchair-dependent

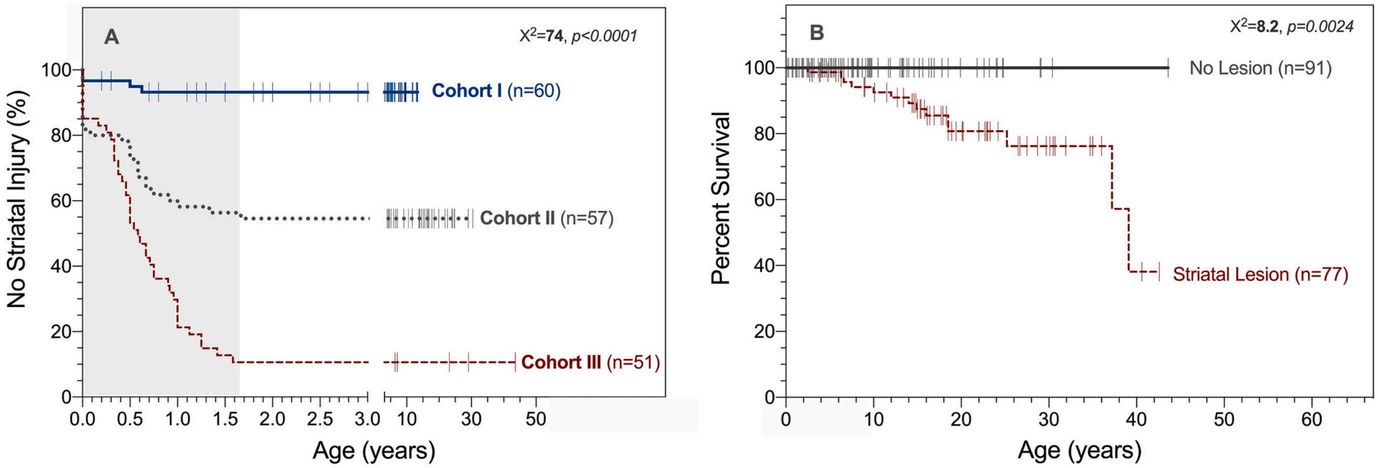

Fig. 5. All Cohorts: Clinical Outcomes. (A) The incidence of striatal degeneration in Cohorts I (blue solid line), II (gray dotted line), and III (red dashed line) was 7%, 47%, and 90%, respectively ($p < .0001$). In 168 patients managed over a span of three decades, no brain injuries were observed after age 19 months (gray shaded area). Note divided abscissa. (B) Striatal lesions were a risk factor for untimely death (red dashed line; median survival 39 years, p = .0024). All 91 patients who escaped neurologic injury are alive and functionally independent (gray solid line).

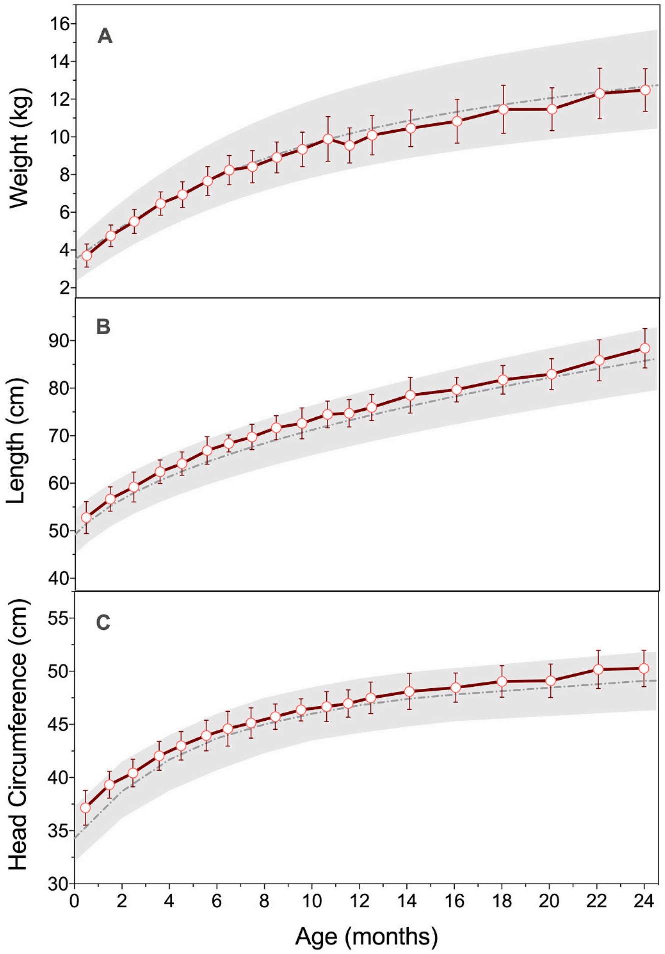

Fig. 6. Cohort I: Growth from Birth to Two Years. Average weight (A), length (B), and head circumference (C) were normal during the first two years of life for children in Cohort I (red circles, mean ± 1SD). Gray shaded areas represent World Health Organization reference intervals for females (3rd to 97th centile).

for mobility, required gastrostomy feeding, and were rendered dysarthric or mute. Intellect was typically preserved, but dystonia often posed an insurmountable barrier to the use of assistive communication devices.

3.2.3. *Medical and surgical management of dystonia*

For GA1 patients with neurologic injury, diazepam was the mainstay of medical management, acting as both a central muscle relaxant and anxiolytic to reduce dystonia. Diazepam was typically administered three of four times daily at doses titrated to balance therapeutic benefit against side effects of sedation and sialorrhea. Tolerance was common with this agent; after years of exposure, patients could require 1.5 mg/kg•day or more to sustain efficacy. Enteral baclofen was rarely used as adjunctive therapy. Levodopa and trihexyphenidyl, sometimes used to treat movement disorders in children [24], were ineffective against GA1-associated dystonia. Four patients gained moderate relief from continuous intrathecal ($n = 2$) or intraventricular ($n = 2$) baclofen [25,26]. One adult patient who underwent internal pallidal deep brain stimulation reported subjective relief of musculoskeletal pain but exhibited no objective improvements in dystonia rating scales or motor function.

There were 35 orthopedic procedures performed among 16 (21%) individuals (1–5 operations per patient) to manage musculoskeletal complications such as scoliosis ($n = 11$; Fig. 8b) and hip dislocation/subluxation (13 hips in 8 patients). The average age at first operation was 13.8 (range 6–25) years (Figs. 8c) and the most common procedures were posterior spinal fusion and proximal femoral varus derotation osteotomy and/or pelvic osteotomy (Fig. 8d). During the postoperative period, pain and anxiety often exacerbated dystonia in a self-reinforcing cycle, sometimes escalating to life-threatening status dystonicus.

4. Discussion

4.1. *Pathophysiology and prevention of striatal degeneration*

The GCDH-deficient brain accumulates glutaryl-CoA, GA, and 3HGA proportional to its rate of lysine uptake [27,28], which is highest during embryonic life and early infancy [29,30]. Studies in cell culture and transgenic *Gcdh−/−* mice reveal the potential for these compounds to activate excitotoxic cascades, amplify oxidative stress, inhibit respiratory complexes, depress sodium-potassium ATPase activity, and disrupt the maturation and function of astrocytes [31–39]. One or more of these mechanisms might precipitate striatal degeneration, but none fully explain its temporal and histological selectivity. The maturation of medium spiny neurons must also play a role, rendering them uniquely vulnerable to histotoxins during a circumscribed phase of development [40,41].

Neuroimaging studies indicate how this biochemical state might affect the developing brain. Asymptomatic infants with GA1 have reduced fluorodeoxyglucose uptake in the lentiform nuclei, reduced cerebral blood flow (CBF), and signs of cerebrovascular congestion [9,42] that coincide with normal developmental surges of synaptogenesis, excitatory neurotransmission, substrate utilization, and perfusion [43–45]. As this normal developmental sequence unfolds, medium spiny neurons in the striatum—with their unique constellation of channels, receptors, and enzymes [40]—potentially experience a mismatch between energy supply and demand during a time when they are unusually sensitive to excitotoxic and oxidative forms of injury [46–48].

Based on the foregoing model, protecting the striatum from GCDH deficiency is predicated on minimizing its exposure to neurotoxins during a discrete phase of development [41]. In principle, this is accomplished by manipulating dietary amino acid content to reduce cerebral lysine influx while administering L-carnitine to clear glutaryl-CoA from brain cell mitochondria (Fig. 1, [16,27,49–51]. The provision of 'emergency' therapy in the form of IV dextrose and saline is intended to stabilize energetically fragile brain tissue during intervals of physiologic stress [41].

Beginning in 1989 [52,53], NBS allowed us to detect pre-symptomatic Amish babies with GA1 and decrease their risk of neurologic injury from 90% (Cohort III) to 47% (Cohort II), representing a sevenfold reduction of relative risk. The use of Lys^-Arg^+ metabolic formula in Cohort I further reduced the incidence of striatal degeneration to just 7% (14-fold reduction of relative risk) [16]. Our experience aligns closely with that of Boy et al., who found that NBS for GA1 in Germany reduced the incidence of neurologic injury from > 90% to 36% and, when coupled to lysine-restricted diet, to 7% [54]. Taken together, these data show that: (1) the neuroprotective effects of emergency IV therapy and Lys^-Arg^+ metabolic formula are *additive*; (2) metabolic formulas devoid of lysine might be to some degree interchangeable; (3) similar outcomes can be expected in cohorts that differ with regard to the distribution of pathogenic *GCDH* genotypes; and 4) the same basic elements of therapy can be deployed in various settings to comparable effect.

4.2. *Elements of effective therapy*

Newborn screening with tandem mass spectrometry (TMS) detects GA1 with a sensitivity and specificity of at least 93% and 99%, respectively [15,55,56]. In certain populations like the Amish, targeted allele detection enables molecular diagnosis within hours of life [12]

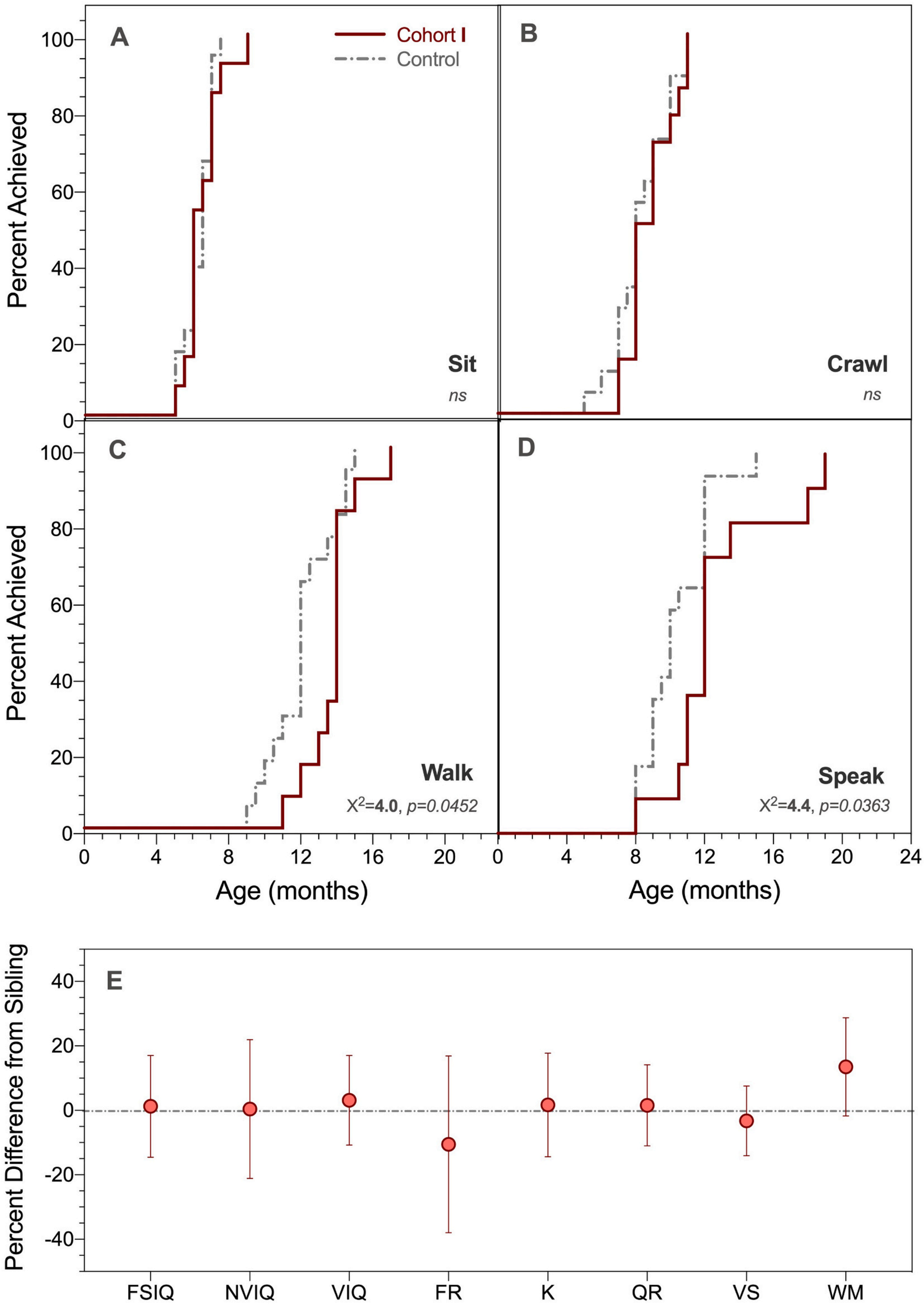

Fig. 7. Cohort I: Developmental and Cognitive Outcomes. Uninjured children from Cohort I ($n = 56$) who took Lys$^-$Arg$^+$ metabolic formula during the first two years of life (red solid lines) achieved independent sitting (A) and crawling (B) as expected relative to their unaffected siblings (gray dashed lines) but walked independently (C) and used their first articulate words (D) about two months later. Ten subjects from Cohort I (red circles, ± 1SD) who were old enough for cognitive testing (median 7.0, range 5–12 years) scored similar to their age-matched control siblings (median 8.9, range 6.0–17.1 years) on FSIQ and SB-5 subscales [verbal IQ (VIQ), non-verbal IQ (NVIQ), working memory (WM), and knowledge (K), as well as visual-spatial (VS), quantitative (QR) and fluid (FR)]. The horizontal dashed line represents absolute equivalence (i.e., 0% difference relative to an age-matched sibling).

but this strategy does not appear to improve outcome over standard TMS-based methods with ~8-day turnaround. Pre-symptomatic diagnosis not only reduces the risk of neurologic impairment but also its potential severity. In the absence of NBS for GA1, ~85% of striatal lesions present as acute, pan-striatal cytotoxic edema that culminates in extensive neuronal loss, generalized dystonia, and profound functional impairment (Cohort III). In the post-screening era, ~50% of children with striatal degeneration present with insidious motor delay; the

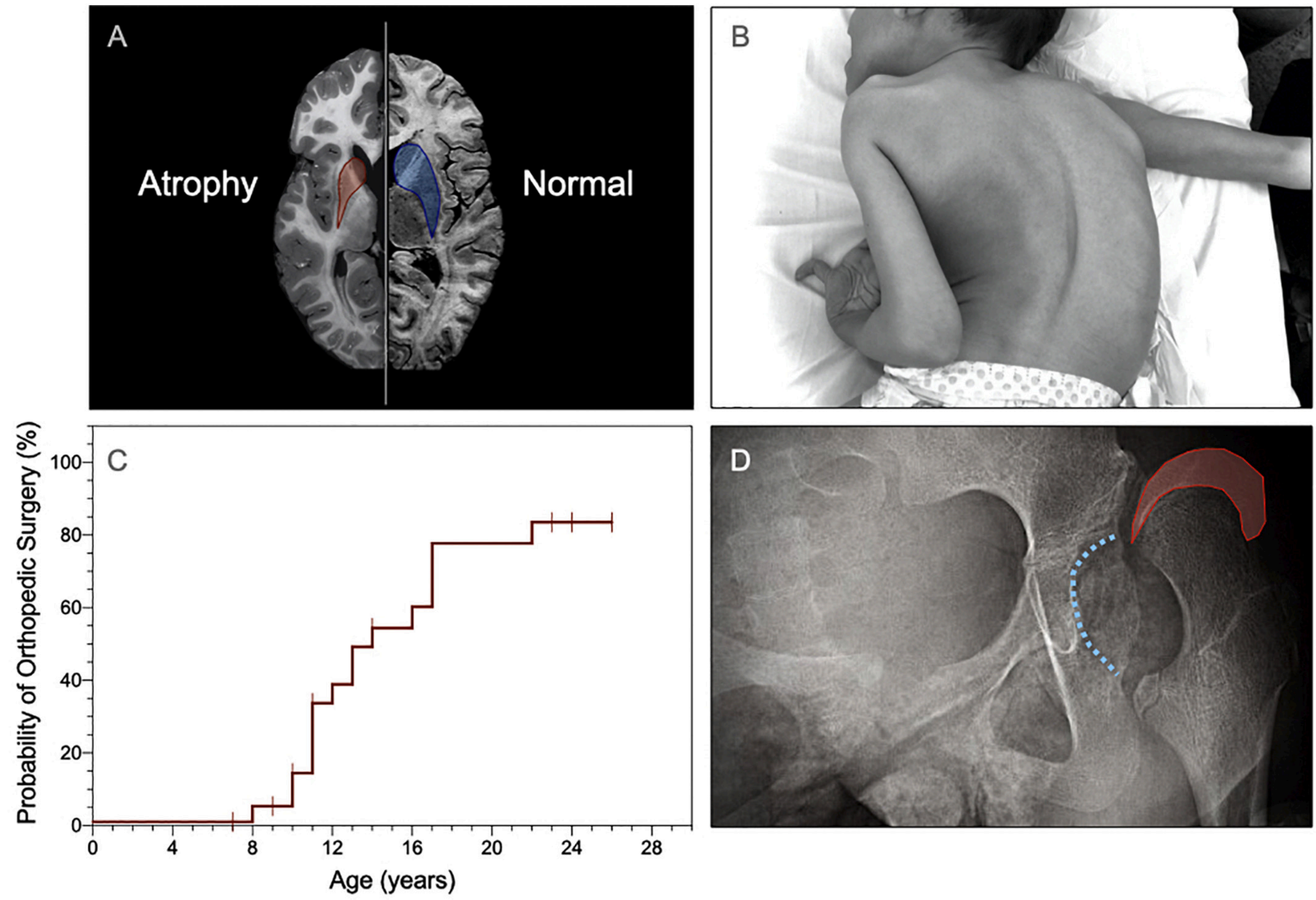

Fig. 8. Morbidity. (A) An axial brain section from a deceased GA1 patient shows characteristic atrophy of the striatum in a dorsolateral to ventromedial gradient (left hemisphere, red shading). A normal age-matched brain is shown for comparison (right hemisphere, blue shading). (B) Striatal lesions typically cause severe, generalized dystonia. (C) The large majority of patients with dystonia require one or more orthopedic surgeries to address common musculoskeletal complications such as scoliosis and hip dislocation. (D) A radiograph of the left hip shows the femoral head (red shading) dislocated from the acetabulum (blue dotted line) in an adolescent with GA1.

associated brain lesions are typically confined to the dorsolateral putamen, visible months or years before a conspicuous movement disorder, and predictive of a better functional outcome [9,10].

Following the diagnosis of GA1 by NBS, use of a metabolic formula devoid of lysine and enriched with arginine establishes desired plasma amino acid concentrations ranges that can be maintained throughout the transition from formula to table foods. Because transport K_m values for lysine (K_m = 70 μmol/L) and arginine (K_m = 56 μmol/L) at the blood-brain barrier are similar [21], they exhibit meaningful transport competition within concentration ranges induced by $Lys^{-}Arg^{+}$ metabolic formula. Based on our calculations, dietary management with $Lys^{-}Arg^{+}$ formula could decrease the brain's toxin exposure by as much as 40% during its vulnerable phase of development, but this also has potential to interfere with normal brain growth [57–59]. Mindful of this risk, we monitor infants with GA1 every month. In general, those who adhere to a strict therapeutic diet during the first two years of life exhibit normal growth, achieve developmental milestones on time, and have normal cognitive function later in childhood. Other investigators report similar outcomes, supporting the overall safety of current GA1 treatment guidelines [60–63].

Rigid insistence on many aspects of feeding may not be necessary to ensure good outcome, provided medical food is balanced appropriately with other dietary components and offered ad libitum. We ask parents to quantify all dietary ingredients but allow them to combine these once daily into a single mixture, which they offer in rhythm with each baby's natural appetite and feeding cues. Following the transition to table foods, metabolic control can be maintained while also allowing for some flexibility (e.g., ± 20%) in daily protein intake. Outpatient monitoring is streamlined around just three parameters—physical growth, psychomotor development, and the plasma Lys/Arg ratio.

As long as diet conforms to accepted guidelines [63,64] and is adjusted for incremental weight gain, normal development can be achieved with minimal or no specialized biochemical testing. Indeed, several families from Cohort I lived in geographically remote, resource-limited households and were unable to adhere to the demanding clinical monitoring schedule. In between a more limited number of outpatient visits, parents collected accurate growth measurements at home and communicated these to our clinical team in a way that allowed their children to have timely dietary adjustments and excellent neurologic outcomes.

There remains some controversy about the appropriate duration of prescription dietary therapy. In our experience over three decades, no neurological crises occurred after age 19 months, but the true window of striatal vulnerability remains unknown. In one large international cross-sectional study (n = 279), 95% of all brain injuries occurred before age 24 months but one was reported at 70 months. This unusual case is often cited to support the recommendation for strict dietary management until age six years [63,64]. Mindful of the potential for long term complications, we advise that GA1 patients > 19 months remain on dietary therapy. Nevertheless, most choose to quit diet of their own accord (Fig. 4A). Although we observed no overt consequences of dietary non-adherence through adolescence and early adulthood, we recognize this might lead to complications later in life (see below).

4.3. Knowledge gaps

Intravenous infusion of dextrose, saline, and L-carnitine during illness is a widely accepted treatment strategy for GA1 [52,63,64]. We know very little about how this intervention protects the brain, but it is empirically successful; throughout 153 hospitalizations we attended over three decades, no GA1 patient developed neurologic regression while on inpatient IV therapy. With regard to the efficacy of IV infusions, *timing* matters. Emergency IV therapy need not and should not be delayed to accommodate transport to a tertiary academic center; the infusion protocol can be safely administered at almost any hospital with basic pediatric services. Over the years, we have successfully collaborated with community hospitals throughout the United States to establish convenient, affordable, and timely access to emergency IV therapy for children with GA1.

Pathophysiological concepts that inform IV therapy continue to evolve. Following its initial discovery, GA1 was viewed as an episodic intoxication syndrome in which systemic proteolysis generates circulating neurotoxins that cross the blood-brain barrier to poison the brain [52,65]. According to this paradigm, infusion of dextrose and saline were intended to suppress generation of organic acids and clear them from the bloodstream [6]. We now know this reasoning to be incorrect for a number of reasons: (1) ambient plasma concentrations of GA and 3HGA in patients are much lower than histotoxic concentrations in vitro [33,66,2]) acute metabolic acidosis is seldom observed during catabolic states [9, 3) under all conditions, plasma GA and 3HGA are orders of magnitude below concentrations measured in brain tissue [67,68]; and (4) 5-carbon dicarboxylates do not readily cross the blood-brain barrier [3,4,69]. Finally, the most compelling evidence comes from experimental *Gcdh*$-/-$ mice, which only develop central nervous system intoxication in response to excess dietary lysine [27,28].

Over the last 15 years, attention has shifted to the possibility that dextrose-containing saline infusions somehow stabilize metabolically vulnerable neurons, perhaps by improving their substrate supply [9,42]. This would require that IV therapy augment cerebral glucose delivery by increasing one or both of its determinants: plasma glucose concentration and CBF. Contrary to this idea, we found that infusing glucose in saline at 10–12 mg/kg•min does not reliably increase plasma glucose concentration, nor does it impact hemodynamics in a way that should alter CBF, as indicated by similar blood pressure measurements before and after the initiation of emergency IV therapy.

Like emergency IV infusion, L-carnitine supplementation is a widely accepted practice that lacks a strong evidence base [63,64,70]. Beyond its obvious role in correcting systemic deficiency, treatment with L-carnitine is founded on the premise that it crosses the blood-brain barrier, gains access to the mitochondrial matrix of neurons, and drives an acyltransferase reaction with glutaryl-CoA [2,71]. The human brain imports L-carnitine via a number of sodium-coupled transporters (e.g., SLC22A5 and SLC22A4; https://www.proteinatlas.org/) and, based on K_m values measured in rat brain slices (1920–2850 μmol/L) [72,73], a five-fold increase in plasma carnitine should increase its cerebral uptake by a comparable degree. However, the activity of neuronal carnitine acyltransferases toward 5-carbon dicarboxylate thioesters appears to be quite low [71,74] and the quantitative relevance of mitochondrial 'detoxification' in vivo thus remains unclear.

4.4. Challenges and opportunities

Prior to the advent of TMS-based NBS in the United States, most neurologic injuries from GA1 were catastrophic in nature. We now have neuroprotective therapy that is safe, simple, and highly effective [75–78] but only a minority of developed nations currently screen for GA1 [75]. Among 46 patients in Cohort III with striatal lesions, more than 85% were Gross Motor Function Classification System (GMFCS) level V, requiring a wheelchair for mobility and retaining limited or no ability to maintain a seated or standing posture, control limb movements, speak, or swallow [79]. When using a disability weighting consistent with severe functional impairment [80] and accounting for the attendant increase in mortality, these data suggest that in the absence of a systematic newborn screening and treatment program, each child born with GA1 loses an average of 64 disability-adjusted life years.

The economic impact of NBS for GA1 is also considerable. In developed nations, aggregate direct, indirect, and social costs of caring for an individual with GMFCS V level disability is between $46,000 and $64,000 per annum (corrected to 2016 U.S. dollars). Considering a 39-year median survival for disabled GA1 patients, this extrapolates to a lifetime cost of between US$1.8 and 2.5 million per brain-injured individual. Recognizing both the social and economic implications [64], the U.S. Health Resources and Service Administration includes GA1 as a recommended condition for universal newborn screening for all babies born in the United States and allied territories (https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html).

Although important evidence gaps remain, current protocols prevent more than 90% of brain injuries and are unlikely to face competing strategies in the near future. A rough power calculation shows why; to be proven superior, any novel intervention needs to be tested in parallel against a cohort of equal size managed according to the current standard of care [63,64]. A statistically meaningful difference between 93% and 97% efficacy would require at least 250 infants in each treatment group. Given the potentially grave neurologic consequences of such a randomization, it would pose a serious ethical challenge and most parents would be reluctant to participate.

4.5. Treatment of GA1 beyond early childhood

A strong confluence of evidence from multiple sources now supports the use of dietary therapy to prevent striatal degeneration from GCDH deficiency. In contrast, only scattered and conflicting data inform treatment of GA1 beyond early childhood. The natural age-related decline in treatment adherence brings this problem into sharp focus (Fig. 4A). In patients aged 8 to 71 years, symptoms and signs attributed to GA1 have included headache, nausea, nystagmus, gaze palsy, hyperreflexia, syncope, vertigo, epilepsy, ataxia, tremor, dysmetria, dysarthria, memory impairment, incontinence, orofacial dyskinesia, confusion, dementia, and peripheral neuropathy [81–86]. Unfortunately, none of these clinical phenomena are specific to GA1 and no combination of them has coalesced into a distinctive late onset neurological 'phenotype' of GA1. In at least half of GA1 subjects *without* striatal lesions, magnetic resonance images (MRI) reveal abnormal signals in white matter tracts and deep extrastriatal nuclei [61,81,87]. These likely reflect areas of interstitial edema and vacuolization [9,28,67,68,88–91], but it is not known how such changes correspond to neurological function or if they change in response to dietary treatment.

A similar consideration applies to the potential for non-neurological complications of GA1. For example, reports from experimental animals and humans indicate that GA1 might confer susceptibility to both acute [92–94] and chronic forms of kidney disease [95]. In Cohort I, we observed no signs of renal insufficiency among patients ≤3 years of age (mean serum creatinine 0.27 ± 0.06, range 0.20–0.40 mg/dL; Table 5) and, over the last three decades, had no GA1 patient present with overt renal failure. However, chronic renal insufficiency could go undetected among subjects from Cohorts II and III, for whom we have limited longitudinal renal biomarker data. It is important to reiterate, however, that the presence of a disease process, renal or otherwise, does not assure its response to therapy. The nephropathy of methylmalonic acidemia (MMA) provides an instructive example; there is no indication that progression of kidney disease in MMA is altered by dietary therapy [96].

The possibility of late-onset disease complications must be balanced against the fact that individuals with GA1 can remain healthy well into adulthood after living decades with no specific therapy. Among 91 individuals we studied without striatal lesions, 59% ranging from 2.6 to 43.6 years of age are off prescription medical foods and 52% stopped L-carnitine. Fourteen of them are > 17 years, have been off protein restriction and dietary supplements since early childhood, and report no clinically significant neurological problems or health concerns. Similar stories emerge from false positive NBS results that reveal GCDH deficiency in otherwise healthy mothers [56,83,97,98].

The foregoing discussion should not be misinterpreted as a formal recommendation to stop dietary therapy for GA1 patients older than two years. Rather, we intend only to draw attention to a problem in need of more evidence. To this end, future clinical research should target two related but distinct questions: (1) Is it possible to more sharply define 'non-striatal' complications of GCDH deficiency after age two years, and consolidate these into something like a canonical late-onset GA1 phenotype that they can be reliably anticipated, measured, and tracked in a clinical setting?; and (2) If such a phenotype becomes established, can its natural course be altered by standard dietary therapies (e.g., lysine-restriction and/or enteral L-carnitine)? Separating these ideas is critically important, because the mere existence of a rational therapy does not guarantee its effectiveness [59].

Over the long term, patients' willingness to adhere to dietary therapy should improve if it can be linked to meaningful outcomes. In the absence of such associations, however, we recognize the potential for prolonged but unnecessary therapy to foster stigmatization, maladaptive relationships, and mental distress—a phenomenon termed the 'vulnerable child syndrome', which can have serious long-term psychological consequences [99]. Balancing the potential risks and benefits of treatment for GA1 beyond early childhood therefore represents an ongoing conundrum open to new research, and marks a pressing challenge for clinical investigators.

Declaration of Competing Interest

Each author contributed significantly to two or more of the following study components: conception, design, data generation, data collection, data curation, data analysis, creation of tables and/or figures, creation of text, editing of text/figures/tables, and final manuscript review. This study was funded in part by a research grant from Nutricia North America, the manufacturer of Glutarade Junior and Glutarade Essential metabolic formulas. No author received direct or indirect personal compensation from Nutricia or owns shares in the company. There are no other actual or potential conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2020.09.007.

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