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ORIGINAL PAPER

Favourable long-term outcome after immediate treatment of neonatal hyperammonemia due to *N*-acetylglutamate synthase deficiency

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Abstract
Introduction *N*-Acetylglutamate synthase (NAGS) deficiency is a rare urea cycle disorder, which may present in the neonatal period with severe hyperammonemia and marked neurological impairment.
Case report We report on a Turkish family with a patient who died due to hyperammonemia in the neonatal period. Reduced activity of NAGS and carbamyl phosphate synthetase were found at autopsy. A second child who developed hyperammonemia on the second day of life was immediately treated with arginine hydrochloride, sodium benzoate and protein restriction. After NAGS deficiency was suspected by enzyme analysis, sodium benzoate was replaced by *N*-carbamylglutamate (NCG). A third child who developed slight hyperammonemia on the third day of life was treated with NCG before enzyme analysis confirmed reduced NAGS activity. Neither of the patients developed hyperammonemia in the following years. After the human NAGS gene was identified, mutation analysis revealed that the older sibling on NCG therapy was homozygous for a 971G>A (W324X) mutation. The parents and the younger sibling were heterozygous. Therapy was continued in the older sibling until now without any adverse effects and favourable neurodevelopment outcome. In the younger sibling, therapy was stopped without any deterioration of urea cycle function.
Conclusion NAGS deficiency can be successfully treated with NCG and arginine hydrochloride with favourable outcome. Molecular diagnostic rather than enzyme analysis should be used in patients with suspected NAGS deficiency.

Keywords Hyperammonemia · *N*-Acetylglutamate synthase · Carbamylglutamate · Neurodevelopment

Abbreviations

NAG	*N*-Acetylglutamate
NCG	*N*-Carbamylglutamate
NAGS	*N*-Acetylglutamte synthase
CPSI	Carbamylglutamate synthetase 1
OTC	Ornithine transcarbamylase

Introduction

In mammals, clearance of nitrogen produced by the breakdown of proteins and other nitrogen-containing molecules occurs in the urea cycle. The flux of nitrogen is regulated among other factors by *N*-acetylglutamate (NAG), an activator of carbamyl phosphate synthetase (CPSI), the first enzyme of the urea cycle. Deficiency of *N*-acetylglutamate synthase (NAGS) or any of the urea cycle enzymes may result in the accumulation of ammonia in the neonatal period or, in partial deficiencies, later in life [2]. First-line treatment of hyperammonemia includes a protein-restricted high caloric diet with arginine supplementation to fuel the urea cycle and sodium benzoate and/or sodium phenylacetate or its precursor, sodium phenylbutyrate, as scavengers of excess ammonia [6]. NAGS deficiency (OMIM #237310) can

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specifically be treated by *N*-carbamylglutamate (NCG), a synthetic stable analogue of NAG. However, NAGS deficiency has so far been found in only a small number of patients with hyperammonemia [4] and experience with NCG is still limited. We report on a family with NAGS deficiency and long-term treatment with NCG.

Case report

The first child (TD) of consanguineous Turkish parents (first cousins) was born in 1992 and was admitted to the hospital on day 4 of life with severe hyperammonemia (2,000 μmol/l). On day 22 of life, the girl died due to hyperammonemia with general brain oedema and seizures. Liver biopsy (day 8 of life) revealed normal activity of ornithine transcarbamylase (OTC) and CPSI activity at the reference limit, but there was not enough material to determine NAGS. A repeat analysis after death of the patient showed again normal activity of OTC, decreased activity of arginine-stimulated NAGS (Table 1) and significantly decreased activity of CPSI (20% of reference limit) and a tentative diagnosis of CPSI deficiency was made.

The second child was born in 1993 and was healthy.

The third child (YD), born in 1995, showed increased ammonia concentrations (138–149 μmol/l; reference value <100 μmol/l) on the second day of life and parenteral high caloric diet with arginine was initiated. Because ammonia remained elevated with peak levels of 170 μmol/l, the diet was switched to breast milk with sodium benzoate and arginine supplementation administered orally. Ammonia subsequently normalised, and the child was discharged from hospital on day 17 of life. In the following 2.5 months, she was rehospitalised four times due to vomiting and increased ammonia (peak 393 μmol/l). Treatment consisted of sodium benzoate administered intravenously and protein restriction. Biochemical analysis revealed markedly elevated glutamine (1,332 and 1,961 μmol/l; ref. range 254–823 μmol/l), low leucine (33 μmol/l; ref. range 56–98 μmol/l) and low isoleucine (7 μmol/l, ref. range 31–48 μmol/l). Other amino acids and organic acids, including orotic acid (3 mmol/mol creatinine, ref. range 1.3–8.5 mmol/mol creatinine) were in the reference range. A liver biopsy yielded normal activities of CPSI and OTC but was not sufficient to assay for NAGS activity. Nevertheless, in view of the decreased NAGS activity found in the first child, NCG therapy was started 3 months after birth at an initial daily dose of 1,200 mg (250 mg/kg) in four oral administrations [1]. Sodium benzoate and protein restriction were discontinued, whereas arginine supplementation (250 mg/kg per day) was maintained. A second liver biopsy 1 month later confirmed NAGS deficiency (Table 1). At the age of 14 months, the daily dose of NCG was increased to 2,000 mg to keep it at about 200 mg/kg per day. No hepatic side effects occurred, and levels of amino and organic acids, including arginine and orotic acid, were regularly controlled. During the next 4 years, the mother progressively reduced the dose on her own initiative without deleterious consequences, and the dose was then formally reduced to 800 mg/day (40 mg/kg). In 2003, when molecular analysis of the NAGS gene became feasible, the patient was found to be homozygous for a 971G>A (W324X) mutation [8], proving NAGS deficiency (Table 1). Throughout the years under NCG treatment, ammonia and amino acids had remained in the normal range. However, in September 2008, the girl reduced NCG to 10 mg/kg per day with a subsequent rise of ammonia from 27 to 58 μmol/l. The girl was neurologically asymptomatic, and blood levels of amino acids as well as urine levels of organic acids were within the normal range (orotic acid <1 μmol/l). Nevertheless, NCG was increased to 15 mg/kg and ammonia decreased. Now, the girl is 13 years old with a weight of 65 kg and a length of 162 cm, shows stage of puberty P 3, visits a normal school and behaves like a normal classmate. There is no neurological abnormality.

In the fourth child (AD), born in 1996, slightly elevated ammonia (104–144 μmol/l) was detected on the second day of life, and therapy with NCG (210 mg/kg per day) and arginine was immediately started. Enzymatic testing of a liver biopsy at the age of 4 months demonstrated NAGS activity slightly below 50% of the lower reference limit (Table 1). The daily dose of NCG was periodically increased to maintain it at about 200 mg/kg per day. However, as in the case of his sister, the mother progressively reduced the amount of NCG to about 40 mg/kg per day over the next 3 years. In 2003, molecular analysis of the NAGS gene revealed that the boy was heterozygous for the mutation found in his sister, and therapy with NCG and arginine was stopped (Table 1).

Table 1 NAGS activity in three patients with suspected NAGS deficiency

	NAGS basal (pmol min^{-1} mg $protein^{-1}$)	NAGS activated (pmol min^{-1} mg $protein^{-1}$)	971G>A (W324X) mutation
Reference limit	>34	>144	
Patient TD	78	104	Not available
Patient YD	98	64	Homozygous
Patient AD	12	62	Heterozygous

Determination of NAGS activity and reference limits according to Colombo et al. [5]

Ammonia has remained in the reference range, and the boy is now 12 years old and healthy.

Discussion

Hyperammonemia due to urea cycle disorders may develop in the first days of life and causes neurological damage leading to psychomotor impairment and death. Prompt recognition of hyperammonemia and immediate initiation of first-line treatment are therefore essential. Therapy may later be adjusted when the deficient enzyme has been identified. In contrast to the other urea cycle enzyme deficiencies, which yield pathognomonic amino acid or organic acid patterns, CPS and NAGS deficiencies can only be identified by enzymatic or molecular diagnostic analysis (reviewed in [2]). However, CPS and, to a lesser degree, NAGS are unstable and may yield falsely low activity in repeat analyses. This may have been the cause of the low CPS activity in the second liver sample from the oldest sibling. Moreover, enzyme analysis was not sensitive enough to distinguish between the homo- and heterozygous state of the siblings. Insufficient sensitivity of enzyme analysis to discriminate between homo- and heterozygosity was previously reported [10]. Molecular diagnostic rather than enzyme analysis should therefore be used in patients with suspected NAGS deficiency.

Previous studies had indicated that NCG effectively activates CPSI [9, 13]. However, reports on treatment of patients with NCG were limited at the time when our patients were born and the outcomes were conflicting [7, 14]. Moreover, NCG was not commercially available at that time. Nevertheless, treatment with NCG provided by the hospital pharmacy was started as soon as the diagnosis of NAGS deficiency was suspected. Although it was intended to keep the daily dose at about 200 mg/kg, the mother decreased the dose progressively on her own initiative without deleterious effects. Today, the daily dose of 15 mg/kg is sufficient to compensate for complete deficiency of NAGS activity.

Since treatment of our patient with NCG was started, several other reports have documented the effectiveness of NCG in NAGS deficiency [3, 11, 12], and more recently, a reliable method for directly measuring the effect of NCG on nitrogen metabolism in patients was developed [15]. NCG is generally well tolerated with few side effects and should be administered as early as possible when NAGS deficiency is suspected, even before the definite diagnosis has been made. Molecular in favour of enzyme analysis offers rapid and definite diagnosis of a suspected NAGS deficiency.

Conflict of interest The authors declare no conflict of interest. The work was not sponsored and there is no financial relationship.

References

1. Batshaw ML, MacArthur RB, Tuchman M (2001) Alternative pathway therapy for urea cycle disorders: twenty years later. J Pediatr 138:S46–S55. doi:10.1067/mpd.2001.111836
2. Brusilow SW, Horwich AL (2001) Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 8th edn. McGraw-Hill, New York, pp 1909–1963
3. Caldovic L, Morizono H, Daikhin Y et al (2004) Restoration of ureagenesis in N-acetylglutamate synthase deficiency by N-carbamylglutamate. J Pediatr 145:552–554. doi:10.1016/j.jpeds.2004.06.047
4. Caldovic L, Morizono H, Tuchman M (2007) Mutations and polymorphisms in the human N-acetylglutamate synthase (NAGS) gene. Hum Mutat 28:754–759. doi:10.1002/humu.20518
5. Colombo JP, Krähenbühl S, Bachmann C, Aeberhard P (1982) N-Acetylglutamate synthetase: enzyme assay in human liver. J Clin Chem Clin Biochem 20:325–229
6. Enns GM, Berry SA, Berry GT et al (2007) Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. N Engl J Med 356:2282–2292. doi:10.1056/NEJMoa066596
7. Guffon N, Vianey-Saban C, Bourgeois J et al (1995) A new neonatal case of N-acetylglutamate synthase deficiency treated by carbamylglutamate. J Inherit Metab Dis 18:61–65. doi:10.1007/BF00711374
8. Häberle J, Schmidt E, Pauli S et al (2003) Mutation analysis in patients with N-acetylglutamate synthase deficiency. Hum Mutat 21:593–597. doi:10.1002/humu.10216
9. Hall LM, Metzenberg RL, Cohen PP (1958) Isolation and characterization of a naturally occurring cofactor of carbamyl phosphate biosynthesis. J Biol Chem 230:1013–1021
10. Heckmann M, Wermuth B, Häberle J et al (2005) Misleading diagnosis of partial N-acetylglutamate synthase deficiency based on enzyme measurement corrected by mutation analysis. Acta Paediatr 94:121–124. doi:10.1080/08035250410030937
11. Hinnie J, Colombo JP, Wermuth B, Dryburgh FJ (1997) N-Acetylglutamate synthetase deficiency responding to carbamylglutamate. J Inherit Metab Dis 20:839–840. doi:10.1023/A:1005344507536
12. Plecko B, Erwa W, Wermuth B (1998) Partial N-acetylglutamate synthetase deficiency in a 13-year-old girl: diagnosis and response to treatment with N-carbamylglutamate. Eur J Pediatr 157:996–998. doi:10.1007/s004310050985
13. Rubio V, Grisolía S (1981) Treating urea cycle defects. Nature 292:496. doi:10.1038/292496a0
14. Schubiger G, Bachmann C, Barben P et al (1991) N-Acetylglutamate synthetase deficiency: diagnosis, management and follow-up of a rare disorder of ammonia detoxication. Eur J Pediatr 150:353–356. doi:10.1007/BF01955939
15. Tuchman M, Caldovic L, Daikhin Y et al (2008) N-Carbamylglutamate markedly enhances ureagenesis in N-acetylglutamate deficiency and propionic acidemia as measured by isotopic incorporation and blood biomarkers. Pediatr Res 64:213–217. doi:10.1203/PDR.0b013e318179454b