



Guanidinoacetate methyltransferase (GAMT) deficiency: Outcomes in 48 individuals and recommendations for diagnosis, treatment and monitoring



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ABSTRACT

We collected data on 48 patients from 38 families with guanidinoacetate methyltransferase (GAMT) deficiency. Global developmental delay/intellectual disability (DD/ID) with speech/language delay and behavioral problems as the most affected domains was present in 44 participants, with additional epilepsy present in 35 and movement disorder in 13. Treatment regimens included various combinations/dosages of creatine-mono-hydrate, L-ornithine, sodium benzoate and protein/arginine restricted diets. The median age at treatment initiation was 25.5 and 39 months in patients with mild and moderate DD/ID, respectively, and 11 years in

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patients with severe DD/ID. Increase of cerebral creatine and decrease of plasma/CSF guanidinoacetate levels were achieved by supplementation with creatine-mono-hydrate combined with high dosages of L-ornithine and/or an arginine-restricted diet (250 mg/kg/d L-arginine). Therapy was associated with improvement or stabilization of symptoms in all of the symptomatic cases. The 4 patients treated younger than 9 months had normal or almost normal developmental outcomes. One with inconsistent compliance had a borderline IQ at age 8.6 years. An observational GAMT database will be essential to identify the best treatment to reduce plasma guanidinoacetate levels and improve long-term outcomes.

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1. Introduction

Guanidinoacetate methyltransferase (GAMT) deficiency (MIM 601240) is an autosomal recessive inborn error of creatine synthesis, which results in global developmental delay/intellectual disability (DD/ID). Affected individuals exhibit marked impairment of expressive speech, autistic features, and varying neurological manifestations, including epilepsy and movement disorders [1,2]. GAMT (EC 2.1.1.2) catalyzes the last step of creatine synthesis, facilitating transfer of a single methyl group from S-adenosylmethionine to guanidinoacetate (GAA) to form creatine and S-adenosylhomocysteine [3]. GAA is synthesized from L-arginine and L-glycine via the activity of the enzyme AGAT (Arginine:Glycine Amidinotransferase, EC 2.1.4.1). Biochemical features of GAMT deficiency include creatine deficiency as well as accumulation of GAA in brain and body fluids.

Oral supplementation of creatine (administered as creatine-mono-hydrate) is used to restore cerebral creatine levels [3]. Strategies to reduce GAA levels include substrate deprivation via an arginine-restricted diet as well as competitive inhibition of AGAT activity via high-dose L-ornithine supplementation [4–8]. Sodium benzoate has been proposed as an additional approach to reduce the production of GAA via conjugation with glycine to form hippuric acid which is rapidly excreted by the kidneys [9–11].

Since GAMT deficiency was first described in 1994 [12], more than 50 patients have been reported in the literature [13], but no guidelines have been established regarding the use of the various treatment strategies. Knowledge is particularly limited with regard to the use and effectiveness of those strategies designed to reduce levels of GAA.

We report the results of an international survey among clinicians focusing on therapeutic strategies applied to patients with GAMT deficiency. In addition, we describe single case studies illustrating individual long-term clinical outcomes and the effects of sequential introduction to the various therapeutic strategies on plasma and CSF GAA levels.

2. Patients and results

2.1. International survey

Using Redcap software, we performed an online survey, among 26 clinicians from Canada, the United States, and Europe who were identified as treating patients with GAMT deficiency. The survey included questions about clinical and biochemical features of patients, therapeutic strategies, and clinical and biochemical outcomes. The survey was initiated in July 2011 and preliminary data on 22 patients were presented at the Annual SSIEM Symposium in Geneva (August 30–September 2, 2011). Additional iterations of data collection were necessary because of inconsistencies in the initial design of the RedCap survey (e.g. some sub-menus of core questions did not allow the clinician to choose among all possible modalities; numerous questionnaires came back with incomplete data entries) and by means of propaganda and personal contacts of the investigators, additional patients could be recruited into the survey until May 2013. This study was approved by the Ethical Review Board of British Columbia Children's Hospital, the institution from which the survey was conducted.

2.2. Study population

Data from 48 patients (21 females, 27 males) from 38 families have been collected. 22 patients from 17 families had been reported previously either as single case reports or in case series [1,2,8,14–24].

2.3. Data collection

Biochemical genetic data collection included cerebral creatine, urine/plasma/CSF GAA, and plasma ornithine and arginine prior and in response to treatment, as well as GAMT mutations. Clinical data collection included age and clinical presentation at diagnosis, type and duration of treatment and changes (improvements) of the initial clinical presentations upon treatment. Criteria for clinical presentation [1] included the degree of DD/ID, and type and degree of additional morbidities such as speech/language, behavior, epilepsy, and movement disorder.

Because data from formal neurodevelopmental assessments were not available for most of the patients, clinicians were asked to estimate the degree of DD/ID based on clinical judgment [25] at the time the patient was diagnosed. Criteria assisting the clinician in the estimation of the degree of DD/ID included the number and severity of developmental domains including expressive speech, self-supporting skills (toilet training, food intake) and behavioral comorbidities (autistic, disruptive, aggressive behavior) in patients older than 5 years; and progress in developmental milestones including speech/language, gross/fine motor skills, cognition, and social/personal interaction in children younger than 5 years. Roughly, patients with severe impairment have limited intellectual functions despite special support in school, and are not able to live independently in adulthood, whereas patients with mild impairment are able to achieve intellectual functions up to grade 3 to 6 levels, and are able to live independently in adulthood [26].

Behaviors were classified as hyperactive, aggressive or autistic according to clinicians' judgment. Epilepsy was considered as a) mild if the patient had occasional (e.g. fever induced) seizures and/or was seizure free upon pharmacological treatment; b) severe if the patient had frequent seizures despite pharmacological treatment. Movement disorder was defined as dystonia, chorea, hemiballism, ataxia, and spasticity.

Table 1 shows clinical and molecular features at the time of diagnosis/treatment start and changes of baseline features upon the various treatment modalities in 48 patients with GAMT deficiency.

2.4. Clinical presentation at time of diagnosis

44 patients, all treated later than 9 months of age, including 2 patients who never received treatment, had DD/ID. Based on the criteria outlined above, 25 patients were determined to have severe DD/ID and 23 of them (92%) had additional problems such as epilepsy and/or movement disorder; 19 patients were found to have mild/moderate DD/ID, and 12 of them (63%) had additional problems such as epilepsy and/or movement disorder. Borderline DD/ID was evident in 2 patients treated since 9 months and 3 weeks of age, and normal development was evident in the 2 patients treated since shortly after birth or prenatally.

Of the 35 individuals with epilepsy, 23 had mild and 12 had severe epilepsy. Severe epilepsy only occurred in patients who had severe DD/ID.

Table 1
Clinical characteristics, treatment modalities and outcomes in 48 patients with GAMT deficiency.

Patient # (ref)	Family #	Gender	Mutation	At treatment onset				Treatment modality	Treatment duration	Outcome/improvement on treatment
				Age	DD/ID degree	DD/ID plus	Basal ganglia changes			
1 (24)	1 (sibling P5)	f	c.327G>A/c.522G>A	Prenatal	Normal (nf)	nd		41 mo	Normal (nf)	
2 (28)	2 (sibling P25)	m	c.299_c.311 dup13/c.233T>A	1 week	Normal (nf)	nd	cr, orn-hd, diet-lp, benz	14 mo	Normal (nf)	
3 (15, 24)	3 (sibling P17)	f	c.152A>C/c.526dupG	3 weeks	Normal (nf)	n	cr ¹ orn-hd ² , diet-ar ² diet-pm, benz ³ " interrupted treatment	7 y	Normal 31 mo (f) borderline 8 y (f)	
4	4 (sibling P27)	f	327G>A	9 mo	Borderline (nf)	n	cr, orn-hd, diet-ar ¹ " , diet-lp, benz	21 mo	dd (dd,s)	
5 (2)	1 (index sibling P1)	m	c.327G>A/c.522G>A	10 mo	Mild (nf)	e y	cr, orn-hd, diet-lp	39 mo	dd,e	
6 (28)	5	m	c.327G>A/c.522G>A	11 mo	Moderate (nf)	e y	cr, orn-hd, diet-lp, benz	48 mo	dd (dd,s),e	
7	6	f	Not determined	14 mo	Mild (f)	e y	cr, orn-hd, SAM	7 y 4 mo	dd	
8 (28)	7	f	c.327G>A/c.403G>A	15 mo	Severe (nf)	e y	cr, orn-hd, diet-lp	8 y 6 mo	dd (dd,b),e	
9 (2)	8	m	c.327G>A/c.36_c.37ins26	16 mo	Moderate (nf)	e y	cr, orn-ld, diet-lp	36 mo	dd (dd,s),e	
10	9	m	c.327G>A	17 mo	Moderate (nf)	m y	cr	6 y 6 mo	dd (dd,s,b),m	
11 (16)	10	m	c.503A>C	19 mo	Mild (f)	n	cr, orn-ld	36 mo	dd (dd,s)	
12 (1, 18)	11 (sibling P19)	f	c.327G>A/c.48C>A	21 mo	Mild (nf)	m, e y	cr, orn-hd	6 y 7 mo	m,e	
13 (14)	12	f	c.327G>A	21 mo	Moderate (f)	n	cr, orn-hd, diet-ar	39 mo	dd (dd,s,b)	
14 (3)	13	m	c.327G>A/c.309dup13	22 mo	Severe (nf)	m, ee y	cr, orn-hd ¹ " "	16 y	e,m	
15 (17)	14	f	c.327G>A/c.522G>A	24 mo	Severe (nf)	m, e y	cr, orn-hd, diet-ar	10 y	dd,e,m	
16	15	m	c.526dupG	24 mo	Moderate (nf)	n	cr	44 mo	dd	
17 (1, 18)	3 (index sibling P3)	m	c.152A>C/c.526dupG	30 mo	Mild (f)	e n	cr ¹ , orn-hd ² , diet-ar ² " , benz ³ later diet-pm	10 y	dd (dd,s),e	
18 (1)	16	m	Not reported	36 mo	Severe (nf)	ee n	cr, orn-hd	36 mo	dd (dd,s,b), e	
19	11 (index sibling P12)	m	c.327G>A/c.48C>A	38 mo	Moderate (nf)	y	cr,orn-hd	6 y 7 mo	dd	
20 (19)	17	m	c.491insG/IVS5-3C>G	39 mo	Severe (nf)	m, e y	cr, orn-ld ¹ " , orn-hd, diet-lp ² " , diet-ar	11 y	dd, e,m	
21	18	f	c.327G>A	39 mo	Severe (nf)	m, ee y	cr	48 mo	dd (b)	
22 (21, 22)	19	m	c.497T>C	44 mo	Mild (tbq = 55) (f)	n	cr, orn-hd	7 y 4 mo	dd (dd,s,b) (IQ = 90)	
23	20	m	c.506G>A	48 mo	Mild (f)	n	cr, orn-hd	3 y 9 mo	dd (s)	
24	21	m	c.327G>A/c.133T>A	4 y 6 mo	Severe (nf)	m, e n	cr, orn-hd	20 mo	dd (dd,b), e,m	
25 (28)	2 (index sibling P2)	f	c.299_c.311dup13/c.233T>A	5 y 6 mo	Moderate (nf)	e n	cr, orn-hd, diet-lp	30 mo	dd (dd,s,b),e	
26	22	m	c.522G>A/c.505T>C	5 y 7 mo	Severe (nf)	ee y	cr, orn-hd, diet-lp	30 mo	Stable	
27	4 (index sibling P4)	m	c.327G>A	5 y 9 mo	Moderate (nf)	e n	cr, orn-hd, diet-ar ¹ " , diet lp, benz	21 mo	dd (b)	
28	23 (sibling P37)	f	Not reported	6 y 9 mo	Mild	ee nd	cr, orn-ld, diet-lp	26 mo	dd(b), e	
29 (23)	24 (cousin P47, P48)	m	c.59G>C	8 y	Severe (nf)	m, e nd	cr, orn-ld ¹ " " , diet-pm	7 y	dd, m	
30	25	f	c.327G>A	8 y 6 mo	Severe (f)	ee n	cr, orn-hd, diet-ar	48 mo	dd (dd,s,b), e	
31	26	f	c.327G>A	9 y 3 mo	Moderate (nf)	e y	cr	36 mo	Stable	
32 (20)	27	f	c.590T>C	10 y	Severe (f)	e n	cr, orn-hd, diet-ar 5 y then diet-lp	7 y	dd,e	
33	28	m	c.327G>A	11 y	Severe (nf)	ee y	cr	48 mo	dd (b),e	
34	29	f	Not determined	11 y	Severe (nf)	n	cr	12 mo	Stable	
35	30	m	Not reported	12 y	Severe (nf)	ee n	cr, orn-hd, diet-lp ¹ " "	8 y	id (s,b),e	
36	31	f	c.59G>C/c.521G>A	13 y	Severe (f)	nd	cr, orn-ld ¹ " , diet-lp ² "	6 y	dd	
37	23 (index sibling P28)	m	Not reported	13 y 9 mo	Moderate	ee nd	cr, orn-ld, diet-lp	26 mo	dd(s), e	
38	32	m	c.289C>T	14 y	Severe (nf)	ee n	cr, orn-hd, diet-lp	4 y	id (b),e	
39 (1)	33 (sibling P42)	m	c.59G>C	16 y	Severe (f)	m, e y	cr	9 y	e	
40	34	f	Not reported	18 y	Severe (nf)	ee n	cr, orn-hd, diet-lp ¹ " "	8 y	id (s,b),e	
41 (23)	35	f	c.506G>A	19 y	Severe (nf)	m, e nd	no treatment	0	nd	
42 (1)	33 (sibling 39)	m	c.59G>C	20 y	Severe (f)	e y	cr	9 y	e	
43 (1)	36 (sibling P45)	m	c.59G>C	20 y	Severe (f)	e y	cr	9 y	e	
44	37	f	c.327G>A	21 y	Severe (nf)	m, ee n	cr, orn-hd	11 mo	dd (b), e	
45 (1)	36 (sibling P43)	m	c.59G>C	22 y	Severe (f)	e nd	cr	9 y	e	
46	38	m	c.327G>A	25 y	Moderate (nf)	n	No treatment	0	nd	
47 (23)	24 (sibling P46)	f	c.59G>C	31 y	Severe (nf)	m, e nd	cr, diet-pm	5 y	m	
48 (23)	2 (index sibling P45)	f	c.59G>C	34 y	Severe (nf)	m, e nd	cr, diet-pm	5 y	Stable	

b: behavior; benz: sodium benzoate (100 mg/kg/d); cr: creatine-monohydrate (300–800 mg/kg); dd/id: developmental delay/intellectual disability; diet-ar: arginine-restricted diet (below DRI needing amino acid supplements); diet-lp: diet low protein (meeting DRI without amino acid formula); diet-pm: protein modified diet avoiding protein rich foods such as meat and milk products, no counting of protein intake); e: mild epilepsy; ee: severe epilepsy; (f): formal assessment; m: movement disorder; mo: months; n: no; nd: not determined; (nf): no formal assessment; orn-hd: L-ornithine high dose (>200–800 mg/kg); orn-ld: L-ornithine low dose (100–200 mg/kg/); P: patient; (ref): reference; s: speech; SAM: S-Adenosyl-L-Methionine; y: yes; y: years; ¹: therapy transiently discontinued; ²: therapy permanently discontinued; ³: therapy transiently performed.

13 patients had a movement disorder and in 12 it occurred in association with DD/ID and epilepsy. Patient 24 had intermittent (fever/illness induced) episodes of ataxia lasting for days and slowly remitting after the insult, a presentation not described in GAMT deficiency before.

2.5. Age at diagnosis/treatment onset

The median age at diagnosis was 51 months (range: prenatal/neonatal – 34 years). Patients with mild and moderate DD/ID were younger at treatment onset (median age at treatment start: 25.5 and 39 months) compared to those with severe DD/ID (median age at treatment start: 132 months = 11 years). 4 patients who began treatment prior to 9 months of age had either normal or borderline developmental outcomes. Preliminary descriptive analysis suggests a positive relationship between mean age at treatment onset and the degree of DD/ID (Fig. 1).

2.6. Treatment

Various treatment strategies were employed in various combinations and with various dosages. Creatine-mono-hydrate was given in dosages of 300–800 mg/kg/d. L-Ornithine was given as L-ornithine aspartate or L-ornithine hydrochloride, either in low dosages (orn-ld, 100–200 mg/kg/d) (as traditionally given for treatment of urea cycle defects), or in higher dosages (orn-hd, 300–800 mg/kg/d; 400 mg/kg being the target in most cases). Sodium benzoate was given at 100 mg/kg.

46 patients were treated and received creatine-mono-hydrate as a common denominator. 10 received it as mono-therapy and 2 in combination with the recommendation to avoid protein rich food such as meat and dairy products. 9 patients received a combination of creatine-mono-hydrate with L-ornithine; 25 received a combination of creatine-mono-hydrate with L-ornithine and a medical diet aiming to reduce arginine intake. L-Ornithine was given in a high dose in 27 and

in a low dose in 7 patients. 6 patients received additional sodium benzoate. 1 patient received S-adenosylmethionine in combination with creatine-mono-hydrate and high-dose or low-dose L-ornithine.

Diet was given in 3 different modalities: 1) a very low protein diet restricting L-arginine intake to approximately 250 mg/kg/d and providing between 0.2 and 0.5 mg/kg natural protein together with arginine-free amino acid formula to meet DRIs for protein intake (diet-arg); 2) a low protein diet providing 0.6–1.8 g/kg natural protein together with an arginine-free amino acid formula to meet DRIs for protein intake (diet-lp); 3) a protein-modified diet avoiding protein rich food such as meat and meat products, but without counting protein intake and without arginine-free amino acid formula (diet-pm). 11 patients received diet-arg, and 5 of them changed to a less strict protein restriction (diet-lp, diet-pm) during the course of their treatment. 9 patients received diet-lp, and one of them was switched to diet-arg later on. 3 patients received diet-pm.

Treatment duration was between 11 and 192 months (median 48, mean 64.1).

2.7. Treatment effects

Clinical improvement was achieved in the majority of patients. Patient 37 became more interactive when his seizures were resolved with treatment, but then showed oppositional and sometimes violent behavior. Significant improvement was achieved on psychostimulant medication.

Improvements were noted in several domains of DD/ID (behavior, language and self-supportive skills/activities of daily life', information being mostly based on clinical judgment and third party informants), as well as in epilepsy and movement disorder. Improvements were achieved in patients with severe, moderate and mild DD/ID, irrespective of the treatment modality. Notably, a significant improvement of DD/ID was objectified in patient 22 by an increase of the IQ (Reynell TBQ) of 55 at treatment start (age 44 months) to 90 after 48 months of treatment (age 7 y 8 m).

2.8. Magnetic resonance imaging (MRI)/proton magnetic resonance spectroscopy (MRS)

38 patients underwent a brain MRI/MRS at the time of diagnosis. 18 had T2 signal hyperintensities of the basal ganglia (especially the globus pallidus), in the rest specific MRI findings were not reported. 16 had follow-up MRI upon treatment and basal ganglia changes were reversed in all of them. Brain creatine deficiency was confirmed in all 38 patients on brain MRS at the time of diagnosis. In the 30 patients who had follow-up MRS upon treatment, brain creatine levels were considerably higher compared to baseline levels (no quantitative values available).

2.9. Biomarkers

Urinary and/or plasma GAA levels were determined in all patients prior to treatment and were elevated in all of them. A decrease, but not normalization of elevated GAA levels, was evident in all 35 patients, who had at least 1 measurement upon treatment. GAA levels in CSF were measured in 8 patients prior to treatment. A decrease, but not normalization was evident in the 4 patients who had a second lumbar puncture upon treatment. Longitudinal data on plasma GAA, creatine, ornithine and arginine levels in response to treatments were available from Patients 1, 5, 13, 14 and 44. Fig. 2 shows cumulative data from these patients upon the various treatment modalities. Systematic monitoring of biomarkers in relation to time and modality of treatment occurred in patients 13 and 44. Individual data of both patients are shown in Figs. 3 and 4.

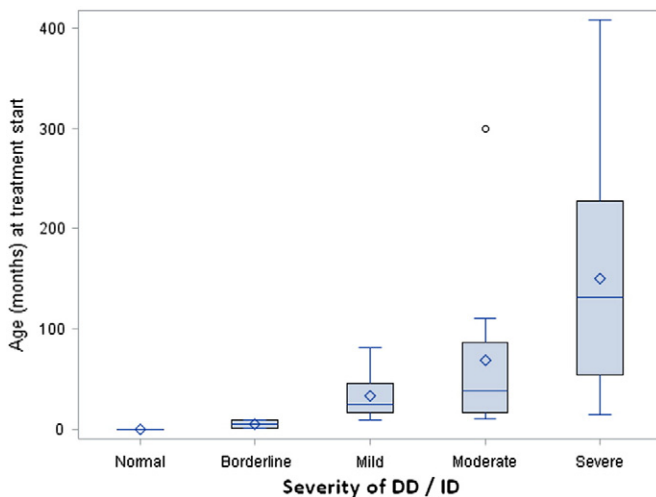


Fig. 1. Mean ages at diagnosis/treatment onset and severity of developmental delay/intellectual disability (DD/ID) in 48 patients with GAMT deficiency. (a) normal development (n = 2): min age = 0 months (treatment started prenatally), max age = 0.23 months (1 week); 25th, 50th, 75th percentile = 0, 0.12, 0.23 months. (b) borderline DD/ID (n = 2): min age = 0.68 months (3 weeks), max age = 9 months; 25th, 50th, 75th percentiles = 0.69, 4.9, 9 months. (c) mild DD/ID (n = 8): min age = 10.0 months, max age = 81 months; 25th, 50th, 75th percentiles = 16.5, 25.5, 46 months. (d) moderate DD/ID (n = 11): min age = 11 months, max age = 300 months; 25th, 50th, 75th percentiles = 17, 39, 87 months. (e) severe DD/ID (n = 25): min age = 15 months, max age = 408 months; 25th, 50th, 75th percentiles = 54, 132, 228 months. More data are required for statistical analysis, exploring the possible causative effect of time of treatment onset on developmental outcomes, controlling for various confounding factors.

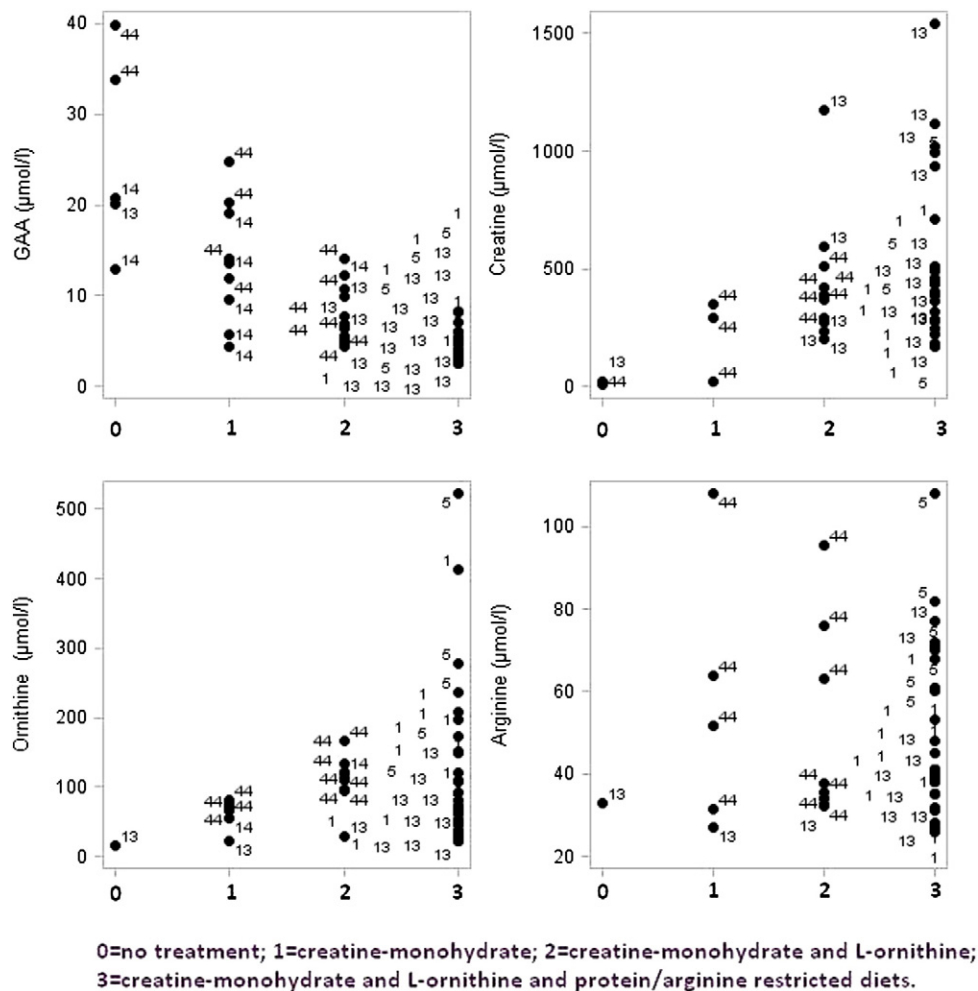


Fig. 2. Cumulative data (scattered blot) on plasma biomarker levels (guanidinoacetate [GAA], creatine, ornithine, arginine) in 5 patients (Patients 1, 5, 13, 14, 44) with GAMT deficiency in relation to various treatment modalities. Numbers 1, 5, 13, 14, and 42 refer to values assigned to respective patients. Patient 1 received creatine-mono-hydrate (300 mg/kg/d), L-ornithine (300 mg/kg/d), and protein restricted diet (1.0–1.4 g/kg natural protein and 0.5–0.7 g/kg/d from arginine free essential amino acid formula). Data represent a 20 month-treatment period. No baseline levels available. Patient 5 received creatine-mono-hydrate (400 mg/kg/d), L-ornithine (400 mg/kg/d), and protein restricted diet (0.5 g/kg natural protein and 0.5–0.7 g/kg/d). Data represent a 20 month-treatment period. No baseline values available. Patient 14 received creatine-mono-hydrate (400 mg/kg/d) as mono-therapy over most of his 16 treatment years and L-ornithine (400 mg/kg/d) for 6 months. GAA levels were measured only occasionally. Treatments of Patients 13 and 44 are described in the respective case studies and in Figs. 3 and 4.

2.10. Mutations

Of the 82 known/reported alleles, 30 were c.327G>A (p.K109K, splice site exon 2), occurring in 26 families. 15 alleles were c.59G>C (p.W20S) and they occurred exclusively in Portuguese (n = 7) families. The remaining mutations occurred in <3 alleles.

2.11. Case studies

2.11.1. Patient 14

2.11.1.1. Potentials and limitations of creatine mono-therapy. Treatment with creatine-mono-hydrate (400 mg/kg/d) was initiated in this male patient with global DD at 22 months. After 12 months of treatment his hemiballistic movements and head drop seizures resolved, and his basal ganglia changes and EEG normalized [3]. He was able to walk at age 4 years, but he remained non-verbal and did not catch up with his cognitive delay. At age 18 years he had severe ID, was not able to speak a single word, and demonstrated hyperactive, erratic behavior. He was toilet trained, fed himself with a spoon and was able to ride a tricycle and to recognize his parents and siblings. Additional L-ornithine (400 mg/kg/d) at the age of 17.5 years did not result in clinical improvement. After six months, plasma GAA and ornithine levels were 12.2 $\mu\text{mol/L}$ (baseline 19.1) (normal < 6.9) and 121 $\mu\text{mol/L}$ (baseline

54) (normal: 23–136 $\mu\text{mol/L}$) respectively. He developed a spontaneous tremor in his hands, which resolved when L-ornithine was discontinued after 6 months.

2.11.2. Patient 44

2.11.2.1. Biomarkers and clinical outcomes after sequential introduction of creatine and high dose L-ornithine. In this female with severe ID diagnosis of GAMT deficiency was established at the age of 21 years. She was not toilet trained, was unable to feed herself, and had progressive dystonia and intractable epilepsy (10 seizures per month). Plasma GAA, creatine, ornithine and arginine levels during 10 months of treatment are shown in Fig. 3. Seizure frequency decreased with creatine-mono-hydrate therapy (400 mg/kg/d). She became seizure-free upon additional L-ornithine (400 mg/kg/d) and caregivers reported improvement in attention and less sleepiness. She developed loose stools and an increased frequency of bowel movements during the first weeks of L-ornithine supplementation.

2.11.3. Patient 13

2.11.3.1. Biomarkers and clinical outcomes after sequential introduction of creatine, high dose ornithine and arginine restricted dietary treatment. In this female patient with global DD, diagnosis of GAMT deficiency was

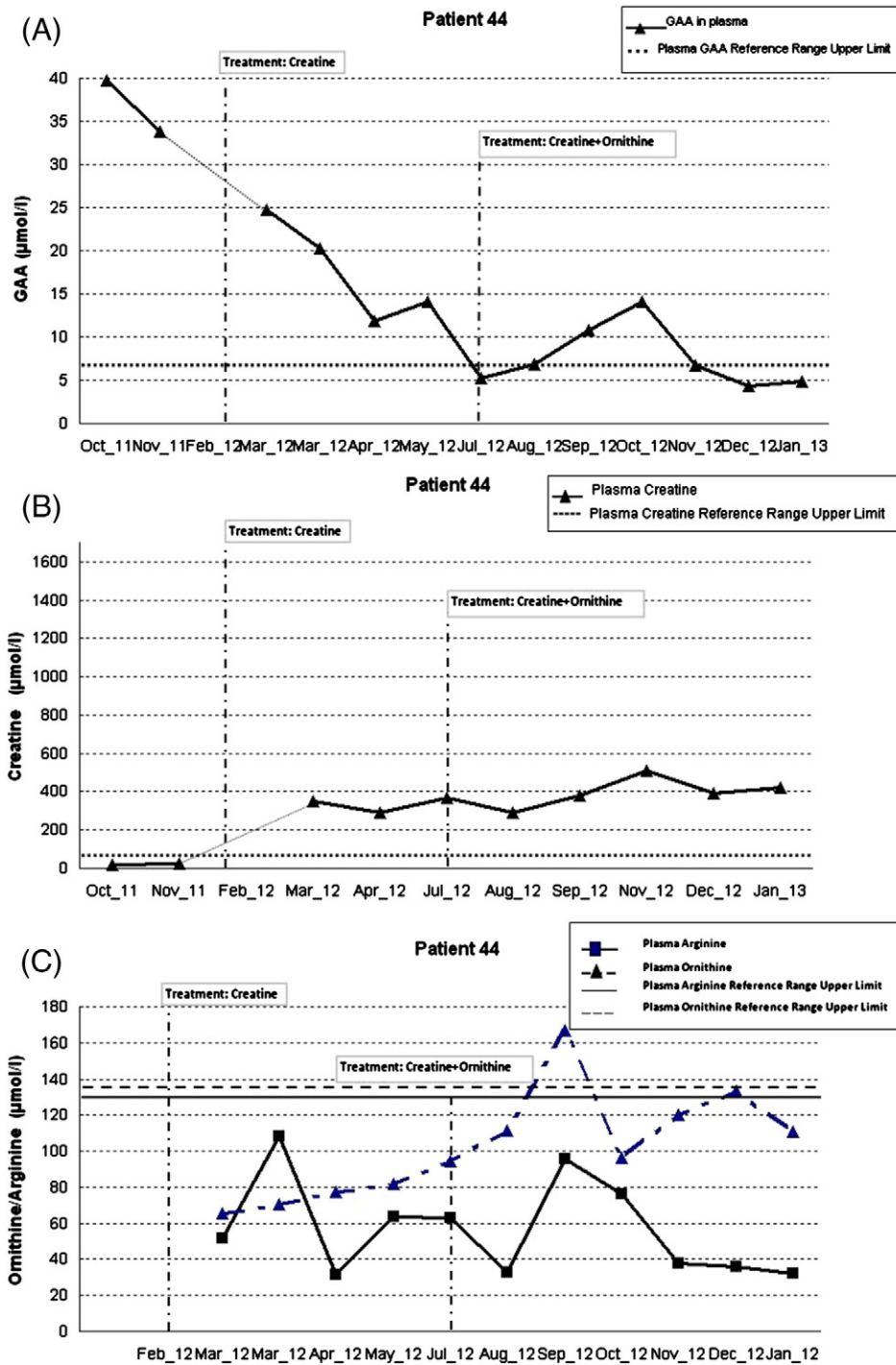


Fig. 3. Patient 44. Effect of the various treatment strategies (creatine-monohydrate [400 mg/kg/d] and L-ornithine [400 mg/kg/d]), on plasma levels of guanidinoacetate (GAA) (A); creatine (B); arginine and ornithine (C).

established at the age of 21 months. Treatments with creatine-monohydrate (400 mg/kg/d), L-ornithine (400 mg/kg/d), and an arginine-restricted diet (260–270 mg arginine/d, 0.3 g/kg/d protein, supplemented with an arginine-free essential amino acid formula for a total protein intake of 1.5 g/kg/d) were sequentially introduced. Brain creatine levels almost normalized after 24 months of treatment with creatine-monohydrate.

Plasma GAA, creatine, ornithine and arginine levels during 46 months of treatment are shown in Fig. 4. Notably, plasma and CSF GAA levels decreased from 20-fold to 2-fold and from 100-fold

to 10-fold respectively, compared to the upper limit of normal (interim data on this patient have partly been published in [14]). Growth continued at the 85th and 90th percentiles for weight and height. At 60 months, after 3 years of this treatment, the caregiver report suggested improved attention and social skills. Non-Verbal IQ was at the 5th percentile (WPPSI-III). Visual motor, global adaptive and single word receptive language abilities were at or below the 1st percentile on standard measures. Recognition knowledge of basic concepts (colors, numbers, letters, shapes) was in the average range (37th percentile).

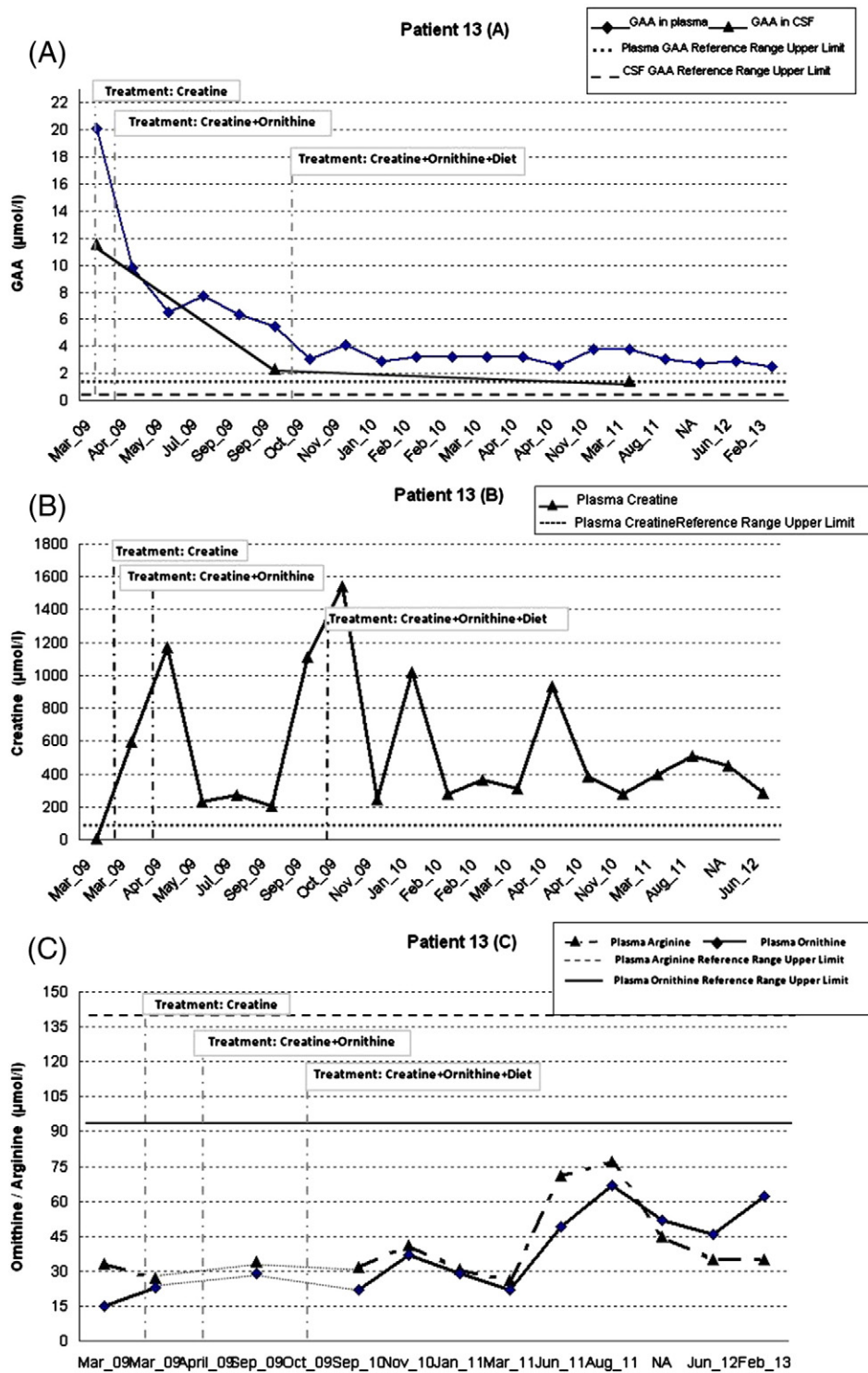


Fig. 4. Patient 13. Effect of the various treatment strategies (creatine-mono-hydrate [400 mg/kg/d], L-ornithine [400 mg/kg/d], arginine-restricted diet [260–270 mg arginine /d; 0.2–0.3 g natural protein/kg/d, supplemented with arginine-free essential amino acid formula for a total protein intake of 1.5 g/kg/d]) on A) guanidinoacetate (GAA) levels in plasma and cerebrospinal fluid (CSF); and plasma levels of creatine (B); arginine and ornithine (C).

2.11.4. Patient 3

2.11.4.1. Challenging compliance and adverse effects of treatment interruption. Treatment was started within the first 3 weeks of life, with creatine-mono-hydrate (400 mg/kg/d), high dose L-ornithine (400–800 mg/kg), an arginine-restricted diet (0.6 g/kg/d natural protein), and sodium benzoate (100 mg/kg). At 32 months her development was age appropriate with an MDI (mental developmental

index) of 91 (normal 100 \pm 14), while her late treated older brother (Patient 17) had global developmental at a comparable age [18]. Interim data on this patient until age 32 months have been published in [15]. At age 4 years, the parents stopped her treatment. Treatment was recommenced at age 5 years when she had a febrile seizure, but compliance was questionable. At age 7 years sodium benzoate and arginine/protein restricted diet were discontinued. Currently, at age 8.6 years her IQ is 71 (HAWIK IV).

Her late-treated brother with the same history of inconsistent therapy has an IQ of 68 at age 13 years. Data on biomarkers (GAA, creatine) are not available.

3. Discussion

The main purpose of this survey was to obtain information about current therapeutic practices and outcome surveillance in patients with GAMT deficiency. Different treatment strategies are used in different centers ranging from creatine-mono-hydrate mono-therapy directed at correcting cerebral creatine deficiency to combinations of one or more other strategies directed at reducing accumulation of toxic GAA.

Creatine-mono-hydrate supplementation had an appreciable effect on the correction of reduced cerebral creatine levels as shown in the 30 patients who had follow-up brain MRS. The 10 patients who received creatine as mono-therapy throughout their treated lifetime, showed mainly improvements in epilepsy and movement disorder. Creatine-mono-hydrate seems to be less efficient for improvement of cognitive/intellectual abilities. Limitations are shown in Patient 14 (case study) who had been on mono-therapy with creatine-mono-hydrate for most of his 16-year treatment period. Although his neurologic signs and symptoms (epilepsy, movement disorder, basal ganglia changes) resolved, at the age of 18 years he had severe ID, was non-verbal and demonstrated autistic/aggressive behavior. Another example which illustrates the limitations of mono-therapy with creatine, is the child reported by Schulze et al. [6], whose intractable epilepsy was only controlled when an arginine-restricted diet with (low-dose) ornithine was given in addition to creatine-mono-hydrate [7].

Additional treatment modalities aiming at reducing GAA accumulation can potentially improve outcomes. The observations in Patients 13 and 44 (case studies) show that a strong reduction of GAA levels is possible through adding L-ornithine to the treatment. While an initial 40–50% reduction of baseline plasma GAA levels was achieved by creatine mono-therapy, a further (36–50%) reduction of plasma GAA concentration was achieved by high dose L-ornithine supplementation (400 mg/kg/d) in both patients. A reduction of AGAT expression by high concentrations of creatine and inhibition of AGAT activity by high intracellular concentrations of L-ornithine is one explanation for these findings [27]. Another explanation is that the high ornithine and the low arginine and glycine concentrations achieved by these treatment modalities, reverse AGAT activity by mass action, thus resulting in decreased production of GAA.

It is currently believed that the GAA reducing effect of L-ornithine is best achieved by dosages of 400–800 mg/kg/d. Lower dosages of L-ornithine (100–200 mg/kg/d), as traditionally given for treatment of urea cycle defects, might not result in high enough tissue ornithine levels required for suppression of AGAT activity. The wide range of plasma ornithine levels observed in our patients during L-ornithine supplementation (from lower to beyond the upper normal range), suggests a fast and complete clearance of peak blood ornithine levels after L-ornithine ingestion. To obtain reproducible data, sample collection should occur at defined intervals from L-ornithine intake.

Dietary protein restriction was used in a proportion of patients to further reduce GAA levels via restriction of arginine, which is an essential substrate for GAA synthesis. In Patient 13 (Fig. 4A) an additional decrease of plasma GAA levels was achieved when an arginine restricted diet was added to the ongoing creatine-mono-hydrate and L-ornithine treatment. The diet employed in this patient implies an arduous protein restriction (allowing for only 0.2–0.3 g per kilo of daily natural protein intake) and adherence is doubtlessly challenging. 5 of the 11 patients who initially started on a similar, arginine restricted diet, continued on a less restricted diet during the course of their treatment. 9 patients were started on a moderate protein restriction (0.8–1.5 g/kg/d) from the beginning, however data allowing the determination of the GAA lowering effect of moderate protein restriction are not available from these patients.

In their most recent study, including 5 patients with GAMT deficiency (4 of them are included in this study as Patients 2, 6, 8, 25), Viau et al. [28] are showing a strong correlation of plasma arginine and ornithine levels. Based on this observation they hypothesize that L-ornithine not only suppresses AGAT activity, but at the same time stimulates arginine production and thus reduces the arginine and GAA lowering effect achieved by an arginine-restricted diet. The authors also show a strong correlation between plasma glycine and GAA levels, suggesting that the glycine lowering effect of sodium benzoate might be a particularly effective treatment strategy.

S-adenosyl methionine (SAM) might enhance residual GAMT activity in mutations affecting the SAM binding site. In our series only patient 7 received SAM, however, enzymatic studies, e.g. in patient fibroblasts, to prove the effect of SAM, have not been performed in this patient.

Concentrations of metabolites in CSF reflect the effects of treatment on the cerebral pathophysiology more directly. In Patient 13, CSF GAA levels decreased by almost 90% from baseline, but despite multiple treatment modalities, the achieved CSF values were still 10 times above the normal range. Because GAA is neurotoxic [29], a more effective reduction of CSF GAA levels may further improve clinical outcomes. This could be particularly achieved by the use of sodium benzoate, which crosses the blood–brain barrier and thus potentially reduces cerebral GAA synthesis by removing glycine from cerebral AGAT activity [30].

Our data suggest that early initiation of treatment favorably impacts neurodevelopmental outcomes. Most patients with severe DD/ID were diagnosed late (median age at treatment start 11 y), whereas those with mild and moderate DD/ID were all diagnosed at a median age of 25 and 38 months. 2 patients who received treatments from birth/prenatally (Patients 1 and 2) were normal at 14 and 41 months of age. Patient 3 who also was treated since birth was normal at 31 months [15]. Her borderline IQ at age 8 years is most likely the result of treatment interruption and ongoing compliance problems. The decline in IQ observed in this patient, suggests that therapy should be continued and monitored, at least throughout childhood, to preserve intellectual functions.

Despite the trend to better outcomes in early treated patients, the median age at diagnosis and treatment start was 60 months. Only 6 children were younger than 12 months when treatment was initiated. Reasons for late diagnosis include: (1) limited awareness of this potentially treatable condition among developmental pediatricians and neurologists; (2) nonspecific clinical presentation (patients may present with developmental delay and seizures only); and (3) limited availability of diagnostic tests (urinary/plasma GAA is a specific diagnostic marker, but its determination requires a particular method which is not universally available). (4) Single observations suggest that urine GAA can be false negative in very young children [16,28].

The beneficial outcomes in the early-treated patients make GAMT deficiency an ideal candidate for NBS. GAMT deficiency is particularly suitable because it has been confirmed in single cases [15,24] that GAA is elevated in dried blood spots of affected newborns. Based on a proof of concept study [31], the British Columbia NBS Program has developed a 3-tiered screening test including a 2-tier determination of GAA in blood spots along with GAMT sequencing in positive cases. Over 40,000 newborns have been screened since September 2012 with good analytical performance, but without a positive result [32]. International collaborations to utilize a standardized, multi-tiered approach to GAMT screening and data collection from a large number of newborns within a foreseeable time period is an important step towards implementation of GAMT newborn screening.

When interpreting the results of this study, the reader must take the following limitations into consideration: First and foremost, the determination of the degree of intellectual impairment was based on clinical judgment rather than formal testing in most of the patients. Although the above defined definitions/criteria were applied by experienced clinicians, one cannot rule out imprecision or bias. Second, particularly in

early treated patients, the observation periods were short and outcomes might be biased in favor of treatment by the occurrence of mild forms of GAMT deficiency, which are hardly predictable by genotype or biomarker association [1]. Third, the small number of pre-symptomatically and early treated patients impedes statistical evaluation; thus the trend towards better outcomes needs to be verified in larger patient numbers.

Data collected in this and in previous studies [1,2] do not allow conclusions about the influence of the genotype on the phenotype and on the ability of the various treatment strategies to modify long-term clinical outcomes. Further experimental studies are an essential step towards personalized medicine, allowing the optimal treatment strategy for each single patient according to the molecular pathophysiology of the individual GAMT mutations.

For most of the patients included in this survey, monitoring of biomarkers, choice of treatment modalities and assessment of clinical outcomes were not done systematically, rather each reflected independent choices of the treating physicians. This lack of standardized protocols for treatment and monitoring is inherent to rare inborn errors of metabolism [33], and creates momentum among the clinicians assembled via this survey, to develop the following consensus recommendations.

1. Diagnosis
 - a. Patients with non-syndromic DD/ID should be screened for GAMT deficiency.
 - b. Determination of urinary GAA and/or of plasma GAA is the preferred screening test.
 - c. Diagnosis is confirmed by GAMT mutation analysis.
 - d. In vivo MRS of the brain is useful in the diagnostic process but not necessary if the diagnosis has been confirmed by biomarker (GAA) and mutation analysis. This test however could serve as a baseline for monitoring the increase of cerebral creatine upon treatment.
 - e. A consistently low urinary creatinine excretion (caused by low body creatine) and nonspecific elevations of urinary organic acids, urinary uric acid or other urinary metabolites normalized to creatinine, are unspecific indicators of GAMT deficiency and should be followed up by specific diagnostic testing [16,34].
2. Treatment
 - a. Oral creatine supplementation with the aim of correcting cerebral creatine deficiency as well as strategies to reduce accumulation of GAA, such as high dose L-ornithine and an arginine/protein restricted diet, are the mainstay of treatment.
 - b. Creatine is given as creatine-mono hydrate at recommended dosages of 400–800 mg/kg/d orally/enterally.
 - c. L-Ornithine supplementation is recommended at 400–800 mg/kg/d (molecular weight = 132.16) orally/enterally. If given as L-ornithine-L-aspartate (molecular weight 265.26), the dosage should be adjusted to provide amounts of L-ornithine corresponding to the amounts given as the free form. L-Ornithine-HCl should not be given in high dosages because it can cause metabolic acidosis [5].
 - d. An arginine-restricted diet, given as 0.3–0.4 g/kg/d of natural protein (containing approximately 250 mg/kg/d of L-arginine) together with an arginine-free essential amino acid supplement to achieve the age related DRI effectively reduces GAA levels [35,36].
 - e. Whether, and to what extent, a low protein diet, given as 0.8–1.5 g/kg/d of natural protein, with or without supplementation of an arginine-free formula, results in reduction of GAA accumulation needs to be established.
 - f. Sodium benzoate conjugates glycine and potentially acts as an additional mechanism to inhibit GAA production via substrate deprivation. Its effectiveness in reducing levels of GAA needs to be established.
 - g. S-adenosylmethionine (SAM), which has been tried in 1 patient (Patient 7), could be indicated in others whose genotype suggests a defect at the SAM binding site of GAMT.
 - h. Patients may also benefit from ancillary services such as speech, occupational and physiotherapy.
3. Safety
 - a. Ingestion of high amounts of creatine may result in the formation of urinary crystals. Thus, supplementation should be monitored via regular urinalysis for the presence of creatine crystals and urinary tract infection. Studies are required to determine whether pharmacologic influence of urinary pH prevents urinary crystal formation.
 - b. High dose L-ornithine supplementation has been associated with tremors and loose stools/frequent bowel movements in 2 patients reported here. L-Ornithine has a bitter taste, and this might have a negative influence on compliance.
 - c. In gyrate atrophy of the choroid and retina (OAT deficiency), permanently high plasma levels of ornithine (800–1.400 µmol/L) are associated with retinopathy. Therefore, monitoring for retinopathy should be considered in patients on high dose L-ornithine supplementation and the concentrations of ornithine in plasma should not exceed 400 µmol/L.
 - d. Dietary restriction of arginine could cause a reduced availability of L-arginine for ornithine synthesis: a reduced flux of ornithine through the urea cycle could result in hyperammonemia. Therefore, L-ornithine supplementation (at least low dose) and regular monitoring of plasma ammonia levels should be considered in patients who receive an arginine-restricted diet [6,7].
 - e. Dietary restriction of arginine raises the risk of nutritional protein deficiency. Thus, treatment must be supervised by a metabolic dietician and include regular monitoring of biochemical (e.g. GAA, creatine, ornithine, arginine) and nutritional parameters (e.g. growth, plasma pre-albumin and plasma amino acids).
4. Biochemical monitoring
 - a. GAA and creatine levels are biomarkers that immediately reflect biochemical treatment effects.
 - b. Plasma is the preferred body fluid for monitoring GAA levels, and should be done in 3–6 month intervals. Shorter intervals should be done every time a new treatment modality is introduced. Plasma GAA levels should be determined within a fixed interval after food and L-ornithine intake (e.g. in the morning after an overnight fast or during the day, depending on the age of the child, 3–6 h after food intake).
 - c. Determination of GAA in CSF should be considered to validate the effectiveness of treatments in the compartment, which reflects metabolism of the brain most directly. An LP should be done in combination with other procedures (e.g. MRI/MRS) which require sedation/anesthesia.
5. Clinical monitoring
 - a. Brain MRI and MRS should be done to evaluate structural (e.g. globus pallidus lesions) and chemical changes (creatine, GAA) and repeated if clinically indicated (persistent seizures, movement disorders, questionable compliance with treatment) as decided by the treating physician.
 - b. Age appropriate neurodevelopmental and behavioral assessments (ideally using formalized tests) should be considered so as to objectively document any delayed skills and behavioral problems.
6. Steps towards evidence informed decision making
 - a. Overall, numerous questions regarding the evidence of the described treatment modalities, still remain to be answered.
 - b. Systematic studies are needed to determine the most effective dosages and combinations of creatine-mono hydrate, L-ornithine, sodium benzoate and dietary protein restriction to correct GAA in plasma, CSF, and brain and optimize outcomes.
 - c. As an approach to achieve this goal we are planning a web-based platform (www.gamtonline.org) including a toolbox with standardized treatment and monitoring protocols linked to a GAMT research database allowing the clinician to choose the treatment strategy most applicable to the individual patient and to longitudinally monitor a minimum set of biomarkers and clinical outcomes.

- d. Experimental research should explore the effects of treatments on cellular pathophysiology as well as new ways i.e. pharmacological inhibition of AGAT, to prevent GAA toxicity.

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