Hyperammonemia due to Adult-Onset *N*-Acetylglutamate Synthase Deficiency

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Received: 04 January 2016 / Revised: 25 March 2016 / Accepted: 29 March 2016 / Published online: 05 May 2016
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Abstract A 59-year-old woman, with a medical history of intellectual disability after perinatal asphyxia, was admitted because of coma due to hyperammonemia after she was treated for a fracture of the pelvis. The ammonia level was 280 μ M. Acquired disorders as explanation for the hyperammonemia were excluded. Metabolic investigations showed an elevated glutamine and alanine and low citrulline, suspect for a urea cycle defect (UCD). Orotic acid could not be demonstrated in urine. DNA investigations were negative for mutations or deletions in the *OTC* and *CPS1* gene, but revealed a homozygous c.603G>C mutation in exon 2 of the *N*-acetylglutamate synthase (*NAGS*) gene (NM_153006.2:c.603G>C), which mandates p.Lys201Asn. This is a novel mutation in the *NAGS* gene.

After the diagnosis of *NAGS* deficiency was made carbamylglutamate was started in a low dose. In combination with mild protein restriction the ammonia level decreased to 26 μ M.

This is one of the first patients in literature in whom the diagnosis of a UCD is made at such an advanced age. It is important for the adult physician to consider a metabolic disorder at every age.

Introduction

In humans, detoxification of ammonia occurs in the liver via the urea cycle, a biochemical pathway consisting of six enzymes and two mitochondrial membrane transporters: *N*-acetyl glutamate synthase (*NAGS*, EC 2.3.1.1), carbamyl phosphate synthetase I (*CPSI*, EC 6.3.5.5), ornithine transcarbamylase (*OTC*, EC 2.1.3.3), argininosuccinate synthetase (*AS*, EC 6.3.4.5), argininosuccinate lyase (*ASL*, EC 4.3.2.1), arginase (EC 3.5.3.1), the aspartate transporter (*citrin*), and the ornithine transporter (*ORNT1*) (Walker 2009; Cartagena et al. 2013). All disorders except for X-linked *OTC* deficiency (OMIM 311250) are inherited in an autosomal recessive manner (Cartagena et al. 2013). The metabolic consequence of a deficiency in the urea cycle is an elevated blood ammonia which may lead to mental retardation, encephalopathy, coma, and possibly death. Urea cycle disorders typically present in the neonatal period or during the first months of life (Nakamura et al. 2014). However, there is a growing knowledge concerning urea cycle disorders that have been recognized in adulthood (Serrano et al. 2010; Roberts et al. 2013). A partial or mild enzyme deficiency may permit an individual to function relatively normally, sometimes for decades, until a stressful medical situation such as infection, starvation, surgery, pregnancy, or trauma occurs, that triggers a hyperammonemic crisis (Summar et al. 2005). Such an underlying urea cycle disorder might be difficult to recognize, because the patients can be ill because of other reasons. However, prompt recognition is critical to prevent a fatal outcome (Blair et al. 2015).

We report a case of hyperammonemic encephalopathy due to late-onset *NAGS* deficiency (OMIM 237310) as a consequence of a novel mutation. We also give an overview

Communicated by: Nancy Braverman, M.D., M.Sc.

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of the patients reported in literature up till now with adult-onset NAGS deficiency.

Case Description

A 59-year-old woman, with a medical history of intellectual disability ascribed to perinatal asphyxia, diabetes mellitus type 2, a cerebrovascular accident, and myelodysplastic syndrome, was referred to our hospital because of coma due to hyperammonemia after she was treated conservatively for a fracture of the pelvis. She had been found confused by the home health care after she had been sent home with pain killers (paracetamol, NSAIDs, and morphine). On presentation at the referring hospital her vital signs were stable, but she had a decreased EMV score of E3M4V2. On examination she had isocoric pupils and pareses of both arms and legs. Laboratory examinations showed an elevated ammonia level of 280 μM ($N < 50$), normal liver enzymes, albumin, and coagulation factors (PT, APTT, and Factor V). Blood gas demonstrated respiratory alkalosis PH 7.52, PCO_2 3.7 kPa, PO_2 15.3 kPa, HCO_3 22.3 mmol/L, BE -0.6 mmol/L. Toxicology screening in urine was negative. Because of further deterioration of her EMV score that night, mechanical ventilation was initiated and she was transferred to our ICU. She was treated with high dose glucose intravenously, laxatives, and sodium benzoate. She became fully conscious at an ammonia level of 100 μM and remained stable on the internal medicine ward using a mild protein restriction, laxatives, and sodium benzoate treatment.

Acquired disorders as explanation for the hyperammonemia were excluded: liver failure, portosystemic shunt, infection with urease positive bacteria, urinoma, and bacterial overgrowth. After exclusion of these acquired disorders, the suspicion of a urea cycle defect (UCD) became stronger. Upon a more thorough anamnestic evaluation, patient reported a self-inflicted protein-restricted diet and she did not eat meat. Thirteen years before this presentation she visited a neurologist with complaints of headache, tiredness, dizziness, dyspnea during exercise, and vision disturbances. No clear diagnosis was made at that time and it was considered to be secondary to the mental retardation. Respectively 5 and 8 years before the current admission, the patient was seen at the hospital because of a delirium during infection. Family history was negative: her son did not have any complaints, although he had learning difficulties and drug addiction problems. She has one healthy sister and two brothers who are also healthy. One sister died at birth because of prematurity. Our patient did not have any problems during pregnancy or delivery of her son.

Three days after admission and treatment at the ICU metabolic investigations didn't show any abnormalities suggestive of a UCD; normal levels of glutamine (777 μM , range 463–797), alanine (449 μM , range 150–450), citrulline (33 μM , range 20–46), arginine (52 μM , range 31–117), and ornithine (72 μM , range 53–153) were measured. All acquired causes as explanation for the hyperammonemia were excluded however and the suspicion of a urea cycle disorder was still very strong. After discharge to the general ward metabolic investigations were repeated after an adequate protein load and this demonstrated an elevated glutamine (1,121 μM , range 463–797) and alanine (845 μM , range 150–450) level and a decreased citrulline (11 μM , range 20–46) and arginine level (22 μM , range 31–117), with a normal ornithine level (61 μM , range 53–153). Orotic acid could not be demonstrated in urine as assayed by specific tandem mass spectrometry analysis. DNA investigations were negative for the *OTC* and *CPS1* genes, but revealed a “homozygous” (not proven, parents unavailable for testing) c.603G>C mutation in exon 2 (NM_153006.2:c.603G>C), which mandates a substitution of lysine by asparagine in the protein: p.Lys201Asn. This is a novel mutation in the *NAGS* gene.

Before the DNA diagnosis of NAGS deficiency was established, the patient was treated with protein restriction (0.5 g per kg bodyweight per day) and sodium benzoate. The combination of protein restriction and a diabetes diet can be a real challenge for the dietician and the patient (Grunert et al. 2013). We decided to give her no dietary carbohydrate restriction. She was readmitted a couple of times because of a hyperammonemic crisis. It turned out that she was not capable of keeping her diet and taking the medication on a regular basis living on her own. After she had been transferred to a nursery home her behavior and cognitive functions markedly improved. Her ammonia levels remained stable around 80 μM . After the diagnosis of NAGS deficiency was made, oral *N*-carbamylglutamate (NCG) was started in a low dose 600 mg bid and sodium benzoate was stopped. With this treatment and a protein load of 0.8 g per kg bodyweight per day the ammonia level decreased to 26 μM and she was doing well.

Discussion

Inherited NAGS deficiency is the rarest of UCD, its true incidence is unknown (Ah Mew and Caldovic 2011). This is the first patient in literature in whom the diagnosis of NAGS deficiency is established at such an advanced age. Retrospectively it is very likely that her earlier medical problems such as the intellectual disability, headache, and

delirium during infection were due to metabolic decompensation of the UCD. In a recent review 35 previously reported cases with confirmed NAGS deficiency were presented, including only five patients that were diagnosed in adulthood (age at diagnosis: 20–57 years) (Cartagena et al. 2013) (Table 1).

NAGS Deficiency

NAGS produces *N*-acetylglutamate (NAG) from glutamate and acetyl coenzyme A (Acetyl CoA). NAG is an essential allosteric activator of mitochondrial carbamyl phosphatase 1 (CPS1), the first enzyme of the urea cycle (Caldovic et al. 2004). Hyperammonemia can result once CPS1 is deprived of its cofactor/activator NAG. Deficiencies of NAGS activity can be inherited as in this case. Sometimes they are acquired by secondary inhibition of NAGS activity in conditions which cause short chain fatty acid accumulation such as some organic acidemias and the use of valproic acid (Cartagena et al. 2013). In addition, conditions of compromised acetyl-CoA formation, such as fatty acid oxidation

disorders, lead to a reduced formation of NAG with subsequent lack of CPS1 stimulation.

Clinical Presentation of NAGS Deficiency

The classical presentation for NAGS deficiency is in the first few days of life (Nakamura et al. 2014). The infant usually presents with vomiting after feeding (as a consequence of the protein load) and lethargy, seizures, and coma can follow quickly. Patients with late-onset NAGS deficiency may present with cyclical nausea and vomiting and chronic headaches (Ah Mew and Caldovic 2011). Almost all survivors of a hyperammonemic coma suffer from developmental delay (Cartagena et al. 2013). Most patients self-select a low protein diet. Symptom onset coincides with a precipitating factor such as infection, surgery, psychological stress, excess protein intake, or a trauma as in this case. Laboratory findings in NAGS deficiency include an elevated plasma ammonia, high levels of glutamine and alanine. Plasma citrulline is frequently low or undetectable and urinary orotic acid is not elevated. The

Table 1 Summary of findings in reported adult cases of confirmed *N*-acetylglutamate synthase (NAGS) deficiency

Patient	Diagnosis/onset of symptoms; sex	Presentation	Genotype	Peak ammonia level	Outcome	References
1	Diagnosis 20 years (1.5 years onset of symptoms); male	Confusion, combative behavior	Partial NAGS deficiency. Liver biopsy: NAGS activity <50% control	>100 $\mu\text{mol/l}$	Critical illness polyneuropathy, cerebral dysfunction, and paraplegia	Hinnie et al. (1997)
2	Diagnosis 33 years (27 years onset of symptoms); female	Seizures, coma during pregnancy	L312P/T431I	4,781 $\mu\text{mol/l}$	Not indicated	Grody et al. (1994) Caldovic et al. (2007)
3	Diagnosis 33 years (5 years onset of symptoms); male	Post-operative combativeness, confusion, seizures	V173E/T431I	621 $\mu\text{mol/l}$	Death	Caldovic et al. (2005)
4	Diagnosis 57 years (40 years onset of symptoms); female	Intermittent staring spells, nausea, recurrent vomiting, lethargy, ataxia, migraine headaches, eventually coma	V350I/L442V	500 $\mu\text{mol/l}$	Normal intellect at 57 years	Tuchman et al. (2008)
5	Diagnosis 38 years (20 years onset of symptoms); male	Episodic confusion, nausea and vomiting	E433G/IVS6+5 G>A	434 $\mu\text{mol/l}$	Short term memory loss	Cartagena et al. (2013)
6	Diagnosis 59 years (46 years onset of symptoms); female	Confusion, coma	Exon 2 (c 603 G>C) in Lys201Asn	280 μM	Behavior and cognitive functions markedly improved	

concentrations of other urea cycle intermediates are low-to-normal (Ah Mew and Caldovic 2011).

The timing of metabolic investigation is important, however, because a strict protein restriction (with a high dose of glucose intravenously as in our patient) can result in a false negative result.

Novel Mutation in NAGS Gene

NAGS deficiency is an autosomal recessive disorder and the last urea cycle disorder for which molecular testing became available. In 2002 the *NAGS* gene was cloned, which was found to be located on the long arm of chromosome 17 within band 17q21.31 (Caldovic et al. 2002). There are 23 mutations in the *NAGS* gene published up to date (Ah Mew and Caldovic 2011; Cartagena et al. 2013). Although at present two mutations occurred in more than one family (Thr431Leu and Trp324Ter), there does not appear to be any mutational hot spot in the *NAGS* gene (Ah Mew and Caldovic 2011).

In our patient, an apparently homozygous mutation c.603G>C in exon 2, which mandates a substitution of lysine by asparagine in the protein: p.(Lys201Asn), was found. The mutation is not found in the online HGMD database and also not in the NHLBI exome variant server. The c.603G>C, p.(Lys201Asn) variant/mutation was not detected in 13,000 control alleles (<http://evs.gs.washington.edu/EVS/>). Parents were not available for analysis to prove homozygosity. Theoretically, a deletion on one allele may have escaped detection. Nevertheless, such a genetic constellation would also lead to NAGS deficiency. The mutation p.Lys201Asn lies in the kinase domain and affects an amino acid residue that is not conserved in evolution. A mutation affecting the adjacent p.Cys200Arg mutation was found in another patient with late-onset NAGS deficiency and has been characterized with some residual enzyme activity (Caldovic et al. 2005; Schmidt et al. 2005). We therefore postulate that p.Lys201Asn is also compatible with residual enzyme activity and a late-onset presentation, although there are currently no enzyme data on this. The clinical presentation and the positive reaction on NCG support this. In summary, this mutation acts as a typical late-onset NAGS deficiency causing mutation.

Management of NAGS Deficiency

A hyperammonemic crisis is an emergency situation and stabilization is most important; treatment includes intravenous glucose, ammonia scavengers, and sometimes hemodialysis for ammonia removal. Therapeutic principles for management of NAGS deficiency, as with all UCDs, include minimizing endogenous ammonia production by limiting protein intake and avoiding periods of catabolic stress; administration of urea cycle substrates that are lacking as a

consequence of the enzymatic defect (arginine and citrulline), and administration of compounds that facilitate the removal of ammonia through alternative pathways (sodium benzoate and sodium phenylacetate) (Walker 2009; Cartagena et al. 2013). However NAGS deficiency is the only inherited urea cycle disorder that can be specifically and effectively treated by a drug (Morris et al. 1998). In patients with NAGS deficiency, a 3-day trial of NCG at a dose of 2.2 g/m²/day was demonstrated to restore ureagenesis and normalize blood ammonia, as demonstrated by isotopic studies (Caldovic et al. 2004). NCG activates CPS1, therefore leading to a reduction in ammonia levels and obviates the necessity for a protein restriction.

Conclusion

Hyperammonemia due to urea cycle disorders can also occur at advanced age. It is important for the adult physician to consider a metabolic disorder at every age. Because of growing knowledge and better diagnostic tools this diagnosis will be made more often, especially in our patient population that is still getting older.

Take Home Message

It is important for the adult physician to consider a metabolic disorder at every age; we describe one of the first patients in literature in whom the diagnosis of a UCD is made at such an advanced age.

Conflict of Interest

A vd Logt, L Kuijtmans, M Huigen, and M Janssen declare that they have no conflict of interest

Compliance with Ethics Guidelines

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the patient.

Details of the Contributions of Individual Authors

A vd Logt and MC Janssen wrote the manuscript. All authors interpreted and discussed the results. The manuscript was read and corrected by all authors.

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