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SHORT COMMUNICATION

A novel intronic homozygous mutation in the *AMT* gene of a patient with nonketotic hyperglycinemia and hyperammonemia

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Abstract

Nonketotic Hyperglycinemia is an autosomal recessive disorder characterized by defects in the mitochondrial glycine cleavage system. Most patients present soon after birth with seizures and hypotonia, and infants that survive the newborn period often have profound intellectual disability and intractable seizures. Here we present a case report of a 4-year-old girl with NKH as well as hyperammonemia, an uncommon finding in NKH. Genetic analysis found a previously unreported homozygous mutation (c.878–1 G > A) in the AMT gene. Maximum Entropy Principle modeling predicted that this mutation most likely breaks the splice site at the border of intron 7 and exon 8 of the AMT gene. Treatment with L-Arginine significantly reduced both the proband's glycine and ammonia levels, in turn aiding in control of seizures and mental status. This is the first time the use of L-Arginine is reported to successfully treat elevated glycine levels.

Keywords Glycine encephalopathy · Genetics · Hyperammonemia · L-arginine · Maximum entropy principle modeling

Introduction

Nonketotic hyperglycinemia (NKH) is an autosomal recessive disorder characterized by defects in the mitochondrial glycine cleavage system (GCS). Most patients present soon after birth with seizures and hypotonia, and many do not survive the newborn period (Toone et al. 2003). Infants that do survive have profound intellectual disability and intractable seizures. Mutations in GCS lead to toxic accumulation of glycine in body fluids. The GCS complex is comprised of three enzymes (P, T and L subunits) and one carrier protein (H subunit) located on four separate chromosomes. About 80% of the mutations in NKH are found on the P subunit, and 20% on the T subunit (Toone et al. 2000). Further studies suggest that mutations on the T subunit may be more common than previously thought (Toone et al. 2003; Applegarth and Toone 2004). The P subunit, glycine dehydrogenase (GLDC), catalyzes the removal of carbon dioxide from glycine and attaches the remainder of the glycine molecule to the H subunit. The T subunit, amino methyltransferase (AMT), catalyzes the production of N5, N10-methylene-H4folate and ammonia in the presence of tetrahydrofolate and the H-subunit. The L protein, dihydrolipoamide dehydrogenase (GCSH), oxidizes the lipoic acid-containing H protein, producing NADH, and therefore providing the metabolic energy for further GCS activity (Walker and Oliver 1986; Kikuchi et al. 2008). Considering that the normal reaction mechanism produces ammonia (Fig. 1a), it is reasonable to assume that defects in the GCS are not likely to produce ammonia excess. Absence of hyperammonemia has been noted across a vast spectrum of NKH cases (von Wendt et al. 1978; Kure et al. 1997; Chang et al. 2012; Madu and Oliver 2013; Iqbal et al. 2015; Belcastro et al. 2016). In this report, we describe a patient with neonatal onset NKH due to a homozygous mutation in the *AMT* gene with an unusual occurrence of hyperammonemia.

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Case report

Our patient is a 4-year-old girl with neonatal onset NKH and intractable epilepsy. She was born uneventfully to consanguineous parents but soon developed lethargy, hypotonia and respiratory distress requiring mechanical ventilation.

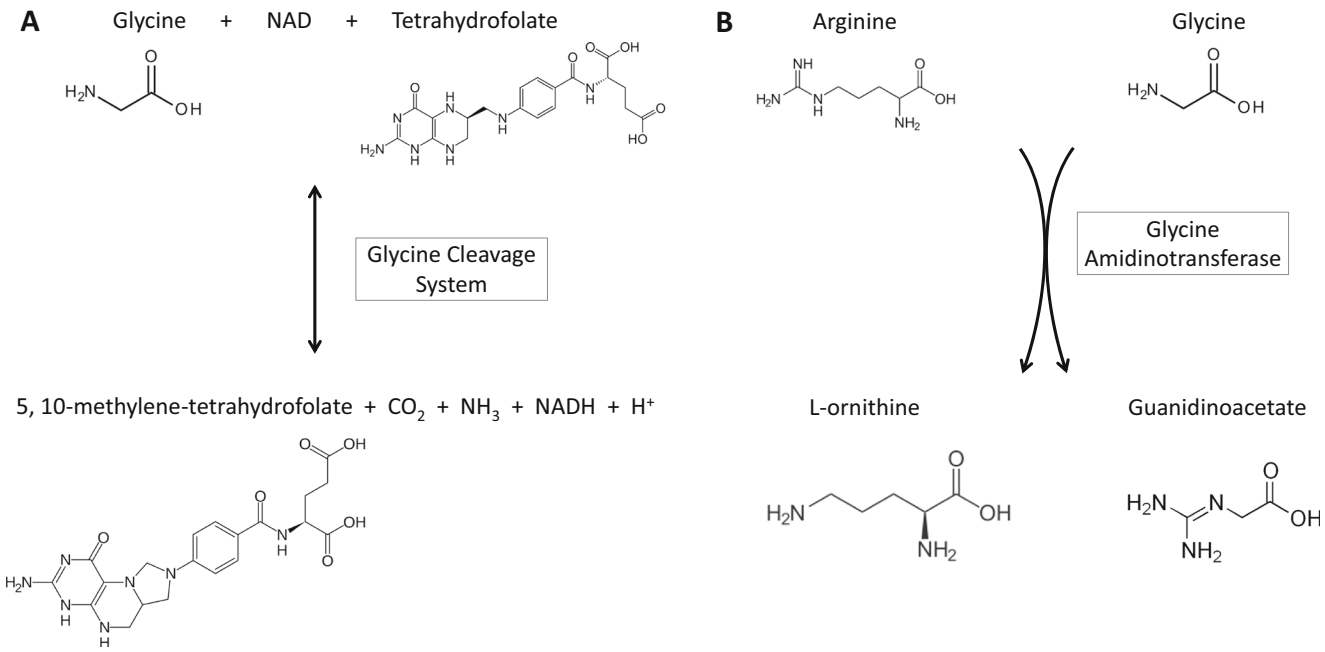
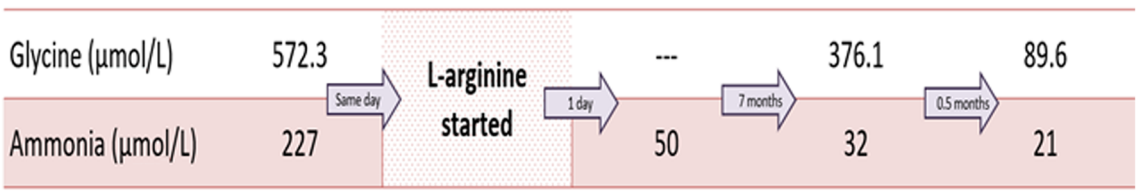


Fig. 1 Breakdown of glycine via the glycine cleavage system under normal circumstances (**a**), and breakdown of glycine, in the presence of L-arginine, via glycine amidinotransferase into L-ornithine and guanidinoacetate (**b**)

Metabolic testing revealed a high CSF glycine (1500 umol/L), an elevated CSF/plasma glycine ratio (0.26) and serum hyperammonemia (70 umol/L) with otherwise normal liver function tests. Periodic laboratory results record persistent hyperammonemia with values of 78, 88 and 64 umol/L throughout the first year, and normal ammonia at 34 umol/L at the third year of life. At four years old, despite standard treatment with sodium benzoate, dextromethorphan, restricted diet, and anti-epileptics, control of her glycine levels and seizure activity was poor. She was hospitalized for worsening seizure frequency and increasing lethargy and noted to have unexplained hyperammonemia of 90 umol/L which increased to 277 umol/L a day later. L-arginine 250 mg/kg/day was started. Serum ammonia and glycine levels were decreased (glycine from 572.3 to 89.6 umol/L; ammonia from 270 to 21 umol/L) (Fig. 2a). Likewise, without adjustments to her anti-epileptics, her seizure control and mental status improved. At five years old, she is unable to track, sit unassisted, smile spontaneously and respond to stimulus. She continues to take L-arginine 250 mg/kg/day in addition to her previous home medications.

a



b

AMT Gene (-1 Position in Intron 7)	Maximum Entropy Consensus Score	Maximum Entropy Variation Threshold Score
Reference/Normal Gene: tctcatgtgtcattctccA**G**gga	9.42	-92.89%
Patient's Gene: tctcatgtgtcattctccA**A**gga	0.67	

Fig. 2 Reduction of glycine and ammonia levels following L-arginine treatment in our patient (**a**), and maximum entropy principle modeling (**b**)

Her ammonia, glycine and seizures have remained better regulated, as assessed with routine serum ammonia and glycine testing.

Genetics

Next-generation gene sequencing of our patient (parents declined testing for themselves) showed a homozygous mutation of NM_000481.3:c.878-1G > A in the *AMT* gene at the −1 position in intron 7 (IVS7), the last base in the splice acceptor site. The same variant in heterozygous form was reported previously in three patients with neonatal onset NKH (Toone et al. 2001, 2003). There are 9 exons in the *AMT* gene (Applegarth and Toone 2001) and mutations at IVS7 lead to a protein product that is missing exon 8 (Toone et al. 2001). Maximum entropy principle (MEP) modeling, a method to predict pathogenicity of splice site mutation, calculates consensus values and variation threshold values (Tsai and Wang 2012). Consensus values predict the probability of a splice site, while variation threshold values predict if mutation to the sequence breaks the splice site (Desmet et al. 2009). The IVS7 mutation, when compared with the wild type sequence, produced a decreased consensus value far below 3 and a decreased variation threshold below 30% (Fig. 2b) (Desmet et al. 2009), strongly suggesting that this mutation deactivates the splice site at intron 7, and can be considered a pathologic mutation (Yeo and Burge 2004; Tsai and Wang 2012). Western blot analysis of three patients with the mutation revealed a translated protein product of 75 kD, while the wild type protein is usually found to be 76 kD (Toone et al. 2001). While some T subunit mutations still allow for residual enzymatic activity (Toone et al. 2003), it is unclear if this is the case with the IVS7 mutation, particularly with the homozygous form as found in our patient.

Discussion

Etiology of persistent, and intermittently worsening, hyperammonemia in our patient is unclear. Only three reports of hyperammonemia and NKH are found in the literature, two of which were transient at birth and one in a case of delayed-onset NKH (Wada et al. 1972; Farriaux et al. 1976; Schiffmann et al. 1992). Information on ammonia levels in previously reported patients with NKH due to compound heterozygous IVS7 mutation is not available. Even if it were available, results may not be generalizable due to differences between the compound heterozygous and homozygous mutations (Toone et al. 2001). Furthermore, to the exclusion of one case, we are not aware of a reported case of NKH, regardless of mutation type, with consistent hyperammonemia (Iqbal et al. 2015; Belcastro et al. 2016). This fits with the proposed model of GCS mechanism and chemical reaction (Walker and Oliver 1986; Kikuchi et al. 2008). The one case that did report consistent hyperammonemia was a delayed onset NKH presenting at 8 months of age, with hyperammonemia starting at 19 months. Liver biopsy and enzyme assay found a deficient GCS pathway but an intact urea cycle. Furthermore, when the patient was given a loading dose of glycine orally, blood ammonia levels rose within three hours, suggesting that the hyperammonemia was secondary to glycine dysfunction (Wada et al. 1972). We hypothesize that high glycine levels due to a defective GCS may be exacerbated due to the potential synthesis of additional glycine by the GCS when the T-subunit is missing or deficient (Walker and Oliver 1986), as it is in our patient. This glycine synthesis is not thought to occur under aerobic physiologic conditions (Kikuchi et al. 2008). It is not known, however, if metabolic stress or anaerobic conditions can induce glycine synthesis aggravating a metabolic crisis. Glycine is derived mainly from serine by serine hydroxymethyltransferase rather than de novo synthesis by reverse reaction of the GCS. NKH patients have normal activity of serine hydroxymethyltransferase, which catalyzes the cleavage of serine to form glycine and N5,N10- methylene-H4folate, despite exceptionally high levels of glycine (Kikuchi et al. 2008).

Elevated ammonia level in our patient might be caused by markedly high glycine levels, the excess of which then gets shunted to other compensatory glycine metabolic pathways (Eagle 1959). However, glycine serum levels can be normal in some cases while CSF is elevated – meaning many cases can be undiagnosed – contributing to a misrepresentation of the true breadth of glycine values (Applegarth and Toone 2001). Therefore, we could not determine if patient's serum glycine levels were significantly higher than the normal for NKH, as no range has been established (only floor cutoff values are known) (Applegarth and Toone 2001). The human T-subunit has been visualized through x-ray crystallography, and known mutations were mapped to the tertiary structure. This analysis showed that most mutations impaired the ability of the active site to hydrogen bond properly with the substrate, thus prohibiting the enzyme from removing the ammonia group and transferring the remaining carbon group to tetrahydrofolate (Okamura-Ikeda et al. 2005). However, the IVS7 mutation was not included in the analysis. L-arginine has a long-established role in the reduction of hyperammonemia due to excess amino acid in body fluids, (Fahey 1957) but it has not been used to reduce glycine levels (Fahey 1957). Addition of L-arginine to our patient's treatment regimen reduced both glycine and ammonia levels, perhaps by reacting with glycine via glycine amidinotransferase to form L-ornithine and guanidinoacetate (Gross et al. 1986) (Fig. 1b).

In conclusion, our case demonstrates that NKH should be considered in neonates presenting with hyperammonemia. Treatment with L-arginine may reduce both ammonia and glycine levels. Further research is needed to elucidate the precise mechanism by which the IVS7 mutation confers pathogenicity, to study the association of hyperammonemia in NKH, to better understand the extent of resulting AMT

protein dysfunction, and to determine if L-arginine is a suitable treatment for NKH.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

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