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Long term follow-up in GAMT deficiency – Correlation of therapy regimen, biochemical and *in vivo* brain proton MR spectroscopy data

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ABSTRACT

GAMT deficiency is a rare autosomal recessive disease within the group of cerebral creatine deficiency syndromes. Cerebral creatine depletion and accumulation of guanidinoacetate (GAA) lead to clinical presentation with intellectual disability, seizures, speech disturbances and movement disorders. Treatment consists of daily creatine supplementation to increase cerebral creatine, reduction of arginine intake and supplementation of ornithine for reduction of toxic GAA levels. This study represents the first long-term follow-up over a period of 14 years, with detailed clinical data, biochemical and multimodal neuroimaging findings. Developmental milestones, brain MRI, quantitative single voxel ^{1}H magnetic resonance spectroscopy (MRS) and biochemical analyses were assessed. The results reveal insights into the dose dependent effects of creatine/ornithine supplementation and expand the phenotypic spectrum of GAMT deficiency. Of note, the creatine concentrations, which were regularly monitored over a long follow-up period, increased significantly over time, but did not reach age matched control ranges. Our patient is the second reported to show normal neurocognitive outcome after an initial delay, stressing the importance of early diagnosis and treatment initiation.

1. Introduction

Guanidinoacetate methyltransferase (GAMT) deficiency (OMIM #601240) is a rare autosomal recessive inborn error of creatine metabolism caused by variants of *GAMT*. Together with arginine glycine amidino transferase (AGAT) deficiency (OMIM #602360) and creatine transporter (CRTR) defect (OMIM #300036), it belongs to the group of cerebral creatine deficiency syndromes (CCDS). Main symptoms of CCDS are rather unspecific and encompass intellectual disability to varying degrees, seizures, speech disturbances and in severe cases movement disorders [1–3]. In GAMT deficiency, characteristic brain MRI abnormalities, such as T2 signal hyperintensities in the basal ganglia, might be detectable, even though majority of patients exhibit normal brain MRI [4,5].

Creatine serves as an essential energy shuttle facilitating manifold functions within neurometabolism [6,7]. Adequate levels of creatine availability are equally provided by diet and endogenous biosynthesis. Creatine biosynthesis requires the amino acids arginine and glycine, the enzymes AGAT and GAMT and the creatine transporter SLC6A8. GAMT catalyzes the transfer of a methyl group from S-adenosylmethionine to guanidinoacetate (GAA), resulting in formation of S-adenosylhomocysteine and creatine as the final synthesis step [8]. Besides the severe effects on brain development and energy metabolism caused by the creatine deficiency itself, the neurotoxicity of accumulating GAA is also considered to contribute to the clinical phenotype of GAMT deficiency, the most severe among CCDS [7,9].

Abbreviations: AGAT, arginine glycine amidino transferase; CCDS, cerebral creatine deficiency syndromes; CRTR, creatine transporter; CSF, cerebrospinal fluid; GAA, Guanidinoacetate; GAMT, Guanidinoacetate methyltransferase; PPM, parts per million; MRS, magnetic resonance spectroscopy.

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The neurometabolic hallmark common in all CCDS is the low cerebral creatine, readily detectable by *in vivo* brain magnetic resonance spectroscopy (MRS). Early proton MRS studies also describe the appearance of GAA at 3.8 ppm in the spectrum from white and gray matter in severely affected patients prior to start of supplementation [5,10].

The elevation of GAA in urine, plasma, and cerebrospinal fluid (CSF) is pathognomonic for GAMT deficiency, while AGAT deficiency leads to low GAA and creatine levels. CRTR deficiency is an X-linked condition and characterized by increased urine creatine/creatinine ratio, which identifies male patients [4,5,11]. For diagnosis of GAMT and AGAT deficiency GAA should be assessed in plasma, as urine analysis can be unreliable [11]. Biochemical findings however should always be confirmed by genetic analysis and diagnosis [4,11].

Treatment in GAMT deficiency consists of creatine supplementation to increase cerebral creatine availability and strategies to reduce GAA levels. Ornithine supplementation inhibits AGAT activity, and some patients additionally receive an arginine or protein restricted diet with the aim of substrate deprivation. Some patients are reported to receive sodium benzoate, which reduces GAA levels through reduction of glycine availability through conjugation [12].

This study aimed to associate the therapeutic creatine and ornithine supplementation regimen with the clinical course and biochemical and quantitative *in vivo* proton MRS data in a patient with GAMT deficiency over a long follow-up period of 14 years.

2. Methods

2.1. Case study

Our female patient was followed over 14 years on a regular basis starting at the age of 15 months. Clinical course, developmental milestones, structural brain MRI, quantitative single voxel ^{1}H MRS and biochemical analyses of urine and plasma were assessed. Molecular genetic studies confirmed the diagnosis. The neurocognitive development was assessed in clinical examinations carried out by experienced pediatric neurologists, albeit neither a scoring system was applied, nor neuropsychological assessments performed.

2.2. Molecular genetic studies

We employed classical sanger sequencing to obtain the coding sequences of *GAMT*. Sanger sequencing at the gDNA of all coding exons of *GAMT* revealed a heterozygous frameshift variant (c.442dupC, p.Gln148ProfsX43) in DNA isolated from blood of the patient and father.

Next, we performed RT-PCR of *GAMT* mRNA followed by sequence analysis of its cDNA to search for the second variant. Hereto mRNA was isolated from the patient and father using blood collected in PAXgene Blood RNA tubes to stabilize and isolate the RNA with the PAXgene blood RNA Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol. While the father expressed both the frameshift variant as well as the wild-type transcript, the patient only expressed the paternal frameshift allele, while no maternal allele could be detected, indicating the presence of a null allele, which produces no RNA transcript. To investigate, if an unstable erroneous spliced *GAMT* transcript could be detected, *GAMT* cDNA analysis was repeated with mRNA isolated from fibroblasts derived from the patient. The fibroblasts were cultured in HAMF10 cell culture medium both in the absence or presence of cycloheximide. The latter condition was applied to prevent nonsense mediated decay. Again, this analysis only showed the mutant transcript and did not reveal an indication of erroneous splicing. Long range PCR excluded an intragenic deletion in exon 2 through 6 but could not exclude a mutation/deletion in the upstream (regulatory) regions.

2.3. Structural brain MR-imaging and ^{1}H-MR-spectroscopy

Each annual follow-up visit included a standard structural brain MRI examination at a 3 T clinical scanner (Magnetom TIM Trio and Prisma Fit, Siemens Healthineers). The structural MR-imaging protocol comprised *e.g.* routine high resolution T1-weighted (T1-w) and T2-w sequences.

At the same examination single voxel proton MR-spectra (64 accumulations) were acquired using a STEAM localization sequence with repetition time (TR)/echo time (TE)/mixing time (TM) = 6000/20/10 ms as described [13,14]. Volume-of-interest (VOI) (4.1 ml) was placed within parieto-occipital white matter (WM) as indicated in Fig. 1. Absolute concentrations (in mmol/l) of the axon-specific marker *N*-acetylaspartate and *N*-acetylaspartylglutamate (tNAA), creatine and phosphocreatine (tCr) reflecting energy metabolism and cell densitiy, choline-containing compounds (Cho) as a marker for myelin turnover and structural integrity, and myo-inositol (Ins) considered an astrocyte marker were determined by LCModel [15] and compared to region- and age-matched healthy controls taken from our local database. Two control groups were established to reflect the long period of follow-ups: age 0–5 years; $n = 4$; 3.4 ± 1.7 years (mean ± 1SD); age 6–14 years; $n = 9$; 10.3 ± 2.5 years (mean ± 1SD). Control ranges comprise mean ± 1SD. Also, GAA was added to the LCModel library in order to obtain the concentration of this specific metabolite (Table 2).

2.4. Biochemical studies

Analysis of GAA and creatin/creatinine in urine was carried out by gas chromatography–mass spectrometry with deuterated internal standards according to previously described protocols [16,17]. Results were compared to healthy age-matched controls from a local database (Table 3).

Plasma levels of GAA were measured with LC-MS/MS and internal deuterated standard in the pediatric metabolism center of Heidelberg University Hospital [18].

3. Results

3.1. Case study

Details of the clinical course, developmental milestones and auxiliary neurophysiological investigations are listed in Table 1. The girl was born at 42 weeks of gestation by caesarean section due to arrest of descent after an uneventful pregnancy. She is the only child of non-consanguineous German parents. At age 6 months physiotherapy was started because of mild positional asymmetry. Within the next 6 months a considerable global developmental delay was noted as she was unable to sit or crawl, nor did she speak single words. The initial untargeted metabolic screening revealed elevated urine GAA, thus she was referred to our clinic at the age of 15 months with suspected GAMT deficiency. At this point, she presented with general muscular hypotonia, sat freely but unstable, began to crawl. Muscle reflexes were at normal levels, she showed targeted grasp, no transfer of objects and only minimal cooing and babbling, while she responded to sound.

Treatment with creatine (400 mg/kg*d) and ornithine (100 mg/kg*d) was instantly initiated at the first visit after confirmation of diagnosis. As recommendations for ornithine supplementation changed, we increased the ornithine dose accordingly to approach the recommended amount of 400 mg/kg*d [4,12]. However, the acceptance of the high intake remained a limiting factor. Treatment continued and dosages were repeatedly adapted to weight (Fig. 2, Table 1). Additional dietary measures such as protein or L-arginine restriction or sodium benzoate intake were not considered necessary. After 3 months of treatment the patient was more active with improved language comprehension, cognition, and motor skills. Over the course of our follow-up motor development, speech and cognition further improved

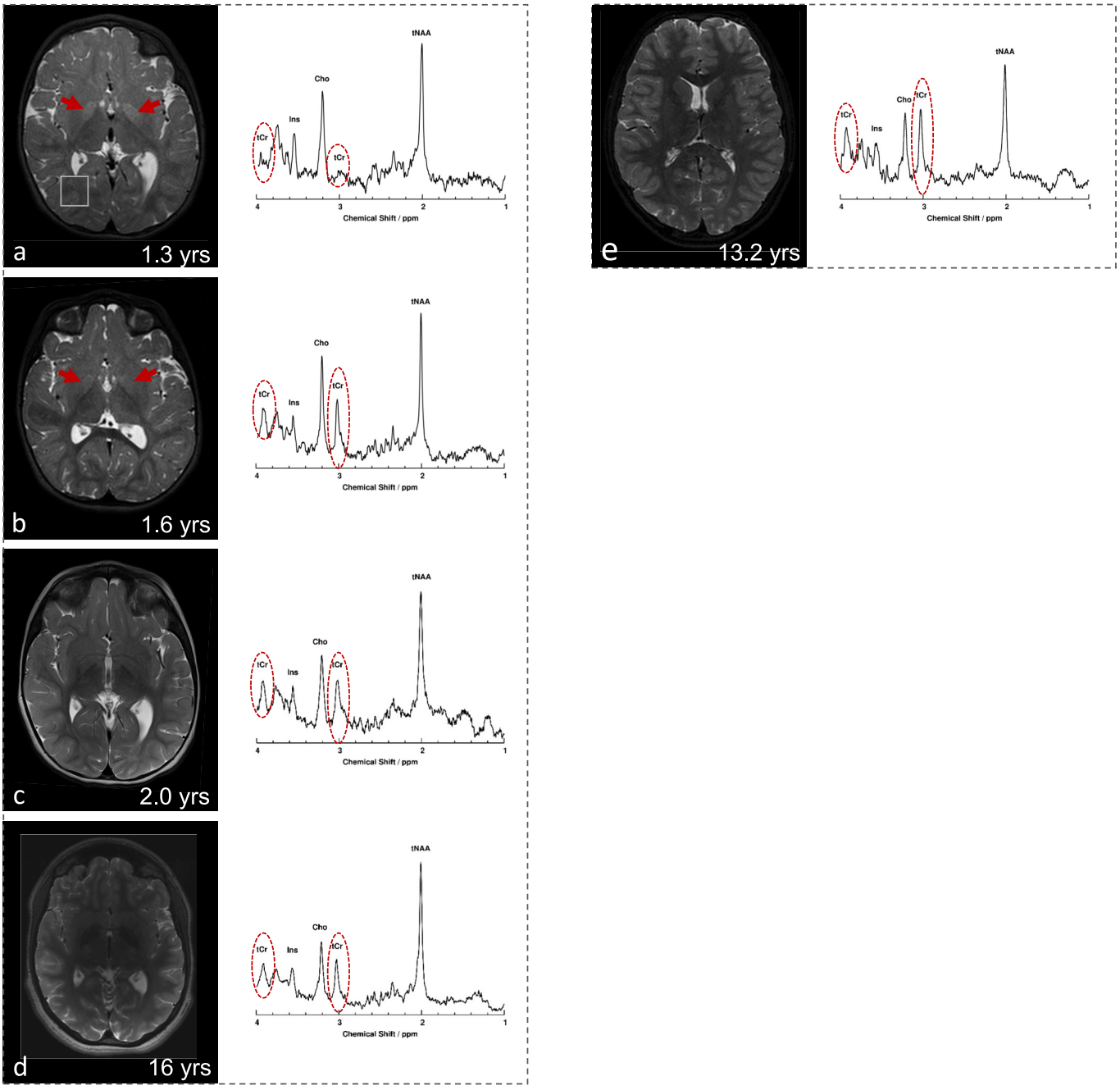

Fig. 1. MRI and MRS over time of follow up.

Table 2
Reference values for MRS (mmol/l).

VOI	age	tNAA	Cho	Ins	GAA
WM PO (n = 4)	0–5 yrs. (3.4 ± 1.7)	6.1 ± 1.2	1.5 ± 0.4	2.6 ± 1.1	0.4 ± 0.4
WM PO (*n* = 9)	6–14 yrs. (10.3 ± 2.5)	7.2 ± 1.2	1.7 ± 0.3	2.9 ± 0.6	0.5 ± 0.5

Values (mean ± SD) were obtained from own data base.
tNAA = sum of *N*-acetyl aspartate and *N*-acetyl aspartyl glutamate; Cho = choline-containing compounds; Ins = inositol; GAA = guanidinoacetate; VOI = volume of interest; WM PO = white matter parieto-occipital; yrs. = years; n = number of controls.

Table 3
Reference values for urine analysis.

Age (yrs)	cr/crn	GAA (μM/mM crn)
0- <6	0.006–1.2	4–220
6- <12	0.01–0.8	4–220
>12	0.011–0.025	4–220

Reference values were established at our metabolic laboratory and used for diagnostics. GAA = Guanidinoacetate; cr = creatine; crn = creatinine

steadily. She regularly received speech therapy (until 9 years of age), occupational therapy and attended an elementary school for special needs with focus on speech development until she was eligible to transfer to a regular high school.

At the last visit the patient was 15.8 years old and revealed only very

Table 1
Clinical, MRS and biochemical data of the patient followed-up over 14 years.

Age (yrs)	Clinical course and Development	Treatment dosages mg/kg*d		MRS (mmol/l)					
		ornithine	creatine	tCr	tNAA	Cho	Ins	GAA	cr/crn (Urine)
1.3	Hypotonia, free sitting. Delay of fine motor skills. No (speech) syllables. Social smile, turns towards noises. Start with ornithine/creatine.	100	400	0.1*	6.1	1.4	2.5	0.3	10.2
1.6	Stands upright with support. Increased vocalization polysyllabic. Tracks objects with eyes. Start with special needs kindergarten.	100	400	3.4	6.8	2.0*	2.2	0.3	4.9
2	Walks without support, broad based gait. Single words.	100 150 200	400	n.a.	n.a.	n.a.	n.a.	n.a.	7.7
2.8	Advancements of motor skills. Steady increase of active vocabulary, difficult to understand, good receptive understanding. Follows simple directions.	185 200	265 400	3.4	7.4*	1.6	3.1	n.d.	8.2
3.9	Age-appropriate gait runs fast. Improved speech, two-word sentences, inarticulate. Receives occupational and speech therapy.	225 250	350 400	3.7	6.8	1.7	2.8	n.a.	3.9
4.8	Limitations in receptive language.	230 250	365 400	2.7	7.8*	1.6	n.a.	0.5	21.8
6	Dysdiadochokinesis. Mild limitations in receptive and expressive language. Difficulties implementing tasks.	280	380	2.6	7.6	1.6	2.6	1.5*	2.3
6.9	Difficulties with complex movement sequences, gains of fine motor skills Cognition age appropriate, timid in behavior. Special needs (speech) elementary school, speech therapy.	293	377	3.7	7.3	2.3*	3.4	1.3*	6.8
8	Still mild dysdiadochokinesis. Speech and cognition age appropriate.	259 296	333 370	2.7	7.9	1.5	2.9	n.d.	4.1
9	Unchanged.	267 300	334 400	2.3*	7.2	1.5	2.6	0.7	8.7
10	Good endurance, mild dysdiadochokinesis. Mild difficulties finding words, vocabulary not quite age appropriate, dysgrammatism. Good social integration takes longer to understands tasks.	265	353	2.6	8.1	1.5	2.2	0.3	10.1
11	Further improvements in all aspects.	222 345	296 345	2.3	7.4	1.4	2.2	1.1*	9.4
11.9	5th grade regular school.	368	368	3	8.7*	1.7	2.7	n.d.	2.9
12.9	Unchanged clinical exam.	311	311 355	2.3	7.5	1.5	2.7	0.5	15.3
13.9	Unchanged.	268	306	n.a.	n.a.	n.a.	n.a.	n.a.	3.4
14.9	Unchanged.	264	264	n.a.	n.a.	n.a.	n.a.	n.a.	3.5
15.8	Unchanged.	327	327	2.7	9*	1.5	2.4	n.d.	2.6

Values for tCr and urine GAA from corresponding visits are laid out in Fig. 2.
tCr = sum of creatine and phosphocreatine; tNAA = sum of *N*-acetyl aspartate and *N*-acetyl aspartyl glutamate levels; Cho = choline-containing compounds; Ins = inositol; GAA = guanidinoacetate; VOI = volume of interest; WM PO = white matter parieto-occipital; n.a. = not available; n.d. = not determined, yrs. = years.
* >1 SD from control.

mild dysdiadochokinesis and axial muscular hypotonia while attending regular high school (9th grade) without any problems. She experienced no restrictions in everyday life or sports activities in which the patient showed good endurance. Speech and cognition were age appropriate; she received no further therapies.

3.2. Brain MR-imaging and ^{1}H-MR-spectroscopy

A total of 14 MRI examinations and proton MR spectra were recorded over the 14-year follow-up. Initial structural brain MRI at age 15 months revealed bilateral T2-w signal hyperintensities within the globus pallidus, which were no longer detectable at age of 2 years (Fig. 1a-c). In the initial MR-spectrum the tCr peak was undetectable (see Fig. 1a), hence the concentration could not be quantified. After 3 months of treatment, the tCr peak appeared in the spectrum and increased thereafter (Fig. 1b-d). In parallel, the calculated absolute concentration (in mmol/) increased until it reached a plateau and remained slightly below the lower limit of the age-appropriate normal range (Fig. 2a, b).

The GAA peak in WM at 3.8 ppm could not be identified in every MRS measurement. A reliable analysis was achieved only when the SNR were very high. Taken together, the absolute GAA concentrations, which were analyzable remained very low with considerable variations (s. Table 1). At ages 6, 6.9 and 11 yrs. levels of GAA were increased compared to age matched normal ranges established from our own data base. At four different time points the NAA concentrations were measured above the normal age-matched ranges and in two separate examinations the Cho concentrations respectively. All other metabolites remained within normal ranges (Table 1). No metabolite alterations were observed at the times of elevated GAA levels.

Axial T2-w images and MRS from parieto-occipital white matter (white rectangle in (a)) of our patient at ages 1.3 (a), 1.6 (b), 2.0 (c) and 16 (d) years. Of note T2 signal hyperintensities in globus pallidus (arrows) at ages 1.3 and 1.6 years which resolved at age 2.0 years. Creatine peaks at 3.02 and 3.93 ppm were undetectable at age 1.3 years (a) and increased 3 months (b) after start of treatment (red circles). Healthy control at age 13.2 years shown in etNAA = sum of *N*-acetyl aspartate and *N*-acetyl aspartyl glutamate; tCr = sum of creatine and phosphocreatine; Cho = choline-containing compounds; Ins = inositol.

3.3. Biochemical and molecular genetic studies

Initial metabolic workup revealed low plasma creatinine and highly elevated GAA in urine. Biochemical analysis showed a drastic decrease in urinary GAA 3 months after treatment initiation. Over the 14 years follow-up levels remained either just slightly elevated or within the normal range (Fig. 1). GAA and creatine in plasma were obtained at the

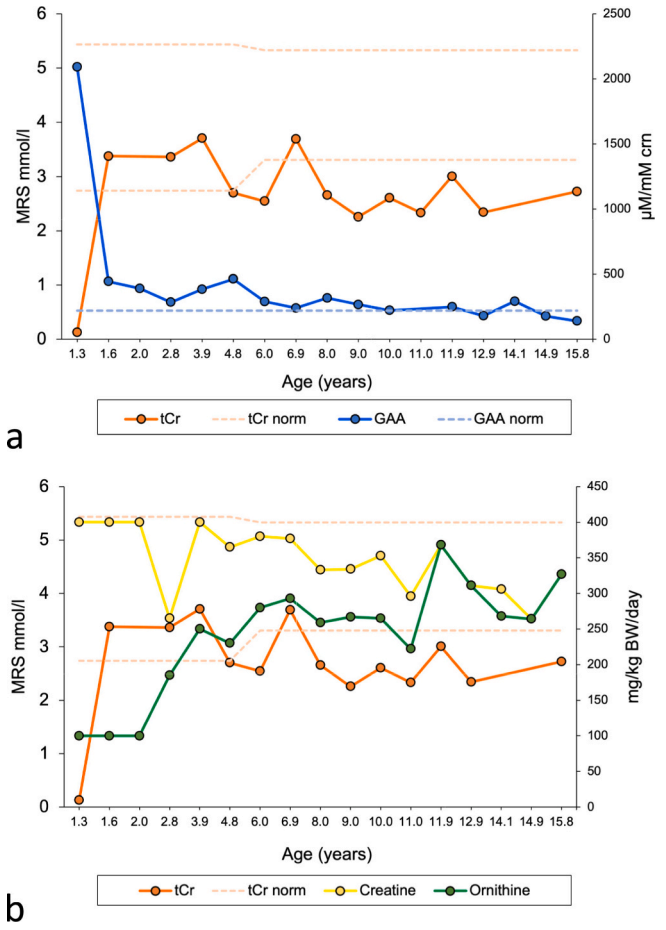

Fig. 2. Correlation of brain creatine concentration with urine GAA and therapy dosages.
a) tCr concentrations obtained from MRS and urinary GAA after treatment initiation b) correlation of creatine and ornithine dosage with tCr concentrations. tCr = sum of creatine and phosphocreatine; GAA = Guanidinoacetat.

last two visits and revealed values slightly above the normal range for GAA with 6.23 and 5.68 µmol/l (normal range 0.5–4.4 µmol/l) and creatine values of 485.4 and 163 µmol/l (normal range 17–150 µmol/l), respectively.

Genetic analysis by gDNA analysis and cDNA analysis confirmed the diagnosis of GAMT deficiency and revealed compound heterozygosity for a *GAMT* frameshift variant predicted to result in a truncated protein (c.442dupC, p.Gln148ProfsX43) and a null allele.

4. Discussion

Our patient presented with the clinical, brain MRI, MRS and biochemical phenotype of GAMT deficiency in early infancy and was diagnosed at the age of 15 months, prompting an immediate initiation of treatment.

Genetic confirmation of the diagnosis remained challenging, as standard sequencing techniques revealed only one heterozygous frameshift *GAMT* variant at gDNA level. RT-PCR of mRNA isolated from blood and fibroblasts showed the presence of a null allele even when nonsense mediated decay was inhibited. This confirms compound heterozygosity for a frameshift variant and a null allele, confirming GAMT deficiency at the genetic level. The variant resulting in a null allele could not be detected at the gDNA level. Most likely the causative variant is present in the transcriptional regulatory regions of *GAMT*.

Long term follow-up over a period of 14 years documented a reversal of clinical symptoms and subsequent almost normal development. To our knowledge, this is the first long-term study from infancy to adolescence that includes clinical, biochemical, and multimodal neuroimaging data in a patient with an extremely favorable clinical outcome.

Several studies indicate that for optimal neurodevelopmental and neurocognitive outcome treatment initiation before the onset of symptoms is desirable. So far, four patients with normal neurocognitive development are published. Stockler-Ipsiroglu et al. [12] report three patients who began treatment shortly after birth while still asymptomatic and showed normal development. Khaikin et al. [19] report one patient who was symptomatic at the age of 1 year when treatment was initiated and achieved normal development. Thus, to our knowledge, our patient is the second reported patient with GAMT deficiency who started treatment after the manifestation of clinical symptoms and still achieved a presumed normal neurodevelopmental outcome without any restrictions in everyday life. Whereas the majority of patients who received therapy only after the onset of symptoms showed improvement, they usually failed to achieve normal neurodevelopmental outcome [19–21].

Of the 48 patients described in Stockler-Ipsiroglu et al. [12] 18 had signal hyperintensities on T2 weighted images in basal ganglia, which reversed in all patients who underwent follow-up brain MRIs under treatment (16). Of the 22 patients described in Khaikin et al. [19], 19 underwent brain MRI studies and of those, 7 had signal hyperintensities on T2 weighted images in dorsal pons, basal ganglia, white matter, enlarged ventricles and/or cerebral atrophy. In line with these observations our patient also showed bilateral brain MRI alterations in the globus pallidus at time of diagnosis in addition to clinical symptoms (Fig. 1). The presence or absence of brain MRI alterations in reported cases could not clearly be linked to clinical severity or a certain phenotype and the reversion of alterations did not guarantee a favorable neurocognitive outcome [19,20].

The initial MRS in our patient revealed creatine depletion in brain tissue as the underlying neurometabolic disturbance. The early detection greatly expedited the diagnosis and thus start of therapy. Short term follow-up already reflected the marked increase of cerebral creatine concentrations as the effect of therapy. Prior studies on quantitative MRS in this disorder also reported a swift, significant increase of Cr concentration within the first 3–4 months after onset of therapy to about 40% or 50% of their respective normal concentrations in agreement with our results [1,8]. The majority of the reports in the literature however provide qualitative MRS data in GAMT deficiency and all concur in a decreased Cr peak before start of supplementation, which subsequently increased [10,19,20]. Interestingly, our long-term study over a period of 14 years revealed that after prompt initial increases, the creatine concentrations reached a plateau and remained slightly below the lower limit of the normal range over time (see Fig. 1). Long-term quantitative MRS data are scarce and only Stoeckler et al. [8] indicated increases of Cr concentration to about 90% of normal values after a follow-up period of 22 months compatible with our observations.

One explanation for the continually lower Cr in the brain could be the relatively low permeability of the blood brain barrier for Cr and endogenous creatine production of CNS cells in healthy individuals. Therefore, dietary supplementation even in high doses might not be sufficient to compensate for loss of creatine synthesis within the brain [22]. The other major metabolites detectable in brain proton MRS namely tNAA, Cho and Ins did not reveal specific and marked abnormalities over time.

In contrast to previous studies also utilizing MRS, GAA could not reliably be detected and quantified by LCmodel in most of our spectra. In the three studies revealing GAA concentrations above the normal ranges no other metabolite abnormalities were detected. Without a distinct peak identifiable in these spectra and no clinical alterations the interpretation of the findings remains difficult. Furthermore, over the time of follow-up, only few NAA and Cho concentrations were found in separate examinations above normal ranges. Again, MRI, clinical course and other biochemical parameter demonstrated no changes hence a severe

underlying cellular of biochemical process seems unlikely. Definite peaks at 3.8 ppm unequivocally reflecting significantly increased cerebral GAA concentrations were only depicted in the early reports about severely affected older patients [1,10]. The young age of our patient with initially mild clinical symptoms, the subsequent effective therapy with normal or only slightly elevated urine and plasma GAA and an almost normal development could be explanations for this. Of note, plasma GAA is only available for the two recent visits. A common problem with comparisons of clinical data in rare diseases is the lack of standardized scores or protocols. Patients are cared for by medical doctors in different institutions with variable routines for monitoring and data collection are in place. Consequently, Mercimek-Mahmutoglu et al. [4] developed a GAMT deficiency severity score, later adapted by Khaikin et al. [19], to classify patients and document treatment success. However, in our opinion, the three categories, namely ID, epilepsy, and movement disorders, did not appeared to be the ideal tool for our patient. Since our patient was diagnosed at the young age of 15 months, she had no history of movement disorders or seizures and she would have been assigned to the mild category at the time of treatment initiation, due to the apparent global developmental delay. The score does not separately consider speech or motor development, muscular hypotony or brain MRI findings, which were the prevalent symptoms in our patient. Also of note, the youngest age of a patient who developed seizures was one year, while they manifested later in most patients [19]. Therefore, it may fail to clearly demonstrate the significant improvement in our patient, including the normalization of brain MRI changes, striking improvement in cognition, speech development, and motor skills. The other patient who regained normal neurodevelopment started treatment at 1 year of age and was assessed with moderate severity at baseline. Overall, Khaikin et al. [19] face similar limitations in their cohort of 22 patients, with lacking objective psychological assessments and very heterogenic treatment responses regarding the improvements in the severity score.

The collection of clinical data (Table 1) illustrates the improvement over the 14 years of follow-up. It is of note, that in the end she could participate in regular school and daily activities without restrictions. During the last follow-up visit the patient was 15.8 years old and revealed only mild dysdiadochokinesis while performing well in regular high school.

Prior studies could not correlate genotype with phenotype or biochemical data (GAA levels in blood and creatine peak in MRS) with clinical severity [4,19]. It is tempting to speculate that because of her clinical presentation and brain MRI findings she would have been at high risk to develop a severe phenotype probably including a movement disorder, seizures, and ID with a later start of therapy.

There are consensus guidelines available for therapy of GAMT deficiency but reported treatment regimens varied between metabolic centers, availability of supplementation and patient compliance (as the taste is unpleasant and the amount high). Khaikin et al. [19] proposed treatment guidelines namely application of creatine and ornithine (each 400–800 mg/kg*d) alone and in cases of severer phenotypes or failure to improve initiation of protein or arginine restricted diet. Our patient received creatine and ornithine with 300 mg/kg*d each (ranged between 265 and 400/100–400). We tolerated dosages slightly below the recommended range to secure compliance of our patient, since especially the ornithine supplement was described as unpleasant to ingest and further increase under positive development was difficult to argue. Because of the extremely favorable clinical outcome, even with rather low doses of creatine and ornithine, additional dietary restrictions were not imposed. Some patients received additional sodium benzoate to reduce the precursor amino acid glycine and thus lower GAA levels, but the impact is controversial [21,23].

Since early, presymptomatic treatment evidently leads to a beneficial clinical outcome and even enables normal neurodevelopment, neonatal screening seems highly desirable. After several studies proved its practicability and local pilot projects were implemented successfully, GAMT deficiency was recently added to the U.S. Recommended Uniform Screening Panel [24–27]. Moreover, new treatment options such as gene and cell therapies, might become available in the future. Recently, some early positive results from AAV-based gene therapy in mice were published [28].

In conclusion, our long-term follow-up of a patient with GAMT deficiency shows correlations between clinical data, biochemical and multimodal neuroimaging results. The early diagnosis facilitated by MRS has most likely led to an exceptionally positive clinical outcome. It underlines the importance of early, ideally presymptomatic, start of treatment and thus the need for widespread implementation of newborn screening of GAMT deficiency.

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Author contributions

LM and SDK conceptualized, designed and wrote the manuscript. SDK, JG, PD, GS, RK and LM finalized the manuscript. LM, SDK, PH and JG acquired and contributed the clinical data. SDK and PD acquired and analyzed the MRI/MRS results. GS and MFO carried out the genetic studies of the patient. RK assisted with the biochemical studies. LM, RK, PH, JG and SDK analyzed and interpreted the datasets. All authors approved the final version of the manuscript. Correspondence: LM.

Ethics approval and consent to participate

The study was approved by the institutional review board of the University Medical Center Göttingen (#19/12/03) and written informed consent was obtained from the parents prior to each MRI examination. The study was in compliance with the Declaration of Helsinki.

Consent for publication

The patient has signed a letter of consent, and it is available if required.

CRediT authorship contribution statement

Lara M. Marten: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Ralph Krätzner:** Data curation, Formal analysis, Resources, Writing – review & editing. **Gajja S. Salomons:** Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Matilde Fernandez Ojeda:** Formal analysis, Investigation, Methodology, Resources. **Peter Dechent:** Data curation, Formal analysis, Validation, Writing – review & editing. **Jutta Gärtner:** Data curation, Funding acquisition, Investigation, Resources, Supervision, Writing – review & editing. **Peter Huppke:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Steffi Dreha-Kulaczewski:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

There is no conflict of interest for any of the authors.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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