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Two Novel Missense Mutations in Nonketotic Hyperglycinemia

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Abstract

Nonketotic hyperglycinemia (OMIM no. 605899) is an autosomal recessively inherited glycine encephalopathy, caused by a deficiency in the mitochondrial glycine cleavage system. Here we report 2 neonates who were admitted to the hospital with complaints of respiratory failure and myoclonic seizures with an elevated cerebrospinal fluid/plasma glycine ratio and diagnosed as nonketotic hyperglycinemia. We report these cases as 2 novel homozygous mutations; a missense mutation c.593A>T (p.D198 V) in the glycine decarboxylase gene and a splicing mutation c.339G>A (Q113Q) in the aminomethyltransferase gene were detected. We would like to emphasize the genetic difference of our region in inherited metabolic diseases once again.

Keywords

nonketotic hyperglycinemia, novel, mutation

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Nonketotic hyperglycinemia (OMIM no. 605899) is a devastating autosomal recessive disorder of glycine metabolism.[1,2] It results in the accumulation of large amounts of glycine in body fluids and severe neurologic dysfunction.[2] Most patients have the neonatal form, presenting in the first few days of life with lethargy, hypotonia, intractable seizures, and severe mental retardation in the following months.[3] The infantile form presents with seizures and varying degrees of mental retardation after a symptom-free period of up to 6 months.[3] In the late-onset form, patients may present either in childhood or in adulthood with progressive spastic diplegia and optic atrophy, although intellectual function is preserved and seizures are not reported.[3] Such patients tend to have a normal life span.[3] The disease is caused by a defect in the mitochondrial glycine cleavage system.[2,4] Nonketotic hyperglycinemia is diagnosed biochemically. A ratio of cerebrospinal fluid/plasma glycine equal to 0.08 or more is consistent with typical nonketotic hyperglycinemia.[5] Diagnosis should be confirmed by genetic studies as mutations within the glycine cleavage system genes.[3] Prenatal diagnosis by glycine cleavage system enzymatic assay in chorionic villus biopsies is not completely reliable and will be replaced by mutation analysis.

The glycine cleavage system has 3 subunits: P protein (also known as glycine decarboxylase), T protein (aminomethyltransferase), and H protein (glycine cleavage system H protein), which are encoded by the *GLDC* (glycine decarboxylase), *AMT* (aminomethyltransferase), and *GCSH* (glycine cleavage system H protein) genes, respectively.[6] Mutations in the *GLDC* gene are responsible for 70% of nonketotic hyperglycinemia cases, whereas mutations in the *AMT* and *GCSH* genes account for 20% and <1% of nonketotic hyperglycinemia cases, respectively.[6] We report 2 novel mutations, one in the glycine decarboxylase and the other in the aminomethyltransferase gene, in 2 neonates with nonketotic hyperglycinemia.

Case Summary

Patient 1

A male infant born to a Turkish consanguineous couple was hospitalized in a neonatal intensive care unit for poor feeding and decreased activity on the first day of life. Same couple had a history of a death of their offspring at the age of 8 months with progressive neurologic deterioration. The neonate became lethargic, with generalized muscular hypotonia, a weak suck, absent deep tendon reflexes and insufficient respiratory drive, which mandated intubation and mechanical ventilation. He was

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Table 1. Cerebrospinal Fluid and Plasma Glycine Values of Both Patients.

	Patient 1	Patient 2	Normal
Glycine (plasma), μmol/mL	743	732	20-400
Glycine (CSF), μmol/mL	113	215	3-21
Plasma/CSF glycine ratio	0.15	0.29	<0.08

Abbreviation: CSF, cerebrospinal fluid.

hospitalized for 50 days. From the age of 4 months, progressive myoclonic seizures were observed. Routine laboratory findings, involving blood glucose, serum ammonia, and blood count, were normal. Electroencephalography (EEG) indicated a continuous burst suppression pattern. Magnetic resonance imaging (MRI) demonstrates the loss of white matter, abnormal signal intensity of the deep white matter, ventriculomegaly, and thinning of the posterior body of the corpus callosum. Cerebrospinal fluid study indicated a normal leukocyte count and protein and glucose levels. The culturing and viral examination of cerebrospinal fluid produced negative results. Cerebrospinal fluid/plasma glycine ratio was 0.15 (normal < 0.08) (Table 1). Thus, the analysis of amino acids in the cerebrospinal fluid and plasma along with the typical EEG pattern prompted a diagnosis of nonketotic hyperglycinemia.

The diagnosis was confirmed by direct sequencing of all exons and intron-exon boundaries of *GLDC*, *AMT*, and *GCSH* using DNA extracted from leukocytes. Sequence analysis was done with Sanger method by ABI 3130 capillary electrophoresis system. The child was homozygous for a sequence modification: the substitution of an adenine by a thymine at position 593 on the c.DNA (c.593A>T, p.D198 V) in the *GLDC* gene. His parents were shown to be heterozygous carriers for this mutation.

This mutation was not given in the public version of the Human Gene Mutation Database and PubMed. Results of in silico analysis of this mutation were as follows: SIFT = 0; Mutation Taster: disease causing (simple_aae, prob: 0.999999999999996); and Polyhen2: probably damaging. These data strongly suggest that this is a disease-causing mutation. As functional analysis was not done, further studies and patients are needed.

Patient 2

A female infant, offspring of a Turkish-origin consanguineous couple, was hospitalized in a neonatal intensive care unit for lethargy and cyanosis in the first day of life. A previous male sibling died on the seventh day of life, after presenting with lethargy and respiratory insufficiency. Sudden onset of general convulsions accompanied by increased heart rate and oxygen desaturation appeared 8 hours after birth. Because of the insufficient respiratory drive, she was intubated and mechanically ventilated. Progressively, overt myoclonic seizures appeared. Routine laboratory tests, including blood glucose, transaminases, serum ammonia, blood gas, C-reactive protein, and blood count were unremarkable. Urine organic acid examination and blood liquid chromatography–mass spectrometry were unremarkable. Seizure frequency increased even under multiple antiepileptic drug treatment and pyridoxine challenge. Conventional EEG indicated a continuous burst suppression pattern. Cerebral MRI demonstrated hypomyelination in both cerebral hemispheres. Because of the intractable seizures, a diagnosis of nonketotic hyperglycinemia was suspected and cerebrospinal fluid examination was performed. Her cerebrospinal fluid/plasma glycine ratio was 0.29 (normal <0.08) (Table 1). Thus, the analysis of amino acids in cerebrospinal fluid and plasma with typical clinical and EEG features led to the diagnosis of nonketotic hyperglycinemia.

The diagnosis of nonketotic hyperglycinemia was then further supported by direct sequencing of all exons and intron-exon boundaries of *GLDC*, *AMT*, and *GCSH* genes using DNA extracted from leukocytes. Sequence analysis was done with an Illumina MISEQ next-generation sequencing system. The child was homozygous for a sequence modification: the substitution of a guanine by adenine at position 339 on the c.DNA (c.339G>A, p.Q113Q) in the *AMT* gene (Figure 1). Her parents were shown to be heterozygous carriers for this mutation. This mutation was not listed in the public version of the Human Gene Mutation Database and PubMed. This mutation does not change the amino acid but it changes the last nucleotide of the third exon and thus predicted to cause a splicing defect. Mutation Taster and Human Splicing Finder evaluation supports this mechanism.

Discussion

The pathogenesis of nonketotic hyperglycinemia is related to central nervous system glycine accumulation as a result of glycine cleavage system deficiency.[7] Glycine leads to overstimulation of inhibitory glycine receptors located predominantly in the brain stem and spinal cord, accounting for the hypotonia and apnea. Glycine is also an obligatory coagonist with glutamate of the excitatory *N*-methyl-D-aspartate receptor (NMDAR).[7] Excess glycine could account for seizures and long-term neurologic defects by leading to overactivation of NMDARs and excitotoxic neuronal damage or by overstimulation of additional glycinergic receptors.[7]

The glycine cleavage system is a multienzyme complex of 3 components: P protein (a pyridoxal phosphate–dependent glycine decarboxylase), H protein (a lipoic acid–containing protein), and T protein (a tetrahydrofolate-requiring enzyme) specific to the glycine cleavage system. These proteins are, respectively, encoded by the *GLDC*, *GCSH*, and *AMT* genes.[8]

Currently, in approximately 70% of nonketotic hyperglycinemia cases, pathogenic variants are in the *GLDC* gene, with no mutational hotspots. Missense mutations comprise 90% of the variants, the others being small-scale insertions/deletions, larger-scale deletions, and mutations that affect splicing.[6,9] More than 150 mutations are associated with nonketotic hyperglycinemia.[2] Kure et al[10] screened 69 nonketotic hyperglycinemia patients examined in different metabolic disease clinics of referring hospitals. *GLDC* or *AMT* mutations were identified in 75% of neonatal and 83% of infantile families, but not in

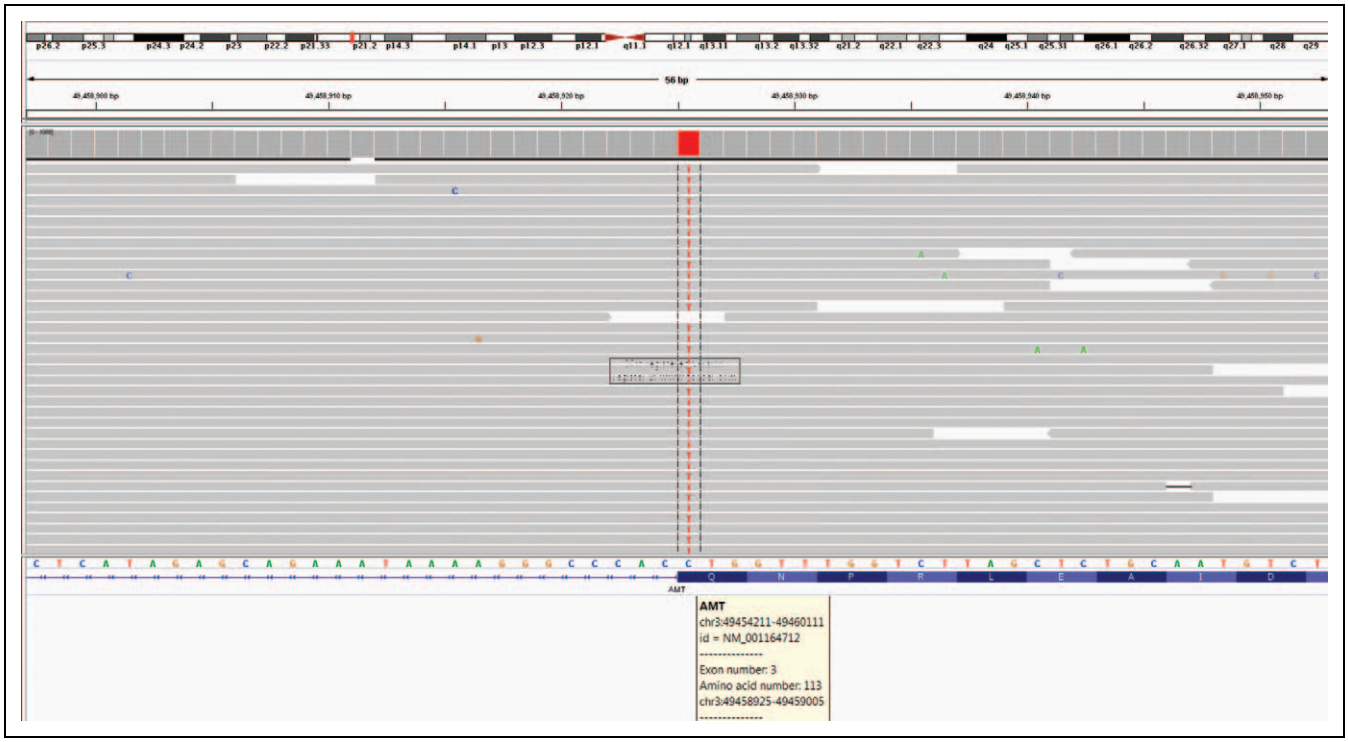

Figure 1. Aminomethyltransferase gene mutation (c.339G>A, p.Q113Q).

late-onset-type nonketotic hyperglycinemia. No *GCSH* mutation was identified in their study.

Different missense mutations in the *GLDC* gene were reported as a cause of neonatal form of nonketotic hyperglycinemia as in patient 1. Chang et al[11] reported a newborn with the classic neonatal form of nonketotic hyperglycinemia. He had a typical presentation of frequent hiccups and myoclonic movements since birth.[11] Genetic analysis demonstrated a mutant allele with a single substitution at nucleotide c.1111C>G in the *GLDC* gene inherited from his mother, resulting in a histidine-to-aspartic acid change at amino acid position 371 (p. His371Asp mutation) in the gene product.[11] The other allele of the *GLDC* gene was deleted, a mutation inherited from the father.[11] Two siblings diagnosed as neonatal nonketotic hyperglycinemia was reported by Korman et al.[7] A novel homozygous *GLDC* c.482A>G(Y161C) missense mutation was identified.[7] Neonatal hypotonia and apnea did not occur but the long-term outcome was poor, with intractable seizures and severe psychomotor retardation.[7]

Mutant proteins encoded by the *AMT* gene were also reported as a cause of neonatal nonketotic hyperglycinemia as in Patient 2. Toone et al[12] described patients from 3 unrelated families with classical symptoms of neonatal nonketotic hyperglycinemia who are heterozygous for a novel splicing mutation (IVS7-1G>A) in the *AMT* gene. Kure et al[13] reported a missense mutation in exon 2 in the *AMT* gene that resulted in an amino acid substitution from histidine to arginine at position 42(H42R). They a found a large Israeli-Arab kindred with nonketotic hyperglycinemia.[13] Fourteen children were affected, and all of the patients had seizures and respiratory failure within 2 days after birth.[13]

Unfortunately, because most reported mutations seem to be rare or private, it is very difficult to predict phenotype from genotype.[14]

In conclusion, we report 2 novel mutations both in the *GLDC* and *AMT* genes in 2 consanguineous Turkish couples. Metabolic studies of suspected patients with molecular analysis can confirm a diagnosis of inherited metabolic diseases such as nonketotic hyperglycinemia, support genetic counseling, and prenatal diagnosis.

Author Contributions

BSY evaluated the patients clinically and wrote the manuscript. DK worked on clinical evaluation. GGM and FI performed the neurologic studies. EK supervised the biochemical studies. SC supervised the molecular studies and assisted in writing the manuscript. NOM acted as the lead clinician in the study, counseled the parents, and assisted in writing the manuscript.

Declaration of Conflicting Interests

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Ethical Approval

As a case report, this project was not submitted for ethics committee approval. Consent from the parents of the infants who are described here were obtained for publication of the case report.

References

1. Childs B, Nyhan WL, Borden M, Bard L, Cooke RE. Idiopathic hyperglycinemia and hyperglycinuria: a new disorder of amino acid metabolism. *Pediatrics*. 1961;27:522-538.
2. Yoon IA, Lee MN, Yoo BH, et al. Two novel missense mutations observed in nonketotic hyperglycinemia. *Pediatr Neurol*. 2012; 46:401-403.
3. Dinopoulos A, Matsubara Y, Kure S. Atypical variants of nonketotic hyperglycinemia. *Mol Genet Metab*. 2005;86:61-69.
4. Kure S, Rolland MO, Leisti J, et al. Prenatal diagnosis of nonketotic hyperglycinaemia: enzymatic diagnosis in 28 families and DNA diagnosis detecting prevalent Finnish and Israeli–Arab mutations. *Prenat Diagn*. 1999;19:717-720.
5. Verrisimo C, Garcia P, Simoes M, et al. Nonketotic hyperglycinemia: a cause of encephalopathy in children. *J Child Neurol*. 2013; 28:251-254.
6. Love MJ, Prosser D, Love RD, et al. A novel glycine decarboxylase gene mutation in an Indian family with nonketotic hyperglycinemia. *J Child Neurol*. 2014;29:122-127.
7. Korman HS, Wexler DI, Gutman A, et al. Treatment from birth of nonketotic hyperglycinemia due to a novel *GLDC* mutation. *Ann Neurol*. 2006;59:411-415.
8. Terek D, Altun Koroglu O, Gunes S, et al. Diagnostic tools of metabolic and structural brain disturbances in neonatal nonketotic hyperglycinemia. *Pediatr Int*. 2012;54:717-720.
9. Kanno J, Hutchin T, Kamada F, et al. Genomic deletion within *GLDC* is a major cause of non-ketotic hyperglycinaemia. *J Med Genet*. 2007;44:69.
10. Kure S, Kato K, Dinopoulos A, et al. Comprehensive mutation analysis of *GLDC*, *AMT*, and *GCSH* in nonketotic hyperglycinemia. *Hum Mutat*. 2006;27:343-352.
11. Chang CY, Lin SP, Lin HY, et al. Non-ketotic hyperglycinemia with a novel *GLDC* mutation in a Taiwanese child. *Acta Paediatr Taiwan*. 2008;49:35-37.
12. Toone JR, Applegarth DA, Coulter-Mackie MB, James ER. Identification of the first reported splice site mutation (IVS7-1G>A) in the aminomethyltransferase (T-protein) gene (*AMT*) of the glycine cleavage complex in 3 unrelated families with nonketotic hyperglycinemia. *Hum Mutat*. 2001;17:76.
13. Kure S, Mandel H, Rolland MO, et al. A missense mutation (His42Arg) in the T-protein gene from a large Israeli-Arab kindred with nonketotic hyperglycinemia. *Hum Genet*. 1998;102:430-434.
14. Applegarth DA, Toone JR. Glycine encephalopathy (nonketotic hyperglycinaemia): review and update. *J Inherit Metab Dis*. 2004;27:417-422.