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Arginine:glycine amidinotransferase (AGAT) deficiency: Clinical features and long term outcomes in 16 patients diagnosed worldwide

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ABSTRACT

Background: Arginine:glycine aminotransferase (AGAT) (*GATM*) deficiency is an autosomal recessive inborn error of creative synthesis.
Objective: We performed an international survey among physicians known to treat patients with AGAT deficiency, to assess clinical characteristics and long-term outcomes of this ultra-rare condition.
Results: 16 patients from 8 families of 8 different ethnic backgrounds were included. 1 patient was asymptomatic when diagnosed at age 3 weeks. 15 patients diagnosed between 16 months and 25 years of life had intellectual disability/developmental delay (IDD). 8 patients also had myopathy/proximal muscle weakness. Common biochemical denominators were low/undetectable guanidinoacetate (GAA) concentrations in urine and plasma, and low/undetectable cerebral creatine levels. 3 families had protein truncation/null mutations. The rest had missense and splice mutations.
Treatment with creatine monohydrate (100–800 mg/kg/day) resulted in almost complete restoration of brain creatine levels and significant improvement of myopathy. The 2 patients treated since age 4 and 16 months had normal cognitive and behavioral development at age 10 and 11 years. Late treated patients had limited improvement of cognitive functions.
Conclusion: AGAT deficiency is a treatable intellectual disability. Early diagnosis may prevent IDD and myopathy. Patients with unexplained IDD with and without myopathy should be assessed for AGAT deficiency by determination of urine/plasma GAA and cerebral creatine levels (via brain MRS), and by *GATM* gene sequencing.

Abbreviations: AGAT, arginine:glycine amidinotransferase; CDD, creatine deficiency disorders; CPK, creatine phosphokinase; CRTR, creatine transporter; CSF, cerebrospinal fluid; EEG, electroencephalogram; GAA, guanidinoacetate; GAMT, guanidinoacetate methyltransferase; *GATM*, glycine amidinotransferase, mitochondrial (gene symbol for AGAT); MRS, proton magnetic resonance spectroscopy; IDD, intellectual disability/developmental delay; IEM, inborn errors of metabolism; MRI, magnetic resonance imaging; P, plasma; U, urine.

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1. Introduction

Arginine:glycine amidinotransferase (*AGAT*, *GATM*) deficiency (OMIM 602360) is an autosomal recessive inborn error affecting the first step of creatine synthesis, and resulting in reduced production of guanidinoacetate, the immediate precursor of creatine [1]. *AGAT* deficiency is one of the 3 known cerebral creatine deficiency disorders (CCD) along with guanidinoacetate methyltransferase (GAMT) (OMIM 601240) and X-linked cerebral creatine transporter (CRTR, *SLC6A8*) deficiency (OMIM 300036) [2]. The common clinical denominator of all CCDs is cerebral creatine deficiency and intellectual disability/developmental delay (IDD). Epilepsy and movement disorder are additionally observed in GAMT and CRTR deficiency. Biochemically, *AGAT* deficiency is characterized by low concentrations of guanidinoacetate (GAA) in body fluids (urine, plasma), while GAMT deficiency is characterized by high GAA concentrations. A high urinary excretion expressed as high urinary creatine to creatinine ratio is characteristic of CRTR deficiency in males.

Since its first description in 2001 [1], less than 20 patients have been identified with AGAT deficiency worldwide. Patients have been described with IDD only [3,4] or with IDD plus myopathy [5–8].

Oral creatine supplementation has proven effective in replenishing cerebral creatine levels [9], improvement of developmental scores [4] and myopathy [5–8]. Early diagnosis and treatment seems to be particularly efficient in improving outcomes [4,10].

Thus far, patients have been reported as single cases and at different stages of their outcomes. We evaluated clinical, biochemical and molecular characteristics as well as long term outcomes of 16 patients diagnosed worldwide.

2. Patients and results

2.1. International survey

We invited physicians/scientists worldwide, known to treat patients with AGAT deficiency from published literature and/or from personal contacts, to participate in a survey on long-term outcomes of their AGAT patients. All physicians agreed to participate in the study. We developed a questionnaire to assess clinical and biochemical features, therapeutic strategies and clinical and biochemical outcomes. The survey was initiated in February 2014 and data collection was completed in August 2015. This study was approved by the Ethical Review Board of British Columbia Children's Hospital, the institution from which the survey was conducted (Institutional Ethics Review Board approval # H11-01392).

2.2. Study population

Data from 16 patients (9 female, 7 male) out of 8 families were collected. 11 patients from 5 families (P1–P11) had previously been reported either in single case reports or in case series [1,3,4–8,10]. 5 patients are newly described. Table 1 summarizes features and outcomes of the 16 patients studied.

2.3. Presentation at diagnosis

Patients were diagnosed between age 3 weeks and 25 years (median 8 years).

2.3.1. Intellectual disability/developmental delay (IDD)

Formal neuropsychological assessments were done in 12 patients, employing Bayley Scale of Infant Development, Clinical Evaluation Language Fundamentals (CELF), Leiter International Performance Scale (LIPS IQ), Griffith Developmental Scale, Wechsler Intelligence Scale for Children and Wechsler Adult Intelligence Scale (WAIS-III/IV).

P4, who was diagnosed at 3 weeks of age, was asymptomatic. The remaining 15 patients, diagnosed between 16 months and 25 years of age, presented with various degrees of IDD with IQ/DQs between 35 and 60.

Language delay was consistently reported in all but the early treated P4. P6 did not speak a word by age of 2 years. P1 and P2 began speaking their first words at 30 months. P8 spoke in monosyllables at age 6 years. P8 and P9 had slow motor speech, impaired syllable transition and impaired written speech when diagnosed at age 25 and 22 years. P15 began speaking at age 2 years, but lost the ability to speak in broken sentences at age 14 and speaks single words only at age 19.

Behavioral/neuropsychiatric problems were reported in P1, P2, P3 and P15 including autistic-like behavior with poor social contact, short attention span, and repetitive movements of the hand. P15 showed evidence of a complex psychiatric disorder with dysthymic affect, visual and auditory hallucination and excessive sleep at the age of 15 years.

Seizures were observed only in P3 and P16. P3 experienced a single fever induced seizure at 18 months, while P16 experienced one episode of seizure with no treatment required further on.

Myopathy, in addition to IDD, was noted in 8 patients/5 families (P6, P7, P8, P9, P10, P11, P13 and P16) diagnosed between 6 and 23 years. They all had low muscle mass and mild/moderate proximal muscle weakness. An EMG, done in 6 patients, showed a myopathic pattern with low amplitude polyphasic waves in P6, P8, P10, P11 and P16, and was normal in P7. Creatine phosphokinase (CPK), measured in 5 patients, was moderately elevated (232–500 U/L; normal <159) in P6 and P7, and was normal in P8, P10 and P11.

Muscle biopsy, done in 4 patients, showed slight preponderance of type 2 fibers and tubular aggregates in P6 and P7, fiber size variation and slight atrophy of both type 1 and 2 fibers in P8, and no structural abnormalities in P10.

P11 (family 5) and P16 (family 8), diagnosed at 5 and 6 years, are the youngest patients to present with myopathy as a prominent feature. P6 had general muscle weakness at age 6 years, with a positive Gower sign, walking with wide-based gain, and unable to stand on toes and to run. He had oropharyngeal dysphagia with recurrent aspiration and poor weight gain in spite of adequate calories provided via g-tube. His EMG showed 50% reduction in amplitude of peroneal muscle action potential. His muscle biopsy was myopathic with fiber size variation, including round atrophic fibers, scattered hypertrophic fibers and rare split fibers. Mitochondrial enzymes such as SDH, COX and ATPases did not show obvious staining abnormalities, however, ultrastructural studies showed significant subsarcolemmal accumulation of mitochondria, many of which show abnormal shapes and abnormal cristae. Echocardiogram showed mildly dilated left ventricle with normal function.

2.4. Cerebral MRI/MRS

A pretreatment MRI/MRS of the brain was performed in 12/16 patients P1, P2, P3, P4, P5, P6, P7, P8, P10, P11, P12 and P16. MRI revealed normal results for cerebral anatomy and structure, and MRS showed absence or marked reduction of brain creatine in all.

2.5. Biomarkers

GAA and creatine concentrations in urine and plasma are shown in Table 2. Urine and plasma GAA ranged from undetectable to various levels below the respective lab specific lower normal range.

Plasma amino acids and (re)-methylation/transsulfuration markers were investigated in P8 and P9 to test whether deficient AGAT activity leads to accumulation of it substrates (arginine and glycine), and whether reduced availability of GAA as substrate for methylation via guanidinoacetate methyltransferase (GAMT) leads to changes in pathways involved in methyl group consumption. Normal results were obtained for fasting plasma concentrations of glycine, arginine (precursors), as well as, for methionine, S-adenosyl-methionine and total homocysteine (5.3 and 5.9 μmol/L: normal 5.1–13.9), while S-adenosyl–

homocysteine was low (13.5 and 14.2 nmol/L: normal 20–32). Cysteine and cystathionine ([re]-methylation and transsulfuration) were normal as well.

Metabolites resulting from activities of 3 other methyltransferases such as glycine and sarcosine (glycine N-methyltransferase), phosphoethanolamine and phosphatidylcholine (phosphatidylethanolamine N-methyltransferase) and dimethylglycine and betaine (betaine homocysteine methyltransferase), were essentially normal. Findings exclude upregulating of these enzymes by a potentially increased methyl group availability caused by reduced consumption through GAMT.

2.6. Mutations

Among the 32 alleles of the 16 patients from 8 different families, 9 different mutations were identified. Consanguinity was confirmed in families 4, 5 and 6. Except for family 6, with compound heterozygous missense mutations, all patients are homozygous for a distinct mutation (Table 1). Parental studies have been performed in all families and each parent is a carrier. 5 families harbor truncation null mutations (nonsense, splice, or frame-shift), expecting no residual enzyme activity.

Families 5, 6 and 8 had missense mutations. We applied molecular structural modeling of these mutant proteins to support their pathogenicity. The crystal structure of the inactive form of AGAT was analyzed at 1.90 Å resolution (PDB code: 4JDW). The arginine at amino acid position 413 of patient 12 is involved in extensive hydrogen bonding networks with p.P65, p.E76 and p.E77. Change to tryptophan or glutamine creates stereochemical clashes and unfavorable charge environment perturbing local protein structure [11]. The substitution of alanine with helix destabilizing amino acid proline at position 185, disturbs the internal protein packing and the arginine substrate binding in patient 16 (Fig. 1A and C). While substitution of tyrosine at position 203 with serine interrupts the hydrophobic internal tight packing of the protein, thus, its structure and function (Fig. 1B and D).

2.7. Treatment & outcome

All patients were treated with oral creatine monohydrate at dosages from 100 to 800 mg/kg/day. Treatment was started at age 4 months to 25 years. Total treatment duration ranged from 5 to 14 years.

2.7.1. Intellectual disability/developmental delay (IDD)

Most favorable intellectual/developmental outcomes were achieved in P4, P3 (family 1) and P5 (family 2) who started treatment at age 4, 24 and 16 months. Currently at 10 and 16, and 11 years of life, they have achieved age appropriate/excellent educational milestones.

P12 (family 6), who started treatment at 2.5 years, had language based learning disability after 4 years of treatment. Despite deficits in expressive language, non-verbal perceptual reasoning and digit span, the patient was average on visual (matrix) reasoning and processing speed (WISC-IV). Parents are reporting constant progress in his speech development.

Currently, P1 and P2 (family 1), who started treatment at age 4.5 and 6.5 years, have achieved educational milestones in high school with teaching aid at age 20 and 22 years. They have residual expressive speech problems.

P8 and P9 (family 4), with late treatment start, had moderate ID (IQ 53 and 50) when they were diagnosed at age 25 and 22 years. Although no formal assessment is available, the reported functions in social life (family) and at work attest a considerable improvement of cognitive/adaptive functioning after 9 years of treatment.

P7 (family 3), diagnosed at age 12 years, had an improved IQ from 60–70 (mild intellectual disability) after 11 months of treatment, while the sibling (P6), diagnosed at 18 years, did not show an improvement of his IQ (47 prior and 49).

P10 and P11 (family 5) had very low baseline functions (IQ 20–35) and did not show improvement after a 13-month treatment period. Their parents reported improvement in verbal comprehension in the younger sibling (P11) who was started on treatment at age 5 years.

P13, P14, P15 and P16 have been on treatment for ≤3 months, too early to evaluate intellectual outcomes.

2.7.2. Myopathy

Affected patients showed a dramatic improvement in muscle strength, stamina and disappearance of Gower sign. P10 and P11 also demonstrated ability to run, jump and climb stairs easily 13 months after commencing treatment with creatine. The improvement in myopathic signs and symptoms contrasted their pronounced intellectual impairment.

2.7.3. Cerebral creatine levels

Cerebral creatine levels were between 66 and >95% of normal after the various treatment periods (Table 1). In P6 (family 3) the cerebral creatine level did not rise beyond 18% after 11 months of treatment.

Results of brain MRS studies, performed in P1, P2 and P5 at various time points during treatment, are shown in Fig. 2.

3. Discussion

Untreated AGAT deficiency is characterized by 2 main presentations: global developmental delay/intellectual disability (IDD) and myopathy. Mild and moderate IDD with prominent speech delay was a constant finding in all our patients. IDD is a common denominator for all cerebral creatine deficiency disorders (AGAT, GAMT, CRTR) [2], but AGAT deficiency is the only one to present with additional myopathy.

In our case series, myopathy was found predominantly in older patients diagnosed in adolescence and young adulthood. However younger patients can also be affected, as shown in P11 and P16, who had pronounced myopathy as young as 5 and 6 years of age. The occurrence of myopathy in some but not all patients might be due to an ascertainment bias. In younger patients muscular weakness might be interpreted as part of the global DD and special investigations to exclude myopathy might not be performed. Individual genetic susceptibility to early onset myopathy in younger patients might be another explanation.

None of our patients had pyramidal or extrapyramidal signs and symptoms and only 2 patients had occasional seizures. This is in contrast to GAMT and CRTR deficiency where movement disorders (dystonia, chorea, ataxia) and seizures are more frequently observed [12,13].

GAA depletion is the most likely explanation for the unique occurrence of myopathy in AGAT deficiency. Experimental evidence suggests that GAA is an alternative substrate to CPK [14]. In GAMT and CRTR deficiency, GAA phosphate [15] provides an alternative high-energy reservoir in lieu of creatine phosphate, thus preventing myopathy. In AGAT deficiency, GAA phosphate is not formed due to impaired GAA synthesis, thus resulting in total depletion of muscular high-energy phosphates. Mitochondrial histo-morphology alterations, as shown in P16, seem to be a consequence of such cellular energy depletion. Measurement of creatine, GAA, creatine phosphate and GAA phosphate in muscles of AGAT deficient patients should be performed in order to prove this hypothesis.

Biochemically, untreated AGAT deficiency is characterized by the deficiency of GAA and creatine in tissues and body fluids. We found significant reductions of urine and plasma GAA in all patients, while blood creatine levels remained within the low normal range. The latter possibly reflects exogenous creatine from nutritional resources such as meat and dairy products. In contrast, cerebral creatine levels were undetectable/significantly reduced in all patients. Significant reduction of cerebral creatine level in the patient diagnosed in the newborn period (P4) indicates that intrauterine supply via maternal cord blood is not sufficient to prevent cerebral creatine deficiency in the fetal/newborn brain.

GAA is substrate for the last step of creatine synthesis, facilitated by GAMT and requiring S-adenosylmethionine as a methyl group donor

Table 1
Features and outcomes of 16 patients with AGAT deficiency.

Patient Gender (F/M) [ref]	Family	Mutations		Age at diagnosis/ treatment start	Treatment		Interim outcome			[Total treatment duration] & long term outcome
		Allele 1	Allele 2		Creatine-monohydrate	Treatment duration at interim assessment of outcome	DD/ID (IQ/DQ) before/after treatment	Myopathy before/after treatment	Cerebral creatine (% normal) before/after treatment	
P1 F [1]	Family 1 Sibling P2, P4	c.446G>A p.(W149X) *Missense*	c.446G>A p.(W149X) *Missense*	4.5 y	400 mg/kg	16 m	60/72	No/stable	Absent/>95%	[After 14 y of treatment] High school degree; preparing for university entrance exam with aid.
P2 F [1]	Family 1 Sibling P1, P4	c.446G>A p.(W149X) *Missense*	c.446G>A p.(W149X) *Missense*	6.5 y	400 mg/kg	16 m	42/71	No/stable	Absent/>95%	[After 14 y of treatment] High school degree; works as waitress in gastronomy.
P3 M [3]	Family 1a cousin P1, P2, P4	c.446G>A p.(W149X) *Missense*	c.446G>A p.(W149X) *Missense*	2 y	400 mg/kg	15 m	<50/63	No/stable	Absent/90%	[After 14 y of treatment] Attending secondary school with support. Gradual improvement of language and communication skills.
P4 M [10]	Family 1 Sibling P1, P2	c.446G>A p.(W149X) *Missense*	c.446G>A p.(W149X) *Missense*	3 w (diagnosis) 4 m (treatment start)	100 mg/kg	8 m	Normal at birth/normal at 12 m	No/stable	Absent/60%	[After 9 y of treatment] Attending primary school without aid. Total IQ = 100
P5 F [4]	Family 2	c.484+1G>T p.Ala97ValfsX11 *Splice*	c.484+1G>T p.Ala97ValfsX11 *Splice*	16 m	400–800 mg/kg	23 m	43/100	No/stable	Absent/79%	[After 10 y of treatment] Attends advanced academic program in high school
P6 M [5]	Family 3 Sibling P7	c.1111dup p.(Met371AsnfsX6) *Nonsense*	c.1111dup p.(Met371AsnfsX6) *Nonsense*	18 y	100 mg/kg	11 m	47/49	Yes/improved	Absent/18%	[After 7 y of treatment] No update available
P7 F [5]	Family 3 Sibling P6	c.1111dup p.(Met371AsnfsX6) *Nonsense*	c.1111dup p.(Met371AsnfsX6) *Nonsense*	12 y	100 mg/kg	11 m	60/70	Yes/improved	Absent/70%	[After 6 y of treatment] No update available
P8 F [7,8]	Family 4 Sibling P9	c.505C>T p.(R169X) *Nonsense*	c.505C>T p.(R169X) *Nonsense*	25 y	5 g/day; increased to 400 mg/kg	6 m	53/ND	Yes/improved	ND/65%	[After 9 y of treatment] Works in family business.

(continued on next page)

Table 1 (*continued*)

Patient Gender (F/M) [ref]	Family	Mutations		Age at diagnosis/ treatment start	Treatment		Interim outcome			[Total treatment duration] & long term outcome
		Allele 1	Allele 2		Creatine-monohydrate	Treatment duration at interim assessment of outcome	DD/ID (IQ/DQ) before/after treatment	Myopathy before/after treatment	Cerebral creatine (% normal) before/after treatment	
P9 M [7,8]	Family 4 Sibling P8	c.505C>T p.(R169X) *Nonsense*	c.505C>T p.(R169X) *Nonsense*	22 y	5 g/day; increased to 400 mg/kg	6 m	50/ND	Yes/improved	ND/66%	[After 7 y of treatment] Married; works in a store.
P10 F [6]	Family 5 Sibling P11	c.608A>C p.Tyr203Ser *Missense*	c.608A>C p.Tyr203Ser *Missense*	10 y	200–400 mg/kg	13 m	25–35/<35	Yes/improved	Marked reduction/70%	[After 5 y of treatment] Slight improvement in daily activities and language. Specialized education
P11 F [6]	Family 5 Sibling P10	c.608A>C p.Tyr203Ser *Missense*	c.608A>C p.Tyr203Ser *Missense*	5 y	200–400 mg/kg	13 m	25–35/ <35 Improvement in verbal comprehension	Yes/improved	Marked reduction/70%	[After 5 y of treatment] Improvement in language. Attending primary school with support
P12 M [new]	Family 6	c.1237C>T p.R413W *Missense*	c.1238G>A p.R413Q *Missense*	2.5 y	500 mg/kg	4 y	Mild/mod ID*/language based learning disability**	Mild hypotonia/normal	Absent/>95%	[After 6 y of treatment] No update available
P13 F [new]	Family 7 Sibling P14, P15	c.629G>A (p.W210X) *Nonsense*	c.629G>A (p.W210X) *Nonsense*	20 y	10 g/day	3 m	Mild–moderate**/no change	Yes/mild improvement	ND/ND	ND
P14 M [new]	Family 7 Sibling P13, P15	c.629G>A (p.W210X) *Nonsense*	c.629G>A (p.W210X) *Nonsense*	19 y	10 g/day	3 m	Mild–moderate**/no change	No/stable	ND/ND	ND
P15 F [new]	Family 7 Sibling P13, P14	c.629G>A (p.W210X) *Nonsense*	c.629G>A (p.W210X) *Nonsense*	18 y	10 g/day	3 m	Moderate***/no change	No/stable	ND/ND	ND
P16 M [new]	Family 8	c.553G>C (p.A185P) *Missense*	c.553G>C (p.A185P) *Missense*	6 y	200 mg/kg	Just started treatment	55 / ND	Yes/ND	Absent/ND	ND

P: patient; [ref] reference; DD/ID: developmental delay/intellectual disability; IQ/DQ: intelligence/developmental quotient; ND: not determined; m: months and y: years.
P12: *Clinical estimation, no formal test result available and **deficits in expressive language, non-verbal perceptual reasoning and digit span. Average on visual (matrix) reasoning and processing speed (WISC-IV).
P13 and P14: **Clinical estimation, no formal test result available. Both patients live at home but need support. Good self-help skills. Can answer basic questions.
P15: ***Clinical estimation, no formal test result available. Poor social interaction and self-help skills. Lost ability to speak in broken sentences at age 14. Acoustic and visual hallucinations.

Table 2
Guanidinoacetate (GAA) and creatine concentrations in urine and plasma from 16 patients with AGAT deficiency prior to treatment with creatine monohydrate.

	GAA urine			GAA plasma			Creatine plasma	
	Patient µmol/L	Normal µmol/L	%**	Patient µmol/L	Normal µmol/L	%**	Patient µmol/L	Normal µmol/L
P1	2.45	56–698	<10	nd	0.22–3.14	0	122	10–200
P2	2.16	56–698	<10	nd	0.22–3.14	0	95	10–200
P3	1.34	56–698	<10	0.10	0.22–3.14	45	N/A	–
P4	0.54	56–698	<10	0.13	0.22–3.14	59	16.2	18–141
P5	0.22*	53.9 ± 26.9*	<10	0.07	1.34 ± 0.64	5.2	2.3	75.6 ± 21.6
P6	3.5	60–850	<10	N/A	–	–	N/A	–
P7	2.0	60–850	<10	N/A	–	–	N/A	–
P8	nd	80–655	0	nd	0.4–3.7	0	N/A	–
P9	1	80–655	12	0.1	0.4–3.7	25	N/A	–
P10	0.9*	4.0–220	22	0.2	0.35–8.00	57	1	17–109
P11	2.6*	4.0–220	65	0.3	1.00–3.5	0.3	1	5–50
P12	N/A	–	–	<0.01	1.09 ± 0.46	<0.01	1.3	69.4 ± 31.5
P13	nd	7–130*	0	nd	1.10–3.80	0	3.4	9–90
P14	nd	7–130*	0	nd	1.10–3.80	0	2.7	9–90
P15	nd	7–130*	0	nd	1.10–3.80	0	1.7	9–90
P16	1*	22–123*	<5	0.1	0.3–2.1	33%	0.8	20–110

N/A = not assessed and nd = not detectable.
* Value given in mmol/mol.
** % value was calculated from the mean value or from the lowest normal range value.

[16]. Hence, the expectation would be that the nearly absent GAA levels in AGAT deficiency result in reduced utilization of methyl groups provided from S-adenosylmethionine. The latter is of particular significance as up to 75% of the body methyl group transfer is utilized for the formation of creative out of GAA [17]. Studies in P8 and P9 suggest that AGAT deficiency has no major consequences on the methylation/remethylation cycle, methyltransferases and the trans-sulfuration pathway.

In addition to its role in creatine synthesis, AGAT is also a key enzyme in the transamidation of lysine to homoarginine [18]. Knowledge of homoarginine's biological functions is limited, but low blood concentrations of homoarginine have been shown to be associated with an increased risk factor for cardiovascular disease [19]. Recent studies in lymphoblasts of one AGAT deficient patient (P5 in this case series) have confirmed that homoarginine synthesis is reduced in AGAT deficiency [20]. We have not determined homoarginine levels in our patients, but further biochemical studies in these patients will contribute to an understanding of the consequences of AGAT deficiency on homoarginine metabolism.

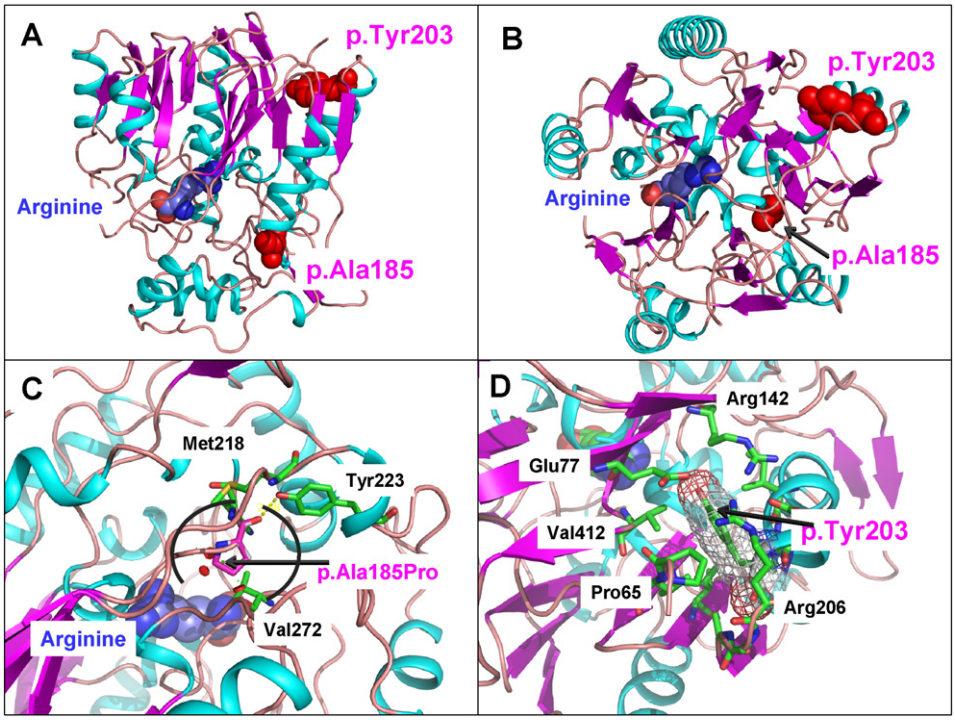

Fig. 1. A ribbon representation of the human AGAT protein, and a structural analysis of novel missense mutations. A. Side view, and B. top view, of human AGAT. The substrate Arginine is modeled as spheres. Positions of p.A185 and p.Y203 are indicated as red spheres. C. The alanine at position 185 is located in the internal part of the AGAT protein close to the substrate arginine binding site. Both the side chain carbon atom and main chain oxygen are well coordinated by the hydrophobic interaction with p.V272 and hydrogen bond with p.T223. The replacement of alanine with proline would create a steric hindrance and disturb the internal protein packing. D. The p.Y203 is also embedded in the internal part of the protein and tightly packed against the adjacent residues. The replacement of tyrosine with serine would collapse the protein structure and its function.

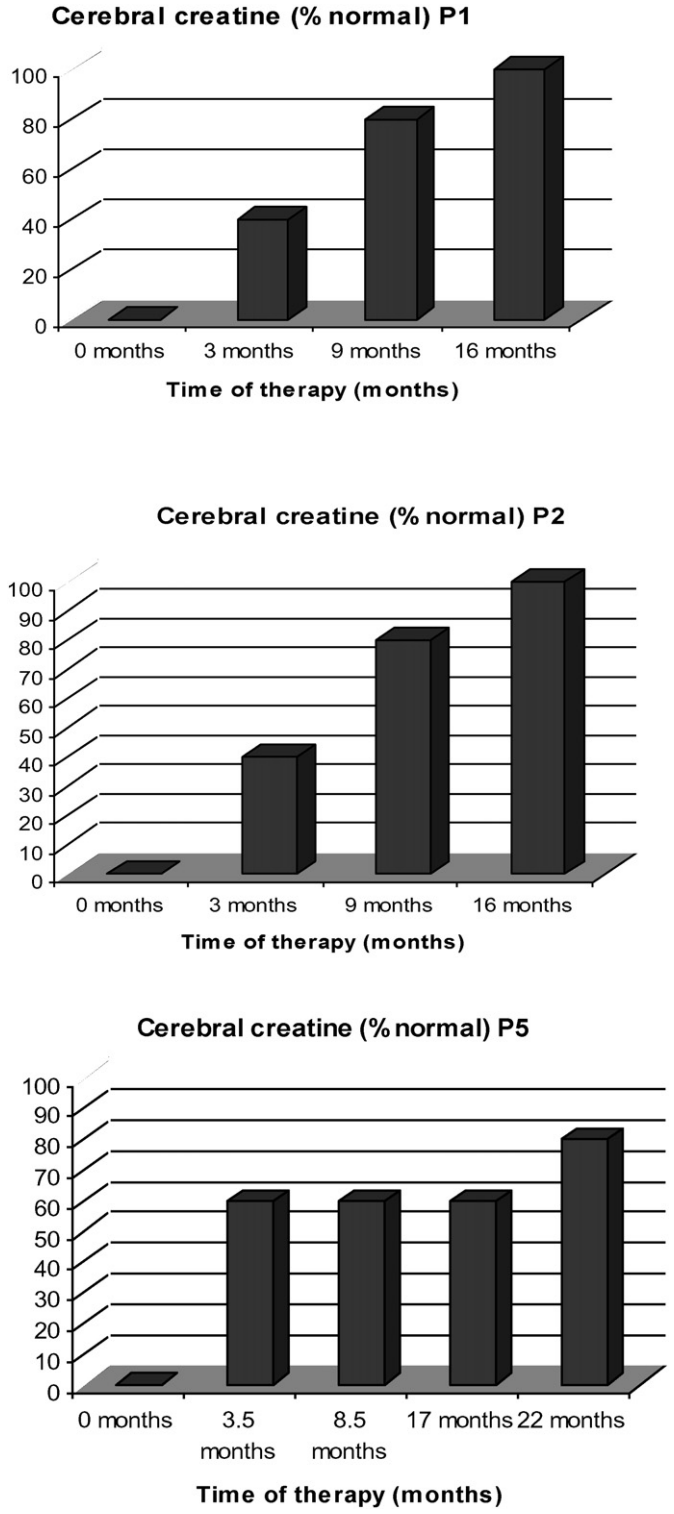

Fig. 2. Changes of cerebral creatine levels (given in % of normal) (y-axis) in 3 patients (P1, P2, P5) with AGAT deficiency upon various time periods of treatment with creatine monohydrate (x-axis) as measured by in vivo brain MRS. P1 and P2 were treated with a dosage of 400 mg/kg/day creatine monohydrate. P5 had several brain MRS during a 22 month treatment period with various dosages of creatine monohydrate ranging from 400 mg/kg/day to 800 mg/kg/day. After 8.5 months of treatment with 400 mg/kg/day of creatine monohydrate, brain creatine levels were at 60% of normal. No further increase of brain creatine was achieved after 9 subsequent months of treatment with the same dose (total 17 treatment months). After 5 months of 800 mg/kg/day (after a total of 22 treatment months) cerebral creatine content was 80% of normal and remained unchanged upon various dosages between 500 and 700 mg/kg/day subsequently.

5 out of 8 families had protein truncation/null mutations, and the rest had missense mutations. Given the low patient number and the heterogeneous clinical presentation of the few patients reported, we were not able to establish any correlation between the genotype and the type and severity of clinical presentation.

Each of the 8 families came from a different ethnic background, including Italian, Chinese, Yemenite/Jewish, Jordanian, Algerian, Haitian, Palestinian and Native American, and each had a distinct mutation. These results suggest that the occurrence of new *GATM* mutations is rare and most mutations are probably ancient.

Treatment with creatine monohydrate resulted in significant augmentation of cerebral creatine levels in most patients. However, as shown via systematic monitoring of cerebral creatine levels in P5 (Fig. 2), a complete normalization was not achieved even when very high creatine dosages were given. A proportion of the cerebral creatine content is derived from intracerebral creatine synthesis [21] facilitated by AGAT and GAMT expression in various brain cell types [22]. Hence, the inability to complete restoration of cerebral creatine content via supplementation might be explained by uncorrected cerebral AGAT deficiency.

In P6, cerebral creatine levels were only 18% of normal after 11 months of oral creatine supplementation. Insufficient compliance, individual requirement of a higher dosage, or additional presence of heterozygous cerebral creatine transporter deficiency are possible explanations for this finding.

Early treatment seems to prevent adverse developmental outcomes. P4 who was started on creatine supplements at the age of 4 months is normally developed at the age of 10 years, while P2 from the same family, who started treatment at the age of 4.5 years, had significant delays at a comparable age [1]. In P5, who started treatment at age 16 months, replenishment of cerebral creatine stores was timely associated with gain of cognitive functions [4]. Although treatment was started beyond the first year of life, this child's neurocognitive functions have totally normalized.

Irreversible brain damage that occurred prior to treatment initiation is the most likely reason why in the late treated patients the cognitive improvement was modest compared to the results achieved by early treatment.

Creatine supplementation had a most favorable effect on muscle weakness and myopathy in affected patients. Although muscle creatine has not been measured in any patient, restoration of muscle creatine content is the most likely pathophysiological explanation for this treatment effect.

Overall, long-term supplementation of creatine monohydrate was effective and well tolerated. Urinary creatine crystals were found in P5 upon a dosage of 800 mg/kg/day, which were reversible upon dose reduction (results not shown). Most patients were given 400 mg/kg/day, but a dosage as low as 100 mg/kg/day of creatine monohydrate seemed to be effective in P4 and P7. Further studies are warranted to determine the optimal effective and safe dosages at various ages and durations of treatment.

The favorable outcome in early treated patients makes AGAT deficiency an ideal candidate for newborn screening. Given that the normal range for GAA is below the detection limit of assays suitable for blood spot analysis, it is difficult to devise a sensitive screening algorithm based on GAA quantitation alone. Multi-analytic algorithms including arginine, ornithine, glycine and creatine as additional markers or a direct AGAT enzyme assay utilizing digital microfluidics technology [23], are possible avenues for the development of a high throughput bloodspot assay for AGAT newborn screening.

In conclusion, AGAT deficiency is a treatable intellectual disability [24]. We recommend that every patient with unexplained IDD and IDD with myopathy be screened for AGAT deficiency via determination of urine and plasma GAA. Whole exome/genome sequencing is an alternative strategy to identify patients. Diagnosis is confirmed by determination of cerebral creatine levels via MRS and *GATM* Sanger sequencing.

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