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REVIEW

N-acetylglutamate synthase deficiency: an insight into the genetics, epidemiology, pathophysiology, and treatment

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Abstract: The conversion of ammonia into urea by the human liver requires the coordinated function of the 6 enzymes and 2 transporters of the urea cycle. The initial and rate-limiting enzyme of the urea cycle, carbamylphosphate synthetase 1 (CPS1), requires an allosteric activator, N-acetylglutamate (NAG). The formation of this unique cofactor from glutamate and acetyl Coenzyme-A is catalyzed by N-acetylglutamate synthase (NAGS). An absence of NAG as a consequence of NAGS deficiency may compromise flux through CPS1 and result in hyperammonemia. The *NAGS* gene encodes a 528-amino acid protein, consisting of a C-terminal catalytic domain, a variable segment, and an N-terminal mitochondrial targeting signal. Only 22 mutations in the *NAGS* gene have been reported to date, mostly in the catalytic domain. NAGS is primarily expressed in the liver and intestine. However, it is also surprisingly expressed in testis, stomach and spleen, and during early embryonic development at levels not concordant with the expression of other urea cycle enzymes, CPS1, or ornithine transcarbamylase. The purpose of NAGS expression in these tissues, and its significance to NAGS deficiency is as yet unknown. Inherited NAGS deficiency is the rarest of the urea cycle disorders, and we review the currently reported 34 cases. Treatment of NAGS deficiency with N-carbamyglutamate, a stable analog of NAG, can restore deficient urea cycle function and normalize blood ammonia in affected patients.

Keywords: urea cycle, urea cycle disorder, N-acetyl-L-glutamate, N-acetylglutamate synthase, hyperammonemia, N-carbamyl-L-glutamate

Introduction

In humans, detoxification of ammonia occurs in the liver via the urea cycle, a biochemical pathway consisting of 6 enzymes and 2 mitochondrial membrane transporters.[1,2] The metabolic consequence of a defect in any step of the urea cycle has been well documented in man.[1–3] A common feature of all urea cycle disorders is elevated blood ammonia which may lead to mental retardation, coma, and possibly death.

N-acetylglutamate (NAG) is the required allosteric activator of carbamylphosphate synthetase (CPS1; EC 6.4.3.16), the first and rate limiting enzyme of urea cycle.[4,5] NAG, in turn, is synthesized from glutamate and acetyl Co-enzyme A[6,7] by the hepatic mitochondrial enzyme, N-acetylglutamate synthase (NAGS; EC 2.3.1.1). In the absence of NAG, the activity of CPS1 is virtually nil,[8,9] thus a deficiency of NAGS (MIM #237310) may result in hyperammonemia.

Herein, we describe the clinical and biochemical phenotype of NAGS deficiency, review the current published mutations in the *NAGS* gene, discuss the epidemiology of NAGS deficiency and review its treatment with N-carbamylglutamate.

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NAGS deficiency

In humans, the only known sequelae of NAGS deficiency result from decreased flux through the CPS1 reaction.[10] Indeed, the clinical and biochemical features of NAGS deficiency are identical to those seen in CPS1 deficiency.

In published cases to date, NAGS deficiency has presented at ages ranging from the neonatal period[11] to the fifth decade of life.[12] Clinical features of NAGS deficiency are those resulting from hyperammonemia, and include poor feeding, vomiting, altered level of consciousness, seizures, and coma. Patients with late-onset NAGS deficiency may present with chronic headaches and nausea. In such patients, acute decompensation has been precipitated by illness,[13] pregnancy,[14,15] or surgery,[16] and symptoms include confusion and combativeness.

Biochemical features of NAGS deficiency include an elevated plasma ammonia and glutamine, whereas the concentrations of other urea cycle intermediates are low-to-normal. As in other proximal urea cycle disorders, plasma citrulline is frequently low or undetectable.[16–29] However, unlike in OTC deficiency,[30] urinary orotic acid is not elevated, as the interruption in the urea cycle occurs before the formation of carbamylphosphate.

Initial diagnoses of NAGS deficiency were based on measurements of hepatic NAGS activity,[11,31] but in some cases, enzymatic assays were not reliable.[32–35] Cloning of the human *NAGS* gene in 2002[36] has allowed molecular testing to become the primary method of diagnosis. Mutations in the coding region of the *NAGS* gene have been identified in all but 1 reported case of NAGS deficiency since 2002 (Table 1).

The *NAGS* gene and transcript

The existence of mammalian NAGS was inferred over 50 years ago after NAG was identified as an obligate cofactor required in the biosynthesis of urea.[4] Nevertheless, the mammalian *NAGS* gene was the last urea cycle gene to be cloned,[36] probably due to the poor conservation of the NAGS protein sequence compared with that of the other urea cycle enzymes.[37] The human *NAGS* gene is located on chromosome 17q21.31 and consists of 7 exons and 6 introns covering slightly less than 5 kb.[36] The human NAGS open reading frame encodes a 528-amino acid protein.[36] A comparison of amino acid sequences of NAGS from 7 mammalian species revealed 3 regions with different degrees of sequence conservation. At the N-terminus is a 50-amino acid-long mitochondrial targeting signal (MTS). This is followed by a 40- to 46-amino acid-long variable segment and a C-terminus conserved segment.[6,36] The MTS has approximately 60% sequence conservation in mammalian NAGS and removal of the MTS results in what is dubbed mature NAGS.[38] The variable segment is poorly conserved in mammalian NAGS and is not required for NAGS enzymatic activity.[39] The rest of the protein, the conserved segment, has 90% sequence identity across mammalian species, and contains the catalytic site and the binding site for the allosteric activator L-arginine.[37,40]

Mutations in the *NAGS* gene

NAGS deficiency is an autosomal recessive disorder, thus affected individuals carry a mutation in each of their NAGS alleles, whereas heterozygous carriers are unaffected. Twenty-two disease-causing mutations in the NAGS coding sequence and in intron/exon boundaries have been reported to date (Table 1). Although at present 2 mutations occurred in more than 1 family (T431I and W324X), there do not appear to be any mutational hot spots in the NAGS gene. This is particularly surprising given that the NAGS coding sequence is GC-rich (67% GC content) and contains 135 CpG dinucleotides.[14] Interestingly, most single base pair replacements in the NAGS coding sequence do not occur in these dinucleotides.[14] Identified deleterious mutations in the NAGS gene include 15 missense, 1 nonsense, 4 frame-shift, and 2 splice-site mutations.[14]

A limited genotype-phenotype correlation may be inferred from affected patients who were homozygous for mutations in the *NAGS* gene. Homozygosity for nonsense or frameshift mutations, predicted to cause truncation of the NAGS protein and thus complete absence of functional NAGS enzyme, resulted in a neonatal presentation in 4 patients.[21,36,41,42] Homozygosity for missense NAGS mutations, depending on the effect of the single amino-acid substitution, may result in either absent NAGS function or diminished but significant residual NAGS activity. The presence of residual enzyme activity, as demonstrated in purified recombinant enzyme, is the likely explanation for a later non-neonatal presentation in some affected patients.[14,16,42] In contrast, a neonatal presentation was observed in patients who were homozygous for missense mutations of conserved residues (eg, S410P) or where a hydrophobic residue was substituted with a polar or charged amino acid (eg, W484R and A518T).[24,28,29,42] Four affected patients were homozygous for missense alterations involving replacement of an amino acid with proline, which is likely to disrupt the NAGS secondary structure resulting in enzyme with little or no activity.[29,43–45] To date, no single amino-acid substitutions have been reported within either the mitochondrial targeting signal or the variable segment

Table 1 Clinical presentation, genotype, N-carbamylglutamate (NCG) treatment and outcome of 34 patients reported to have NAGS deficiency

Family	Patient	Onset of symptoms	Presentation	Genotype	Onset of NCG therapy	Initial NCG dose	Chronic NCG dose	Outcome	Reference
1*	1	FH, 3 d	Asymptomatic		10 d	100 mg/kg/d	180 mg/kg/d at 13 m	Ataxia, spasticity, psychomotor retardation, death at 9 y	Bachman et al[51] Bachman et al[11] Schubiger et al[49]
2	2	6 d	Poor feeding, tachypnea, somnolence		N/A			Death at 8 d	Bachman et al[17]
3*	3	13 m	Decreased level of conciousness following febrile illness		N/A			Death at 13 m	Elpeleg et al[22]
4‡	4	5 w	Seizures following a viral gastroenteritis		N/A			Normal development at 18 m	Pandya et al[27]
	5	FH, 26 d	Diarrhea		N/A			Mild psychomotor retardation and spasticity at 6 m	
5	6	2 m	Frequent episodes of vomiting and lethargy, hypotonia		N/A			Profound psychomotor retardation at 2 y	Burlina et al[18]
6	7	4 y 10 m	Vomiting, lethargy, hepatomegaly		N/A			Normal development at 5 y 7 m	Vockley et al[34]
7	8	3-4 d	Poor feeding, seizures	S410P/S410P	25 d	220 mg/kg/d	80 mg/kg/d at 1 y	Normal development at 1 y	Guffon et al[24] Schmidt et al[29]
	9	FH, 1 d	Asymptomatic		1 d	114 mg/kg × 1 dose		Normal development	Guffon et al[23]
8	10	1.5 y	Confusion, combative behavior		20 y	60 mg/kg/d	50 mg/kg/d at 22 y	Cerebral dysfunction, paraplegia, incontinence at 22 y	Hinnie et al[25]
9	11	3-4 d	Poor feeding, vomiting, cycling movements and fist clenching		after 10 w	100 mg/kg/d	100 mg/kg/d at 20 m	Normal development at 20 m	Morris et al[26]
10	12	13 m	Vomiting, somnolence, hypotonia	A279P/A279P	13 y	100 mg/kg/d	100 mg/kg/d	IQ = 78 at 13 y	Plecko et al[28] Haberle et al[42]
11	13	2.7 y	Paroxysmal crying, lethargy, meat and dairy aversion, "Reye syndrome" after valproate administration		4 y	100 mg/kg/d	100 mg/kg/d	Mental development <2 SD below age-matched controls at 4 y	Forget et al[50]
12	14	4 d	Seizures, coma	c.1036insC/ c.1036insC	36 m	150 mg/kg/d	Not indicated	Psychomotor retardation at 4 y	Elpeleg et al[22]
	15	FH, 1 d	Asymptomatic		3 m	150 mg/kg/d	Not indicated	Psychomotor retardation at 2 y	
13	16	<2 d	Tachypnea, jitteriness	W324X/W324X	N/A			Death at 4 d	Caldovic and Tuchman[20]

(*Continued*)

Table 1 (*Continued*)

Family	Patient	Onset of symptoms	Presentation	Genotype	Onset of NCG therapy	Initial NCG dose	Chronic NCG dose	Outcome	Reference
14	17	2 d	Lethargy, anorexia, vomiting, respiratory distress, coma, seizures	c.1025delC	N/A			Not indicated	Caldovic and Tuchman[20]
	18	2 d	Lethargy, anorexia, vomiting, respiratory distress, coma, seizures					Not indicated	
15	19	3 d	Not indicated	c.1306insT/ IVS3-2A>T	N/A			Death at 3 d	Haberle et al[42]
16	20	3 d	Not indicated	L430P/L430P	N/A			Not indicated	Haberle et al[42] Schmidt et al[29]
17	21	6 d	Not indicated	E433S/E433S				Not indicated	Haberle et al[42]
18	22	3 d	Not indicated	W484R/W484R	N/A			Death at 6 m	Haberle et al[42] Heckman et al[32]
19	23	4 d	Not indicated					Death at 22 d	Haberle et al[42]
	24	FH, 2 d	Asymptomatic	W324X/W324X	3 m	250 mg/kg/d	10–200 mg/kg/d	Normal at 13 y	Gessler et al[41]
20	25	9 y	Attention deficit, learning disabilities, episodes of anxiety and irritability		12 y	100 mg/kg/d	15 mg/kg/d	Not indicated	Belanger-Quintana et al[13]
21	26	4 w	Vomiting, irritability, lethargy	R509Q/IVS4-1G > C	N/A	NCG study 2.2 g/m^2/d	Not indicated	Not indicated	Caldovic et al[16] Caldovic et al[19]
	27	9 y	Lethargy, anorexia, vomiting					Not indicated	
22	28	33 y	After surgery: hypertensive, combative, confused, seizures	V173E/T431I	N/A			Death at 33 y	Caldovic et al[16]
23	29	2.5 m	Vomiting, weight loss, hypotonia	C200R/C200R	4 m	180 mg/kg × 1 dose	Not indicated	Normal development, age not indicated	Schmidt et al[29] Guffon et al[23]
24	30	2 d	Irritablity, poor feeding. By 4 d, drowsiness, tremor, hypotonia	A518T/A518T	4 d	200 mg/kg × 1 dose	Not indicated	Normal development, age not indicated	Schmidt et al[29] Guffon et al[23]
25	21	27 y	Seizures, coma during pregnancy	L312P/T431I	N/A			Not indicated	Grody et al[15] Caldovic et al[14]
26	32	3 d	Seizures, coma	R414P/R414P	4 d	200 mg/kg/d	50 mg/kg/d at 3 y	Normal development at 3 y	Nordenstrom et al[52]
27	33	40 y	Migraine headaches, intermittent staring spells, nausea, recurrent vomiting, lethargy, ataxia, coma	V350I/L442V	N/A	NCG study 2.2 g/m^2/d	Not indicated	Normal intellect at 57 y	Tuchman et al[2]
28	34	3 d	Vomiting, feeding intolerance, episodic confusion	c.278delC/M167V	6 m	350 mg/kg/d	23–140 mg/kg/d	Normal development at 20 y	Corne et al[53]

Notes: Familial hyperammonemia patients were identified prospectively based on a family history of hyperammonemia or N-acetylglutamate synthase deficiency (NAGS); *Older siblings died of hyperammonemia of unknown origin; ‡Older brother died of seizures, liver failure.

of NAGS, suggesting that these regions are perhaps tolerant to missense changes.

Mutations in splice sites were observed in 2 families. Two splice-site mutations involved changes in the consensus acceptor splice sites of introns 3 and 4, which are expected to abolish mRNA splicing.[16,42]

One alteration, G236C, was incidentally discovered in a patient whose DNA was used as wild-type control sample for the NAGS sequencing assay in our clinical laboratory. Whether or not this alteration is disease-causing is unknown, but it was neither identified in a study of common polymorphisms of urea cycle genes,[46] nor found in dbSNP build 133.

Expression of the NAGS mRNA and protein

In NAGS deficiency, disruption of the *NAGS* gene results in reduced or absent NAGS enzyme in tissues in which it is normally expressed. NAGS mRNA is primarily expressed in the liver, but is also expressed in other tissues such as small intestine, spleen, and testis.[20,36] Because the only known function of mammalian NAGS, CPS1, and OTC is to synthesize citrulline, NAGS would be expected to be expressed in the same tissues as CPS1 and OTC. To test this hypothesis, we used RT-PCR to quantify the relative expression levels of mouse NAGS, CPS1 and OTC mRNA in 14 tissues as well as at 4 stages (E7, E11, E15, and E17) of embryonic development.

As expected, liver had the highest expression of NAGS, CPS1, and OTC mRNA, followed by intestine (Figure 1). However, substantial expression of all 3 genes was also seen in the testis (between 3% and 25% of the expression in liver) and much less in the stomach and spleen (between 0.1% and 1.2% of the expression in the liver). Low levels of NAGS mRNA were also detectable in the brain, kidney, and ovary. In all other tissues, expression of NAGS, CPS1, and OTC mRNA were less than 0.1% of the expression seen in the liver. Surprisingly, NAGS mRNA was also expressed at embryonic stage E7, at levels approximately 3.7% of that seen in adult murine liver, in the absence of detectable levels of CPS1 and OTC mRNA.

In contrast, western blot of a panel of 9 mouse tissues revealed the presence of NAGS, CPS1, and OTC proteins in the liver but only CPS1 and OTC in the intestine (data not shown). Although NAGS activity has previously been measured in the intestine,[47,48] NAGS protein was not detectable in the small intestine most likely due to its low abundance in this tissue.

The presence of NAGS mRNA in mouse embryos at E7, as well as testis, ovary, spleen, stomach, kidney, and brain, could be due to illegitimate transcription, or more interestingly, to an as yet undiscovered novel function of NAGS. Additional studies will reveal if this expression pattern is also observed in humans, whether the expression of NAGS mRNA has physiological roles in tissues that do not express CPS1 and OTC, and whether absence of NAGS in these tissues contributes to the pathophysiology of NAGS deficiency.

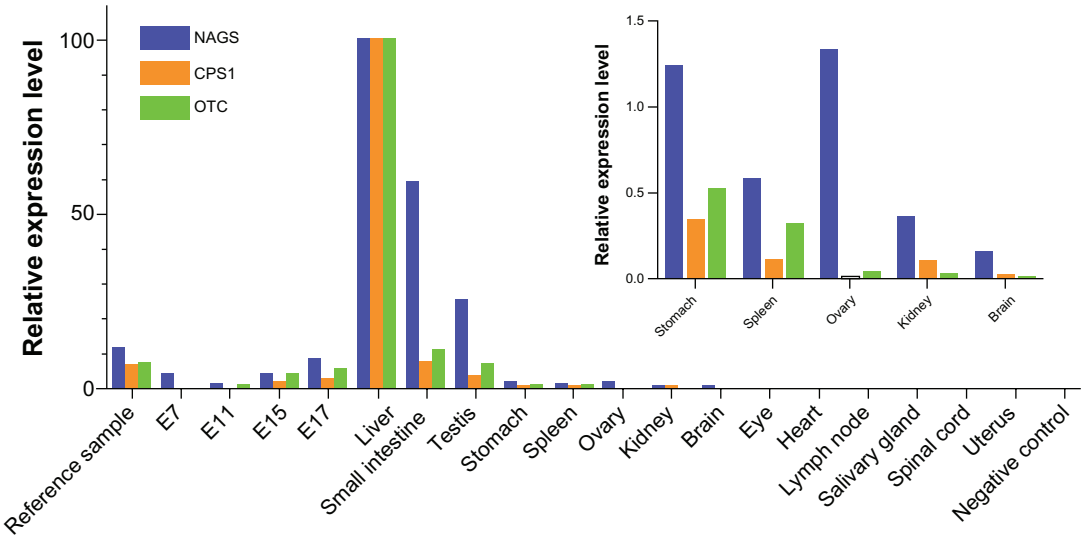

Figure 1 Relative expression levels of mouse NAGS, CPS1, and OTC mRNA in mouse tissues and stages of embryonic development. Insert shows relative expression of NAGS, CPS1 and OTC mRNA in the stomach, spleen, ovary, kidney and brain. Expression of NAGS, CPS1, and OTC mRNA was measured using quantitative real-time PCR and normalized to their mRNA abundance in liver. 1 μg of total mouse RNA from ovary, testis, brain, eye, heart, kidney, liver, lymph node, submaxillary gland, spinal cord, spleen, stomach, uterus, intestine, 7-day embryo, 11-day embryo, 15-day embryo, 17-day embryo was reverse transcribed to cDNA using random primers. Real time PCR was carried out using primers designed to anneal to different exons to avoid amplifying genomic DNA.

Epidemiology and incidence of NAGS deficiency

Inherited NAGS deficiency is the rarest of urea cycle disorders,[2] and the true incidence of NAGS deficiency is not known. To date, there are 34 reported patients from 28 families with NAGS deficiency. In the 2 decades before identification and cloning of the human *NAGS* gene,[36] suspected diagnoses of NAGS deficiency were reported in only 11 families.[11,17,18,22,24–28,34,49–51] Identification and cloning of human *NAGS* gene now allows accurate molecular diagnosis of the condition, and NAGS deficiency has since been reported in an additional 16 families.[2,13,14,16,19–21,23,29,32,41,42,52,53] Nearly half of patients with NAGS deficiency are homozygotes, rather than compound heterozygotes, for mutations in the NAGS gene and these families indicated the existence of consanguinity[22,23,25,26,29,42,52] or a known common ancestor.[20]

Several explanations could account for the low incidence of NAGS deficiency, compared with other urea cycle disorders.[2] First, even mutations resulting in significant impairment of NAGS enzymatic function may allow for the production of sufficient CPS1 cofactor to maintain adequate flux through CPS1 and thus preclude hyperammonemia. Additionally, in a comparison of the sequences of urea cycle enzymes across phyla,[54–56] NAGS is the least conserved.[57] Thus, the NAGS structure may be more tolerant of amino acid substitutions. As a result, only individuals with rare amino acid substitutions that virtually abolish enzymatic function, either due to abolished substrate binding and catalysis or disruption of NAGS structure, will present with symptoms of NAGS deficiency. Alternatively, it is possible though unlikely that another enzyme is able to synthesize limited amounts of NAG, and that mutations in both NAGS and this second "moonlighting" enzyme are required to reduce CPS1 activity sufficiently to cause hyperammonemia. Finally, NAGS could potentially have other functions besides ammonia detoxification and a complete deficiency of NAGS may result in reduced embryonic survival. As described above, NAGS mRNA is curiously expressed in mouse spleen and testis (Figure 1 and Caldovic et al[58]) and also at mouse embryonic day 7, in the absence of significant CPS1 or OTC expression, thus positing another possible function of NAGS or NAG.

Treatment of NAGS deficiency with N-carbamylglutamate

Before the discovery of the CPS1 enzyme, Grisolia and Cohen determined that a derivative of L-glutamic acid, N-carbamylglutamate (NCG), was necessary for the biosynthesis of citrulline.[59] While it was only later determined that N-acetylglutamate was the natural co-factor to the CPS1 enzyme,[4] this earlier discovery was fortuitous as it would subsequently provide an important avenue of treatment for patients with NAGS deficiency.[11]

In contrast to NAG, which is hydrolyzed in vivo by acylamino acid acylase,[60] NCG is acylase-resistant.[61] Because both NAG and NCG can function as activating co-factors of CPS1, NAGS deficiency is the only inherited urea cycle disorder that can be specifically and effectively treated by a drug. In patients with NAGS deficiency, a 3-day trial of oral NCG at a dose of 2.2 g/m^2/day was shown to restore ureagenesis and normalize blood ammonia, as demonstrated by [^{13}C] and [^{15}N] isotopic studies.[2,19]

Oral NCG has successfully rescued neonates with NAGS deficiency during hyperammonemic crisis.[23,52] Published data on appropriate NCG dosing are limited. The initial NCG dose for treatment of acute hyperammonemia ranged in neonates from 25 mg/kg (100 mg/kg/day in 4 divided doses) to 200 mg/kg,[23] compared with 15 mg/kg (60 mg/kg/day in 4 divided doses)[25] to 180 mg/kg[23] in those with late-onset NAGS deficiency who presented after the first month of life.

In patients receiving NCG as part of long-term chronic therapy, the lowest reported daily dose required to prevent hyperammonemia was 15 mg/kg/day in both neonatal[41] and late-onset NAGS deficiency.[13] NCG therapy appears to correct the metabolic defect in such patients, who no longer require ammonia-scavenging agents.[13,23,25,41,49,52] In fact, dietary protein was liberalized to 2–3 g/kg/day in some patients,[24,52] but 1 patient became mildly ataxic after ingestion of more than 3.5 g/kg/day.[52] It is possible that a higher daily NCG dose would allow for greater protein tolerance in these patients, since in other NCG-treated patients, protein intake has been entirely liberalized, with no adverse effects.[12]

Extremes of NCG dosing have been associated with adverse effects. One patient, in whom NCG dosing was reduced to 10 mg/kg/day, experienced a rise in plasma ammonia from 27 to 58 μmol/L, which normalized once NCG was increased to 15 mg/kg/day.[41] Another patient who received a dose of 650 mg/kg experienced tachycardia, sweating, bronchial hypersecretion, elevated body temperature, and restlessness.[51]

Some NAGS-deficiency patients on NCG have experienced breakthrough hyperammonemia during episodes of acute illness.[26,49] Hyperammonemia while on NCG may reflect inadequate dosing. However, protein restriction

during illness may be prudent if poor oral tolerance prevents the administration of NCG. Withdrawal of protein from the diet may have helped to prevent hyperammonemia in 1 patient.[26]

The advantage of treating NAGS deficiency with NCG is that NCG increases ammonia elimination by activating in vivo enzymes, whereas ammonia scavenging agents act stoichiometrically and response to scavengers is frequently suboptimal. All 3 affected neonates who presented with acute hyperammonemia and were administered NCG in a timely fashion along with standard therapy, had normal psychomotor development at 12 and 13 months of age.[23,52] In contrast, some affected neonates who initially received conventional therapy alone, including ammonia scavenging agents and dialysis, have exhibited psychomotor retardation.[21,50,51]

It has been suggested that all hyperammonemic newborns with a suspected diagnosis of a urea cycle disorder should receive a therapeutic trial of NCG, which may provide a life-saving therapeutic option for patients with NAGS deficiency, and provide additional benefit in some cases of CPS1 deficiency.[23] A rapid response to NCG may help to diagnose some cases of NAGS deficiency,[23] though not all cases respond quickly.[52]

Other conditions with N-acetylglutamate deficiency

Secondary deficiencies of NAG may be observed in conditions associated with a depletion of intramitochondrial Coenzyme-A, acetyl-CoA, or glutamate, or inhibition of the NAGS reaction. A reduction of hepatic NAG has been hypothesized as the mechanism of hyperammonemia in the organic acidemias (eg, propionic academia,[62,63] methylmalonic academia,[62] isovaleric acidemia[64]), hyperinsulinism-hyperammonemia syndrome,[65] and in valproic acid treatment.[66,67] Exogenous benzoate may also decrease the intra-mitochondrial NAG concentration.[68] Treatment with NCG may effectively treat hyperammonemia in these disorders.[12,65,68–75] Indeed, 3-day administration of NCG has been shown to increase ureagenesis and decrease plasma ammonia in propionic academia.[70]

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Disclosure

The authors report no conflicts of interest.

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