Identification of Two Novel Mutations in Aminomethyltransferase Gene in Cases of Glycine Encephalopathy

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Abstract

Keywords

- ▶ aminomethyltransferase gene
- ▶ clinical exome
- ▶ nonketotic hyperglycinemia

In this study, we report three cases of nonketotic hyperglycinemia (NKHG) diagnosed biochemically and molecularly. Clinical exome analysis in two families revealed two novel mutations in the aminomethyltransferase (AMT) gene, that is, c.14_15insT (p. Ser6LysfsTer22) and c.259–2A > T, both of them adversely affecting the protein. This is the first report of AMT gene mutations in NKHG from India. Prenatal diagnosis in the first family showed an unaffected fetus in the third pregnancy. The role of AMT protein is pivotal for the synthesis of 5,10-methylene tetrahydrofolate, the first metabolite in one-carbon metabolism that regulates DNA synthesis, repair, and methylation.

Introduction

Nonketotic hyperglycinemia (NKHGG, MIM no. 605899) is a rare autosomal recessive inborn error of glycine metabolism with the accumulation of large quantities of glycine in all the tissues including the brain and hence referred to as glycine encephalopathy, which is a life-threatening metabolic disease.¹ Glycine is not an essential amino acid and is produced from serine and is degraded by means of glycine cleavage system (GCS), a mitochondrial enzymatic complex located in the brain, liver, and placenta, which is composed of four proteins, that is, P, H, T, and L. GCS plays a pivotal role in one-carbon metabolism as it initiates the first step of the pathway. The P-protein (glycine decarboxylase [GLDC] gene, MIM no. 38300) catalyzes decarboxylation of glycine and transfers the amino-methyl group to lipolate on the H-protein (GCS protein H [GCSH] gene, MIM no. 238330). The T-protein (aminomethyltransferase [AMT] gene, MIM no. 238310) releases ammonia and forms 5,10-methylene tetrahydrofolate, after which the reduced lipolate is reoxidized by the L-protein² (– Fig. 1).

Around 72% of NKHG patients are reported to have a causative mutation in *GLDC*, 24% in *AMT* with no muta-

tions reported in *GCSH*.³ In 4% of patients, no mutations are identified and were considered as variant NKHG. Besides being a precursor of multiple compounds, glycine is a brain neurotransmitter. Glycine is both an excitatory neurotransmitter, modulating the N-methyl-D-aspartate (NMDA) receptor in the cerebral cortex, and an inhibitory neurotransmitter acting upon specific receptors in the brainstem and spine. Glycine is a neurotoxin and its accumulation in the plasma and cerebrospinal fluid (CSF) is diagnostic of NKHG. An increased CSF/plasma glycine ratio (> 0.08) is the typical biochemical finding in NKHG.⁴

The neonatal form of NKHG is severe and contributes to 85% of total cases of NKHG and it is the only inborn error that can manifest soon after birth.⁵ Infantile NKHG manifests in two forms, that is, the infantile attenuated form and the infantile severe form, both contributing equally in terms of prevalence (50% each).⁵ The newborn presents usually with lethargy, gross hypotonia, hiccups, and myoclonic jerks leading to apnea and often death. Profound intellectual disability and intractable seizures occur in the surviving infants. NKHG is underdiagnosed due to early neonatal

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Fig. 1 Assembly of glycine cleavage system. The role of four proteins, namely, P, H, T, and L in the glycine cleavage system. The P-protein catalyzes decarboxylation and transfers the aminomethyl group to lipoate on the H-protein. The T-protein adds methylene moiety to tetrahydrofolate to form 5,10-methylene tetrahydrofolate and releases ammonia. The reduced lipoate is reoxidized by the L-protein.

death. The infantile form presents with developmental delay, seizures, hyperactivity, and chorea.⁶

The worldwide incidence of NKHG is estimated to be approximately 1 in 250,000 births.⁷ A very high frequency of NKHG was reported in Finland with an estimated incidence of 1 in 55,000 newborns overall, rising to 1 in 12,000 in northern Finland.⁸ However, no prevalence data are available from India. Herein, we report the clinical and biochemical findings in three patients and genetic mutations in two of the three cases of NKHG from South India.

Patients and Methods

Case 1

A 6-day-old female child born of a consanguineous marriage (1st cousin) presented with gross hypotonia in all the limbs, encephalopathy, and seizures. Baby, second in birth order, was full-term and delivered by cesarean section. The birth weight was 2.9 kg with no hypoxia. The neonate was lethargic since birth with poor feeding and sluggish neonatal reflexes. She developed seizures on the third day of life. Clinical evaluation revealed gross generalized hypotonia. Transcranial magnetic stimulation (TMS) study revealed normal acylcarnitine profile with high glycine level (1,080 μ M). Plasma and CSF amino acid analysis were suggestive of NKHG (**→ Fig. 2**) and the neonate expired on the ninth day of life. No genetic analysis was done in the proband and parents came for genetic counseling in the next pregnancy. The Guthrie card was retrieved from the newborn screening laboratory, and mutation analysis was performed by exome sequencing. A homozygous frameshift mutation in *AMT* gene, that is, c.14_15insT (p.Ser6LysfsTer22), was identified in this

proband. Parents were heterozygous carriers for the *AMT* mutation (► **Fig. 3**). Prenatal diagnosis was done in the third pregnancy and the fetus was unaffected.

Case 2

A 10-day-old neonate, first child, born to consanguineous parents (first cousin), was admitted with seizures, encephalopathy, and gross hypotonia. Newborn screening identified high glycine (1,867 μM) in the dried blood spot which was confirmed by quantitative amino acid analysis both in the plasma and CSF, wherein CSF/plasma glycine ratio was found to be 0.9. The neonate expired on the 12th day of life. No mutation analysis could be performed as the family was lost to follow-up.

Case 3

An 8-month-old male child born of a consanguineous marriage (first cousin) was seen in the pediatric neurology department for epileptic spasms, swallowing difficulty, excessive drowsiness, spasticity, exaggerated Mongolian spots, and *Klebsiella pneumoniae* sepsis. He was treated for infantile epilepsy. The child manifested symptoms from the second day of life with neonatal encephalopathy and hypotonia. Seizures started from the second month of life and became intractable at 6 months of age. TMS testing was done and glycine was found to be high (1,276 μ M). Genetic analysis revealed c.259-2A > T homozygous mutation in the AMT gene (**Fig. 4**). At 8 months of age, the child was referred to the metabolic specialist in view of uncontrolled seizure activity. Serum lactate and blood ammonia levels were normal. The child was started on sodium benzoate, dextromethorphan, along with antiepileptic medication. A repeat TMS after 30 days revealed a glycine level of 849 nmol/mL.

Fig. 2 Plasma and cerebrospinal fluid (CSF) glycine levels in a normal control and nonketotic hyperglycinemia. (A) Normal control glycine levels in plasma and CSF were 302 and 3.39 $\mu\text{mol/L}$, respectively, with CSF/plasma glycine ratio being 0.01. (B) Nonketotic hyperglycinemia case glycine levels in the plasma and CSF were 1,454 and 206.8 $\mu\text{mol/L}$, respectively, with CSF/plasma glycine ratio being 0.14.

Parents were carriers for the *AMT* gene. At 8 months of age, the child was grossly delayed in development.

Results

In all the three cases, plasma and CSF glycine levels were high with an increased ratio of CSF/plasma glycine > 0.8 . Clinical manifestations occurred in the neonatal period in all the three

cases with fatal outcome in two neonates. The surviving neonate developed seizures at 2 months of age and was treated with antiepileptic medication. Treatment with sodium benzoate, dextromethorphan, along with antiepileptic drugs was initiated with little response. A homozygous single base pair insertion in exon 1 of the *AMT* gene resulting in a frameshift and premature truncation of the protein 22 amino acids downstream to codon 6, that is, p.Ser6LysfsTer22, was

Fig. 3 Sequence chromatogram and alignment to the reference sequence showing the variation in exon 1 of aminomethyltransferase (*AMT*) gene. *AMT* (chr3:49459869_49459870insA; c.14_15insT; p.Ser6LysfsTer22) detected in heterozygous condition in the parents of index patient, father (A) and mother (B).

Fig. 4 Integrative Genomics Viewer (IGV) file depicting homozygous aminomethyltransferase (AMT) c.259-2A > T mutation in the AMT gene in the proband. A homozygous 3' splice site variation in intron 2 of the AMT gene (chr3:49459007; T > A; Depth: 216 x) that affects the invariant AG acceptor splice site upstream of exon 3 (c.259-2A > T; ENST00000273588) was detected.

detected in case 1. Carrier testing of the parents revealed both were asymptomatic carriers. A homozygous 3' splice site variation in intron 2 of the AMT gene that affects the variant AG-acceptor splice site upstream of exon 3 (c.259-2A > T) was detected in case 3. The AMT variant has not been reported in the 1000 Genomes database⁹ and has a minor allele frequency of 0.0008% in the Exome Aggregation Consortium database.¹⁰ This mutation was found to be damaging based on in silico prediction of MutationTaster2.¹¹ The reference base is con-

served across species. Both the parents were carriers for the mutation.

The clinical variation database (<https://www.ncbi.nlm.nih.gov/clinvar/>) described several mutations in AMT gene (► **Supplementary Table S1**, available in online version only).^{12,13} However, among them, 25 (14 exonic and 3 intronic single nucleotide variants, 7 deletions, and 1 indel) are reported either to be pathogenic or likely pathogenic and are associated with NKHG (► **Fig. 5**).

Fig. 5 Pathogenic and likely pathogenic mutations reported in the aminomethyltransferase (AMT) gene. Location and type of mutations across the AMT gene documented in the ClinVar database of [ncbi.nlm.nih.gov](https://www.ncbi.nlm.nih.gov/clinvar/): 14 exonic, 3 intronic single-nucleotide variations (SNVs), 7 deletions, and 1 indel. On the top, two novel mutations reported by us were indicated based on their location in the AMT gene.

Genotype–phenotype correlations between reported AMT versus GLDC mutations showed a lower incidence of hiccups (odds ratio: 0.06, 95% confidence interval: 0.003–0.79, $p = 0.03$) in AMT mutants compared with GLDC mutants, whereas both mutations are associated with hypotonia, poor sucking, apnea, and seizures.

Discussion

Nonketotic hyperglycinemia mostly presents in the neonatal period and develops into progressive encephalopathy within the first 6 hours to 8 days of birth. Neonate presenting with symptoms of lethargy, hiccups, and poor sucking associated with gross hypotonia, apnea, myoclonic seizures, and coma, glycine encephalopathy should be suspected. Most of the mutations are missense/nonsense mutations (63.11%), the rest are gross deletions (15.57%), splicing mutations (9.02%), small deletions (8.20%), small insertions (3.28%), and small indels (0.82%).⁸ De novo mutations occur in approximately 1% of individuals with NKHG.⁵

There are very few case reports from India, out of which in only one case mutation analysis was reported harboring a novel mutation, c.2296G > T (p.Gly766Cys), in exon 19 of the *GLDC* gene.^{14,15} Our report is the first to describe *AMT* gene mutations in two families. Both are novel mutations, one is a splicing mutation of c.259–2A > T in intron2 of the *AMT* gene and a single base pair insertion in exon 1 of the *AMT* gene resulting in a frameshift and premature truncation of the protein. Both the mutations have not been reported in the Human Gene Mutation Database. The splice mutation reported by us is adjacent to already reported *AMT* mutation, c.259–1G > C.¹⁶

In this article, we report an insertion frameshift mutation and a splice site mutation which is novel and pathogenic. Genotype–phenotype correlations between reported AMT versus GLDC mutations showed a lower incidence of hiccups in AMT mutants compared with GLDC mutants, whereas both mutations are associated with hypotonia, poor sucking, apnea, and seizures. This is consistent with the current study showing the absence of hiccups in both the patients with AMT mutation.

The *AMT* gene has been localized to 3p21.2–p21.1 and is approximately 6 kb in length with nine exons. The 5′-flanking region of the gene lacks typical TATAA sequence by fluorescence in situ hybridization.¹⁷ The 1,209 base pair open reading frame encodes 403 amino acid precursor protein, and the deduced amino acid sequence of the mature peptide show 90 and 68% homology to that of bovine and chicken counterpart, respectively.¹⁸ The protein encoded by AMT catalyzes the release of ammonia and the transfer of a methylene carbon unit to tetrahydrofolate. In case 1, a stop codon mutation is present and therefore the synthesis of T-protein stops leading to T-protein deficiency. T-protein (a tetrahydrofolate-requiring enzyme) is a component of the GCS. Because of T-protein deficiency, glycine accumulates in the central nervous system, predominantly in the brain stem and spinal cord. Excess glycine is agonistic

to glutamate of the excitatory NMDAR, which accounts for seizures and long-term neurologic defects. Treatment is aimed to reduce plasma concentration of glycine with sodium benzoate and blocking of glycinergic receptors, most commonly at the NMDAR site with dextromethorphan, ketamine, and felbamate. However, in case 3, a limited response was seen with these medications. Presently, the child is on the ketogenic diet. Prognosis for neonatal NKHG is uniformly poor. Those who survive are left with serious neurological sequelae. In a study of 124 patients, 26 patients (21%) died in the neonatal or early infantile period while 56 patients (45%) had severe disease.¹⁹ In our case 3, the child is now 12 months old with global developmental delay.

To conclude, in view of the severity of NKHG and its associated high mortality rate, genetic counseling is extremely important in these families. Identifying the disease-causing mutations in index cases is important along with carrier testing of parents and at-risk relatives for prenatal testing. In case 1, we could offer prenatal testing in the next pregnancy and the fetus is unaffected.

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