REVIEW

DOI: 10.4274/imdn.galenos.2026.2026-3

Inherit Metab Disord Nutr 2026;2:38–52

Current Perspectives on Non-ketotic Hyperglycinemia

Hatice Güneş

University of Health Sciences Türkiye, İzmir Faculty of Medicine, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Department of Pediatric Metabolism, İzmir, Türkiye

Abstract

Non-ketotic hyperglycinemia (NKH) is a rare autosomal recessive neurometabolic disorder caused by defects in the glycine cleavage system, leading to glycine accumulation in plasma and cerebrospinal fluid (CSF) and a severe early-onset encephalopathy. This review summarizes current knowledge of NKH pathophysiology, clinical spectrum, diagnostic strategies, treatment options, prognostic factors, differential diagnosis, and future perspectives. Diagnostic evaluation relies on detecting elevated CSF and plasma glycine with an increased CSF/plasma glycine ratio, confirming genetic variants in *GLDC* and *AMT*, and, when available, functional studies. Therapeutic strategies remain largely symptomatic and palliative. Current treatments aim to reduce glycine levels and modulate N-methyl-D-aspartate (NMDA) receptor overactivation, mainly using sodium benzoate, dextromethorphan, and other NMDA receptor antagonists, combined with a ketogenic diet and intensive supportive care and rehabilitation. The prognosis of classic NKH is generally poor.

Keywords: AMT, glycine encephalopathy, glycine cleavage system, GLDC, ketogenic diet, non-ketotic hyperglycinemia, neonatal encephalopathy

INTRODUCTION

Non-ketotic hyperglycinemia (NKH) is a rare autosomal recessive neurometabolic disorder caused by defective activity of the mitochondrial glycine cleavage system (GCS), resulting in accumulation of glycine in plasma and cerebrospinal fluid (CSF) without ketosis. Elevated glycine levels lead to profound central nervous system dysfunction, particularly during the neonatal period.[1]

The estimated incidence of NKH varies geographically, but is generally reported as 1:76,000 live births.[2-4] It is more prevalent among individuals of Finnish descent.[5] The classic neonatal form is the most severe phenotype, characterized by hypotonia, lethargy, intractable seizures, and respiratory failure. Attenuated forms of NKH, presenting with later onset and milder neurological symptoms, have also been reported, underscoring the considerable phenotypic variability observed in this disorder.

Despite advances in molecular diagnostics and supportive care, the prognosis remains poor for most patients with classic NKH. Current therapies are largely symptomatic and aim to reduce glycine levels or attenuate modulate N-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity. However, emerging molecular approaches are under investigation and may offer future therapeutic potential.

This review provides a comprehensive overview of the pathophysiology, genetic basis, clinical spectrum, diagnostic evaluation, and management strategies for NKH.

GLYCINE METABOLISM AND PATHOPHYSIOLOGY

Glycine is a non-essential amino acid synthesized in the body from serine and threonine. It participates in numerous physiological reactions and primarily serves as a neurotransmitter in the brain. In the brain, glycine acts as an excitatory neurotransmitter via the NMDA receptor, which is involved in learning and memory, and as an inhibitory neurotransmitter via the glycine receptor (GlyR), which helps regulate motor and sensory functions in the brainstem and spinal cord. The GCS catabolizes glycine.[6] The dual role of glycine as an excitatory and inhibitory neurotransmitter is crucial for maintaining the balance of neural activity, and

Address for Correspondence: University of Health Sciences Türkiye, İzmir Faculty of Medicine, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital, Department of Pediatric Metabolism, İzmir, Türkiye

E-mail: drhaticegunes82@gmail.com **ORCID ID:** orcid.org/0000-0002-6940-0964

Received: 01.04.2026 **Accepted:** 13.10.2026 **Publication Date:** 03.11.2026

Cite this article as: Güneş H. Current perspectives on non-ketotic hyperglycinemia. Inherit Metab Disord Nutr. 2026;2:38–52

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Child Nutrition Metabolism Association.
This is an open access article under the Creative Commons AttributionNonCommercial 4.0 International (CC BY-NC 4.0) License.

disruptions in glycine signaling have been linked to neurological disorders such as epilepsy and schizophrenia.[7,8]

The pathophysiological process of the disease likely begins early in utero; therefore, irreversible glycine-induced brain damage has probably already occurred by the time the patient presents with symptoms. GlyRs regulate distinct processes during both prenatal and postnatal brain development. During embryonic development, studies indicate that GlyRs help regulate neurotransmitter release and cell migration. Postnatally, GlyRs initially mediate excitation and promote neurotransmitter release and synaptic communication. Later, GlyRs function as inhibitory receptors and contribute to synaptic inhibition. The importance of GCS in neurogenesis and morphogenesis is supported by the presence of congenital malformations such as corpus callosum dysgenesis in NKH.[6,7]

Recent NKH models, including mouse and zebrafish models and human induced pluripotent stem cell (iPSC) systems, have elucidated complementary aspects of disease pathophysiology. Hypomorphic and *GLDC-deficient* mouse models reproduce glycine accumulation, impaired folate-mediated one-carbon metabolism, altered serine balance, hydrocephalus, electroencephalogram (EEG) abnormalities, and energy-metabolism defects and demonstrate that AAV9-mediated *GLDC* gene therapy can normalize glycine levels and restore folate profiles. Zebrafish models extend the phenotypic spectrum to include craniofacial and renal developmental abnormalities, and human iPSC-derived astrocytes reveal metabolic rewiring accompanied by altered glial differentiation. Additional rat and oligodendroglial models link glycine exposure to myelin disruption, glutamatergic imbalance, and oxidative stress. Together, these model systems show that NKH involves glycine toxicity, disrupted one-carbon and serine metabolism, impaired energy regulation, and glial-white matter vulnerability.[9-14]

GCS and One-Carbon Metabolism

The GCS is a four-protein complex consisting of the P-, T-, H-, and L-proteins, with the H-protein bearing a lipoyl group that shuttles reaction intermediates.[7,8] The P-protein, encoded by *GLDC*, initiates glycine decarboxylation and transfers the aminomethyl group to the H-protein. The P-protein, encoded by *GLDC*, initiates glycine decarboxylation and transfers the aminomethyl group to the H-protein. The T-protein, encoded by *AMT*, transfers the resulting one-carbon unit to tetrahydrofolate, generating 5,10-methylenetetrahydrofolate, whereas the L-protein regenerates the oxidized form of the H-protein. Thus, rather than functioning solely as a glycine-degrading pathway, the GCS supplies one-carbon units that contribute to folate-dependent cellular metabolism.[7]

The interaction among glycine degradation, serine metabolism, and folate-mediated one-carbon metabolism is summarized in Figure 1. GCS-mediated glycine cleavage generates 5,10-methylenetetrahydrofolate, whereas serine hydroxymethyltransferase catalyzes the reversible interconversion of serine and glycine using this one-carbon pool. Consequently, impaired GCS activity may affect not only glycine

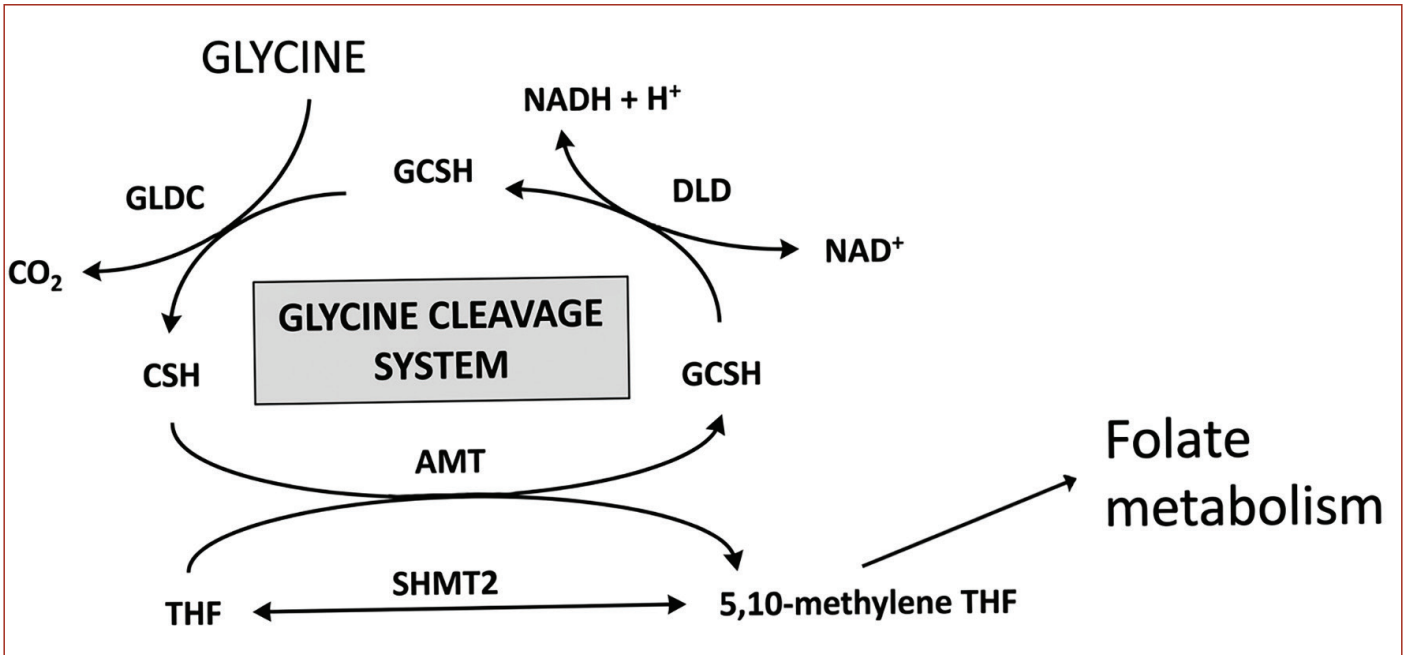


Figure 1. Glycine cleavage system.

homeostasis but also the availability of one-carbon units and serine.[15-17]

Metabolic Consequences of GCS Deficiency

Reduced GCS activity causes glycine accumulation and may also compromise folate-bound one-carbon metabolism. In *GLDC-deficient* mouse models, abnormal folate profiles, reduced levels of one-carbon-carrying folates, growth impairment, and developmental abnormalities have been reported. Formate supplementation partially restored folate balance, improved embryonic growth, and prevented neural tube defects, supporting a functional link between impaired GCS-derived one-carbon supply and abnormal development.[12]

Recent metabolic profiling further demonstrates that GLDC deficiency is associated with elevated cortical glycine, reduced folate one-carbon charging, secondary reduction in methionine, and decreased levels of alternative one-carbon donors such as L-serine and formate. Region-specific decreases in D-serine and alterations in sphingolipid species suggest effects on myelination and neuronal support.[13] Together, findings from animal and cellular models indicate that NKH pathophysiology extends beyond isolated glycine accumulation to involve interconnected disturbances in glycine, serine, and one-carbon metabolism, with potential consequences for astrocyte maturation, glial function, and neuronal homeostasis.[13]

Mechanisms of Neurological Injury

The neurological manifestations of NKH likely result from the combined effects of excessive glycine signaling, metabolic disturbances, and abnormal neurodevelopment rather than from a single pathogenic mechanism. Elevated glycine levels are proposed to bind to and overactivate NMDA receptors, disrupting glutamatergic neurotransmission and increasing Ca^{2+} influx into the postsynaptic neuron. Elevated intracellular Ca^{2+} activates phospholipases and nitric oxide synthase, leading to the production of reactive oxygen and nitrogen species. Glycine causes oxidative damage to lipids and disrupts the antioxidant system, ultimately resulting in oxidative stress. Glycine also impairs the citric acid cycle and the electron transport chain, and inhibits mitochondrial creatine kinase and Na^{+},K^{+}-ATPase activity.[10,18]

MOLECULAR GENETICS

Pathogenic variants in the genes encoding the GCS proteins disrupt glycine metabolism. *GLDC* mutations affect the P-protein and account for about 70-80% of classic NKH cases; missense variants are particularly common in *GLDC*, reflecting the gene's large size and functional complexity. Mutations in *AMT* affect the T-protein and account for approximately 20% of NKH cases.[19] The major genetic causes of classic NKH, their relative contributions, and the principal variant classes are summarized in Table 1. The predominance of *GLDC* variants is particularly relevant because this gene also exhibits substantial allelic and functional heterogeneity.

No clinically significant differences have been observed between individuals with biallelic pathogenic variants in the *GLDC* and *AMT* genes. GCS activity is a decisive predictor of the severity of the clinical course of NKH. Biallelic variants that completely abolish GCS activity (e.g., exon copy number changes, frameshift, non-sense, and splice-site variants) usually result in absence of residual activity; however, some exceptional GLDC variants (e.g., *c.2203-2A>G* and c.2999delG) are associated with severe NKH. In contrast, biallelic variants that preserve some GCS activity are expected to be associated with a milder phenotype and better prognosis; attenuated NKH is typically observed when at least one allele retains residual activity. Because the functional effects of missense variants are difficult to assess, predicting the genotype–phenotype relationship remains challenging. Taken together, the type of genetic variant and the extent of residual GCS activity largely determine NKH severity and clinical outcome, although exceptions exist.[20,21] Functional studies of GLDC variants have demonstrated a broad spectrum of residual activity, ranging from undetectable to greater than 10% of wild-type activity; even very low residual activity may suffice to produce an attenuated phenotype in some individuals.[20] As illustrated in Table 2, the relationship between genotype, residual GCS activity, and clinical phenotype is best understood as a continuum rather than a binary distinction between severe and attenuated disease. Nevertheless, genotype-based prediction of disease severity remains imperfect. In a cohort of 124 patients, the presence of one allele with residual activity was necessary but not sufficient for an attenuated outcome, and individuals with the same genotype had divergent clinical outcomes.[22]

Table 1. Mutation spectrum in non-ketotic hyperglycinemia.

Gene	Encoded protein	Approximate frequency	Mutation types
GLDC	P-protein (glycine decarboxylase)	~70–80%	Missense, non-sense, splice-site, frameshift, deletions, CNV
AMT	T-protein (amino methyltransferase)	~20%	Missense, non-sense, frameshift, splice variants
GCSH	H-protein	Rare (<1%)	Missense, small deletions
DLD	L-protein	Very rare	Missense variants

CNV, copy number variation.

These observations suggest that residual GCS activity provides important prognostic information but does not fully account for phenotypic variability. Accordingly, genotypes should be interpreted together with biochemical parameters, age at onset, neuroimaging findings, neurological phenotype, and longitudinal clinical course.

GCSH mutations affect both glycine metabolism and mitochondrial energy production. The H-protein functions within the GCS and is also required for protein lipoylation, a process essential for energy metabolism enzymes such as pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase.[9] The clinical phenotype depends on which function is more strongly affected by the mutation.

CLINICAL MANIFESTATIONS

NKH is classified as classical when caused by GCS mutations, and as variant (formerly termed atypical) when due to defects in lipoic acid metabolism.

NKH presents with varied symptoms, ranging from severe neonatal encephalopathy to milder forms in later childhood. The disease is divided into severe (classic) NKH and attenuated NKH, depending on clinical severity and neurological outcomes.[20,23]

Severe (Classic) NKH

Neurological impairment, the hallmark of NKH, results from glycine accumulation in the central nervous system. Elevated glycine acts as a co-agonist at NMDA receptors, leading to excitotoxic neuronal injury and epileptic encephalopathy. In early infancy, hypotonia, increased lethargy, coma, apnea, myoclonic jerks, seizures, recurrent hiccups, and pinpoint pupils may be observed in the severe form and may require ventilator assistance during the initial 10 to 20 days.[3,24] Basic skills such as smiling, purring, and turning can be acquired; however, developmental stages such as sitting, reaching, grasping objects, and communicating cannot be learned. Spastic quadriparesis and trunk hypotonia are often observed. Seizures gradually increase during infancy and become treatment-resistant. In addition, cortical vision loss, microcephaly, and nutritional problems may accompany the clinical picture.[3,23,25] Children who survive the neonatal period often fail to achieve developmental milestones or lose previously acquired skills over time.

Attenuated NKH

Patients with attenuated NKH demonstrate better cognitive and neuromotor development than those with classical NKH. However, during infancy, hypotonia, coma, and seizures may occur. Brain malformations seen in the classical type are not detected in this form. Seizures can usually be controlled with one or two anticonvulsants. According to the degree of intellectual development, those with a DQ of 50-80 are classified as "attenuated good"; although hyperactivity, behavioral problems, and seizures are not seen in this group, late-onset chorea may develop. Those in the DQ range of 20-50 (attenuated intermediate) show moderate development; hyperactivity, chorea, and speech retardation are frequently observed. Those with a DQ <20 are classified as having poor, attenuated NKH and typically present with autistic features, absence of speech, and spasticity.[20,22] The behavioral phenotype of attenuated NKH includes intellectual disability, impaired motor and communication skills, and pronounced adaptive behaviors. ADHD-like traits frequently occur in these cases.[26]

Variant NKH

Variant NKH has an overlapping phenotype with classical NKH and results from mutations affecting lipoic acid biosynthesis. It is classified within the broader group of lipoate synthesis disorders. Lipoate is not only the cofactor for GCS but also for pyruvate dehydrogenase, 2-ketoglutarate dehydrogenase, and branched-chain ketoacid dehydrogenase. These disorders are also known as multiple mitochondrial dysfunction syndromes. Disorders affecting active pyridoxal-5-phosphate result in decreased activity of many pyridoxal-P-dependent reactions,

Table 2. Genotype–phenotype correlation in non-ketotic hyperglycinemia.

Genetic finding	Enzyme activity	Clinical phenotype	Age of onset	Neurological outcome
Null mutations (non-sense, frameshift) in *GLDC*	Absent or minimal	Classic severe NKH	Neonatal	Severe developmental delay, refractory epilepsy
Compound heterozygous variants (one severe + one milder variant) in *GLDC*	Low residual activity	Intermediate NKH	Neonatal or infantile	Variable neurological impairment
Missense variants with residual activity in *GLDC*	Partial activity	Attenuated NKH	Infantile/childhood	Milder developmental delay, variable epilepsy
Pathogenic variants in *AMT*	Reduced activity	Classic or intermediate NKH	Neonatal or infantile	Variable severity
Variants in *GCSH*	Variable	Rare phenotypes	Variable	Limited data

NKH, non-ketotic hyperglycinemia.

including the GCS; an example is pyridox(am)in 5′-phosphate oxidase deficiency (caused by PNPO mutations).[23]

Biochemically, variant NKH is characterized by markedly elevated plasma glycine, mildly to moderately increased CSF glycine, and a lower or borderline CSF-to-plasma glycine ratio than in classical NKH. GCS activity is reduced, although mutations in *GLDC, AMT*, or *GCSH* are typically absent. Instead, impaired protein lipoylation may be observed.[23]

The diagnosis should be considered in patients with moderate CSF glycine elevation, absence of *GLDC/AMT/GCSH* mutations, and additional findings such as elevated lactate or alanine, which suggest broader mitochondrial involvement. In such cases, sequencing of genes involved in lipoate synthesis and Fe–S cluster biogenesis is recommended (*NFU1, BOLA3, IBA57, LIAS* and *GLRX5*).[27]

Clinically, variant NKH manifests a heterogeneous phenotype depending on the underlying gene defect. Presentations may range from childhood-onset spastic paraplegia with relatively preserved cognition to severe early-onset neurodegeneration. Features such as leukodystrophy, cardiomyopathy, optic atrophy, and elevated lactate are more suggestive of variant NKH than classical NKH.[28]

DIAGNOSTIC APPROACH

Diagnosing NKH is a multistep process that integrates clinical suspicion, biochemical markers, neuroimaging, and genetic or enzymatic confirmation.[20] In contemporary clinical practice, diagnostic evaluation relies primarily on biochemical assessment of glycine metabolism, particularly the simultaneous measurement of plasma and CSF glycine concentrations and the calculation of the CSF-to-plasma glycine ratio, followed by molecular genetic confirmation. Enzyme activity studies and the 13C-glycine breath test provide functional information on GCS activity and have historically contributed to diagnostic evaluation, but they are not the primary basis of contemporary diagnosis.

Biochemical Analysis

Elevated plasma glycine is typically the initial finding; however, this alone is insufficient for diagnosis. Plasma glycine may be falsely elevated in conditions such as valproic acid treatment, administration of glycine-buffered intravenous immunoglobulin, neonatal herpes infection, and branched-chain organic acidemias. Urinary glycine excretion may also increase. However, isolated hyperglycinuria may be observed in renal transport disorders and should not be interpreted as evidence of NKH. Concomitant elevation of CSF glycine is required, and plasma and CSF glycine concentrations should be measured simultaneously by quantitative amino acid analysis.[20]

CSF-to-plasma glycine ratio: This ratio is 0.02 or lower in healthy individuals. Although the diagnostic threshold for classic NKH is generally considered to be greater than 0.08, it may be lower (0.04-0.08) in variant NKH.[29] However, the ratio is meaningful only when CSF glycine is elevated. Blood contamination, as reflected by a high CSF protein concentration, may falsely elevate results. Valproate inhibits glycine degradation and increases plasma and CSF glycine concentrations, as well as their ratio. Rare cases of attenuated NKH may have normal CSF glycine concentrations.

In NKH, the urinary organic acid profile is expected to be normal; this finding distinguishes NKH from organic acidurias associated with ketotic hyperglycinemia.[29]

GCS Activity Measurement

GCS activity assays represent an earlier functional approach to diagnosing NKH. Measurement of GCS activity in a liver wedge biopsy is the most precise method, but it is reserved for selected cases because it is invasive and unavailable in many centers. Because the enzyme is highly sensitive, rapid sample processing and deep freezing are essential. This approach may be used for prenatal diagnosis, particularly when the familial mutation is unknown. However, because the false-negative rate is approximately 1%, it is not routinely recommended.[20,30]

Although enzyme analysis in cultured lymphoblasts has been investigated, the results are unreliable because enzyme activity values overlap between controls and patients with NKH.[31]

Although enzyme activity assays provide functional information on GCS activity, their invasive and technical requirements, together with the limitations of certain assay methods, have restricted their role in routine diagnostic evaluation.

13C-Glycine Breath Test

The 13C-glycine breath test is another functional method historically used to assess GCS activity. Normally, hepatic GCS metabolizes glycine, generating CO_2. When glycine is labeled with [1-13C], its breakdown produces $13CO_2$, which is exhaled and can be measured to evaluate GCS activity *in vivo*.

$NH_2\text{-}CH_2 \rightarrow 13COO \rightarrow 13CO_2 + NH_3$

The patient receives 10 mg/kg (maximum 100 mg) of [1-13C] glycine orally. A baseline breath sample is collected before administration, followed by breath samples collected at intervals between 15 and 300 minutes. An infrared analyzer measures the change in $13CO_2$ ($\Delta 13CO_2$). Results are expressed as cumulative percentage recovery, accounting for the administered dose and the patient's height and weight. Because 13C is non-radioactive, it can be used safely in children.

In healthy individuals, the mean 13C recovery within 5 hours is approximately 24%, whereas it is substantially lower in patients with NKH (approximately 8%). Thus, the test can readily distinguish patients with NKH from healthy individuals. Although the test is non-invasive, safe, and easy to perform, results may vary in patients with organic acidemias (e.g., methylmalonic and propionic acidemias), and the patient's metabolic status may affect the findings.[32,33] Although the 13C-glycine breath test provides functional information on GCS activity, it is not part of the primary biochemical-molecular diagnostic pathway used in contemporary clinical practice.

Neuroimaging and Functional Tests

Neuroimaging and electrophysiological studies provide complementary information, particularly to support the clinical diagnosis and assess disease severity and prognosis.

Magnetic resonance imaging (MRI): MRI findings vary with age. When symptoms begin within the first 3 months, diffusion restriction is usually detected in the posterior arm of the internal capsule, anterior brainstem, posterior tegmental tracts, and cerebellum. After three months, the restriction in the infratentorial regions decreases; between 3 and 14 months, spread toward the motor cortex and supratentorial white matter can be observed . Additionally, the corpus callosum may be thin and short, but does not completely disappear. In some infants, retrocerebellar cystic enlargement may occur in association with hydrocephalus. Atrophy, by contrast, occurs with advancing age in severe cases of NKH, while it is usually not observed in mild cases.[23,28,29]

Studies have found a significant relationship between the appearance of the corpus callosum and the clinical phenotype; the former is considered one of the first objective indicators of severe NKH (sensitivity 60-70%). This relationship has greater predictive value during infancy. After one month, no patient with severe NKH had a normal-appearing corpus callosum. The diffusion-limitation pattern is valuable for diagnosing NKH, but its severity is insufficient to distinguish the phenotype. NKH is characterized mainly by a brain growth disorder; spongiosis (microvacuoles) does not have prognostic significance. Early MRI findings are important for diagnosis, particularly for differentiating it from transient NKH.[29]

MR spectroscopy (MRS) offers a non-invasive method for measuring glycine levels in the brain. Detecting a significant glycine peak at 3.6 ppm can aid in diagnosis. At a short echo time (TE = 35 ms), the glycine signal at 3.55 ppm overlaps with myo-inositol. At an intermediate echo time (TE=135 ms), the glycine signal at 3.6 ppm separates and is clearly visible. While a significant glycine peak is seen in severe NKH, the glycine peak is lower in attenuated NKH and is sometimes even difficult to distinguish.[20] Magnetic resonance spectroscopy reveals the NAA/myo-inositol-glycine ratio as a prognostic indicator for adverse outcomes.[30]

EEG: The "burst suppression" pattern is common in the neonatal period, but this can also be observed in atypical cases. This pattern consists of periodic high-amplitude activity at 1-3 Hz on a background of low activity and shows no spatial or temporal differentiation. The prognostic value of EEG is limited because changes such as hypsarrhythmia and burst-suppression patterns, which are common in severe forms of NKH, can also occur in attenuated NKH.[31,32]

Molecular Genetic Testing and New Diagnostic Approaches

It is an autosomal recessive disease. The molecular basis of classical NKH is largely attributed to two main genes: *GLDC* (9p24.1) and *AMT* (3p21.31). GCSH (16q24) encodes the H-protein, mutations in this gene are extremely rare (1<% of cases) and are usually associated with "variant NKH" or combined bioenergetic defects. Furthermore, mutations in the *SLC6A9* gene, which encodes the glycine transporter, result in GLYT1 encephalopathy, leading to a similar clinical picture despite normal GCS activity.[15,19,33]

Genetic heterogeneity is high in NKH; most mutations are private. Missense mutations are the most common type. In addition, non-sense (early stop), splicing (clipping zone), and small insertions/deletions (indels) were observed. Copy Number Variants: Approximately 20-21% of mutations in the *GLDC* gene are large deletions or duplications that are undetectable by traditional sequencing methods. Therefore, methods such as MLPA or microarray should be used alongside sequencing for a complete molecular analysis. Next-generation sequencing: today, multiple-gene panels or whole-exome sequencing have become the primary tools for diagnosing NKH and similar inherited metabolic diseases.[19,20,33]

Advances in functional genomics have improved the interpretation of genetic variants and the assessment of genotype-phenotype relationships in NKH. Recent reports have demonstrated that synonymous GLDC variants, which may initially be classified as variants of uncertain or low clinical significance, can cause aberrant splicing and loss of normal gene function when evaluated using functional minigene assays. These findings highlight the importance of incorporating functional evidence into variant classification, particularly for non-canonical or apparently synonymous variants.[34,35] *In silico* prediction alone may be insufficient for variant interpretation in NKH; functional assays, particularly splicing assays and residual enzyme activity studies, can substantially refine pathogenicity classification.

Molecular diagnostics provide important information for predicting disease severity. In severe cases of NKH, mutations in both alleles that eliminate enzyme activity (e.g., non-sense, frameshift, or large deletions) are detected. The attenuated form, on the other hand, is characterized by mutations that maintain residual enzyme activity in at least one allele (p. Ala389Val, p. Ala802Val in *GLDC*; p. Ile106Thr in *AMT*). These patients often show more favorable neurodevelopmental outcomes.[19] Kuseyri Hübschmann et al.[36] reported that genotype-based predictions indicate that two mild variants lead to attenuated phenotypes, whereas two severe variants result in severe disease. The presence of one mutation with residual enzyme activity is necessary but not sufficient for an attenuated outcome. However, divergent outcomes for the same genotype indicate that other genetic or non-genetic factors contribute to disease severity.[22] Genotype-phenotype discordance limits molecular prediction, as identical genotypes produce divergent outcomes, and 206 of 255 *GLDC* missense mutations remain uncharacterized.[22,37]

Molecular testing fails to identify defects in the *GLDC* or *AMT* genes in approximately 5% of cases. These conditions should be classified as variant NKHs resulting from mutations in genes involved in the synthesis or transport of lipoic acid, a GCS cofactor (including *LIAS, BOLA3, GLRX5, NFU1, IBA57, LIPT1*, *and LIPT2*). Such cases commonly present with additional clinical features, such as pyruvate dehydrogenase deficiency and lactic acidosis, alongside elevated glycine levels.[19,23] In addition, plasma alanine, serum lactate, and pyruvate may increase. An increase in 2-ketoglutarate can be detected in urinary organic acids. Those with *NFU1* mutations also have elevated levels of 2-aminoadipic acid and 2-ketoadipic acid. In addition, pyruvate dehydrogenase (PDH) activity has consistently been reduced. Deficiencies of respiratory chain complexes I and II are seen in iron-sulfur cluster synthesis disorders (*NFU1, ISCA2, IBA57, BOLA3*). Amounts of Lipoyl-H protein, the E2 subunit of PDH and 2-ketoglutarate dehydrogenase, can be shown to be decreased by Western blotting.

Although the disease is autosomal recessive, *de novo* mutations have been reported in about 1% of cases. This significantly reduces recurrence risk when the parents are not carriers; therefore, molecular confirmation of parental carrier status is recommended. Prenatal diagnosis through chorionic villus sampling and pre-implantation genetic testing are possible in families with known mutations.[20]

Diagnostic standards remain CSF/plasma glycine and GCS genetics, but several newer tools are refining earlier detection and severity prediction. Functional assays of missense variants (enzyme activity, protein stability, structural modeling) help classify variants as severe vs. hypomorphic and predict phenotype, suggesting which patients may have an attenuated course.[22,38] A 2025 microRNA sequencing study identified 10 candidate miRNAs differentially expressed in NKH patients versus heterozygous carriers and controls, the first such profiling in *AMT/GLDC*-related disease, proposed as future blood-based early biomarkers and a framework for novel diagnostic strategies.[39]

Some integrative severity prediction models combine age at onset, CSF and plasma glycine thresholds, and genotype and pathogenicity scores to stratify NKH as severe versus attenuated, achieving high AUCs (0.87-0.97).[36,40] The following were suggested as diagnostic criteria for severe NKH: onset of symptoms within the first week, CSF glycine >230 µM, brain malformation on MRI, two non-missense mutations, and glycine index >3. In attenuated NKH, symptom onset after 4 months, CSF-to-plasma glycine ratio ≤0.08, no use of antiepileptic drugs, and at least one functional allele are determinants. For early diagnosis, these markers provide 78% accuracy for the severe form and 49% for the milder form.[22,31] Although high CSF glycine correlates with the severe form, low CSF glycine does not rule it out.

Beyond the traditional CSF/plasma glycine ratio, CSF serine and threonine levels provide additional diagnostic evidence; the CSF serine/threonine ratio discriminates classic NKH from transient NKH, in which the glycine ratio is elevated but the serine/threonine ratio remains normal. Untreated NKH patients show a specific alteration in D-serine metabolism, with a greater reduction in D-serine than in L-serine and a decreased D/L-serine ratio, reflecting a perturbation of the serine shuttle and one-carbon metabolism.[41]

New diagnostic methods that combine genetics, functional assays, and omics and imaging tools are improving the detection of NKH, and further research is needed to achieve precise and widely accessible diagnostics.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of NKH can be challenging, particularly in neonates who present with hypotonia, seizures, apnea, feeding difficulties, or altered consciousness, as these manifestations overlap with those of several metabolic and genetic encephalopathies. The differential diagnosis includes urea cycle disorders, organic acidemias, maple syrup urine disease, disorders of amino acid metabolism, mitochondrial disorders, and other causes of neonatal epileptic encephalopathy. Distinguishing NKH from these conditions requires integrating clinical presentation with targeted biochemical investigations rather than relying on a single abnormal laboratory parameter. Table 3 summarizes the principal clinical and biochemical features that distinguish NKH from these major mimics.

For practical diagnostic purposes, the distinction between NKH and other neonatal metabolic encephalopathies should not

Table 3. Disorders showed elevated plasma glycine.

Condition	Distinguishing features vs. NKH
Organic acidemias (propionic, methylmalonic, isovaleric acidemia)	Metabolic acidosis, ketosis, abnormal urine organic acids; NKH shows no ketoacidosis and normal organic acids despite high glycine
"Variant NKH" due to lipoate defects (*LIAS, BOLA3, NFU1, GLRX5,* some *GCSH*)	Hyperglycinemia plus combined deficiency of pyruvate/α-ketoglutarate dehydrogenase, lactic acidosis, multisystem disease, leukodystrophy; not an isolated GCS defect
Glycine transporter defect (*SLC6A9*)	Hyperglycinemia with abnormal CSF/plasma ratio but primary transport defect; may have overlapping neonatal encephalopathy, requires genetic confirmation

NKH, non-ketotic hyperglycinemia; CSF, cerebrospinal fluid; GCS, glycine cleavage system.

rely solely on plasma glycine concentration. The combined assessment of plasma and CSF glycine, particularly the CSF-to-plasma glycine ratio>0.08 (often ≥0.15 in severe NKH), provides an important discriminator, while concomitant evaluation of ammonia, lactate, acid-base status, ketones, plasma amino acids, and urine organic acids helps distinguish NKH from urea cycle disorders, organic acidemias, and other aminoacidopathies.[41,42]

NKH also mimics some clinical situations;

Hypoxic-ischemic encephalopathy (HIE) presents with similar features, including hypotonia, coma, and seizures; unlike NKH, it is not associated with isolated glycine elevation and shows distinct MRI patterns. At intermediate echo times, MRS may be misinterpreted as showing glycine, whereas long echo times help distinguish glycine from myo-inositol and reduce the risk of misdiagnosing NKH in patients with HIE.[43-49]

Infectious encephalitis/meningitis–CSF pleocytosis, elevated protein, systemic infection markers; no characteristic CSF glycine pattern.

Other inborn errors of metabolism (urea-cycle defects, maple syrup urine disease, and mitochondrial disorders) usually present with hyperammonemia, characteristic organic acid abnormalities, or lactate elevation—findings that are absent in isolated NKH. Hyperglycinuria caused by renal glycine transport defects, including familial iminoglycinuria and benign hyperglycinuria, should also be distinguished.[50]

Transient NKH-early hyperglycinemia that normalizes as glycine-cleavage activity improves; milder course. Requires follow-up amino acids and genetics to avoid over-diagnosis of classic NKH.[44]

Clinical "red flags" that should keep NKH high on the list are: neonatal onset within days, profound hypotonia, apnea/ventilation need, hiccups, refractory seizures, and very high CSF glycine with normal organic acids.[28,34] Overall, establishing NKH and excluding mimics depends on combined clinical phenotype, CSF/plasma glycine profile, absence of ketoacidosis/alternative metabolic signatures, neuroimaging, and confirmatory genetic testing for GCS or related genes.[36,34]

TREATMENT STRATEGIES

Treatment remains challenging and is primarily supportive, aiming to reduce glycine levels and mitigate neurotoxicity. Current therapeutic strategies include pharmacological reduction of glycine levels (e.g., sodium benzoate), NMDA receptor antagonists (e.g., dextromethorphan), dietary interventions such as the ketogenic diet for seizure control, and emerging experimental approaches such as gene therapy (Table 4).[28,45,46] Early intervention may improve outcomes in milder cases of classic NKH, but no curative therapy exists.

Glycine-Lowering Therapy:

Sodium benzoate reduces plasma glycine levels by conjugating them to hippuric acid; the target range is 120-300 µmol/L. Measurements should be performed on blood samples taken 1-2 hours after benzoate administration.

Benzoate + CoA → Benzoyl-CoA +Glycine → CoA+ Hippurate

• Dose for attenuated NKH: 200-550 mg/kg/day. For children and adults, the initial dose is recommended to be 5.5 g/m^2 based on body surface area.

• Dose for severe NKH: 550-750 mg/kg/day. In adults, the maximum dose should be limited to 16.5 g/m^2/day.

• Division of dosing: It should be administered at least three doses/day. Six doses per day should generally be preferred for newborns.[20,28,47]

Treatment starts at a low dose and plasma glycine levels are monitored regularly. If the target level is not reached, the dose is increased by 50 mg/kg/day each time, and the plasma glycine level is measured again after 24-48 hours. Follow-up frequency by age group is as follows:

• In infants: Every two weeks,

• In young children: Once a month,

• In older children: Every three months.

Table 4. Treatment options for NKH.

Modality	Primary goal	Evidence/limitations
Sodium benzoate	Lower plasma/CSF glycine	Standard care; improves arousal/seizures in some, no cure; effect greater in attenuated NKH
NMDA antagonists (dextromethorphan, ketamine)	Block glycine-NMDA excitotoxicity	Mixed evidence; no clear added long-term benefit; mechanistic concerns and side-effects
Ketogenic diet	Seizure and glycine reduction	Lowers plasma glycine more consistently than high-dose benzoate; variable clinical impact
Conventional ASMs (e.g., levetiracetam)	Seizure control	Helpful in some refractory cases; no standardized regimen
VNS, other non-drug epilepsy therapies	Refractory epilepsy	Case-level evidence of seizure reduction
Palliative, multidisciplinary care	Quality of life, survival	Crucial in severe NKH; can extend survival and support families
AAV-GLDC gene therapy (preclinical)	Causal correction	Normalizes metabolic profile in mouse model; human trials not yet available

NKH, non-ketotic hyperglycinemia; NMDA, modulate N-methyl-D-aspartate; AAV-GLDC, adeno-associated virus - glycine decarboxylase; ASMs, anti-seizure medications; VNS, vagus nerve stimulation; CSF, cerebrospinal fluid.

Benzoate therapy is effective in the liver and kidneys but does not normalize glycine levels in the brain or CSF; therefore, monitoring CSF glycine levels is unnecessary.[47]

High doses of benzoate (500-750 mg/kg/day) can lead to gastritis, carnitine loss, and toxicity. Overdose may cause hypocalcemia, low plasma glycine, nausea, vomiting, lethargy, coma, seizures, acidosis, and electrolyte disturbances; the threshold for toxicity is plasma benzoate >2.5 mmol/L. Treatment includes discontinuing benzoate, supplementing glycine, and/or performing hemodialysis.[20] The taste of benzoate can cause incompatibilities. In some countries, a 75% granule form is available.

The glycine index evaluates whether plasma glycine concentration is maintained within the target range (120-300 µmol/L). The glycine index helps determine a person's specific benzoate requirement. Especially in patients on a ketogenic diet, the benzoate dose should be reduced as the glycine pool shrinks; the glycine index guides this adjustment.[47]

$$Glycine\ index = \frac{benzoate\ dose\ (mg/kg/day)}{plasma\ glycine\ (\mu mol/l)}$$

A low glycine index indicates that the benzoate dose does not adequately reduce the glycine level; in this case, the dose may need to be increased. A high glycine index, on the other hand, indicates that the benzoate dose reduces glycine below the target range, so dose reduction is recommended because this reduction may pose a risk of toxicity. Target values: plasma glycine ≤300 µmol/L and benzoate level should be at the non-toxic limit (≤2.5 mmol/L).[28,48]

The ketogenic diet (KD) has been explored in NKH primarily as an adjunctive therapy for refractory epilepsy, a major clinical feature, particularly in classical neonatal forms. Given the limited efficacy of standard treatments such as sodium benzoate and NMDA receptor antagonists, KD has emerged as a potential supportive strategy.

The therapeutic rationale for KD in NKH is based on its antiepileptic and neuroprotective effects rather than addressing the disease itself. Ketone bodies may decrease neuronal excitability, modulate glutamatergic neurotransmission (important because glycine acts as an NMDA co-agonist), and enhance mitochondrial energy metabolism.[49] In cases of glucose deprivation, glycine is converted to serine, which is then converted to pyruvate by serine dehydratase to be used as the starting substrate for gluconeogenesis. The ketogenic diet may enhance endogenous glycine utilization through metabolic pathways involved in energy production and gluconeogenesis, potentially influencing glycine homeostasis.[48] Importantly, emerging evidence suggests that the effects of KD in NKH may extend beyond seizure control to include modulation of glycine metabolism. In a multicenter study involving six infants with NKH, the patients initially treated with high-dose sodium benzoate were subsequently switched to KD with low-dose benzoate. KD was associated with an approximately 28% reduction in plasma glycine concentrations compared with high-dose benzoate therapy. In two patients evaluated by serial magnetic resonance spectroscopy, brain glycine concentrations decreased by approximately 30% in the basal ganglia and by 50% in the white matter, although concentrations remained above the normal range. Concentrations of CSF glycine, serine, and threonine also decreased during KD, consistent with increased utilization of these substrates during gluconeogenesis. However, the clinical response was heterogeneous, with seizure reduction observed in about half of the patients. These findings suggest that KD may influence glycine homeostasis, although

biochemical improvement does not necessarily translate into uniform neurological benefit.[48]

More recent clinical evidence from Türkiye further supports a potential role for KD in NKH. In a retrospective comparative study of 14 patients with classical NKH, eight received KD and six received a glycine-restricted diet (GRD). KD was associated with significantly greater reductions in seizure frequency and seizure scores, as well as a reduced requirement for antiseizure medications, compared with GRD. However, neither dietary intervention significantly improved plasma glycine concentrations, motor function, or swallowing ability. These findings are particularly important because they suggest that the clinical antiseizure effects of KD may occur independently of a measurable reduction in systemic glycine concentrations.[50]

Additional reports support KD's potential antiseizure benefit in severe NKH. The ketogenic formula has been associated with complete seizure control and EEG improvement within approximately one month, whereas other cases have reported reduced focal seizures, lower CSF glycine levels, and improved quality of life when KD is combined with conventional therapy.[44,51-55]

Taken together, the available evidence supports KD as a potential adjunctive treatment for seizure control in selected patients with NKH, particularly those with drug-resistant epilepsy.

NMDA Receptor-Targeted Therapy:

In NKH, glycine degradation is impaired, leading to accumulation of glycine in the central nervous system. Glycine overactivates the NMDA receptor, causing neuronal damage through increased calcium entry and excitotoxicity. This causes severe epileptic encephalopathy, especially in classical neonatal NKH.

Dextromethorphan and ketamine are commonly used non-competitive NMDA receptor antagonists. Clinical studies report reduced seizure frequency, partial EEG improvement, and frequent coadministration with sodium benzoate to block glycine co-agonism.[23,28] Early findings demonstrated improved alertness, improved sucking, and partial control of seizures in neonates. A 17-year-old patient treated with high-dose benzoate and dextromethorphan experienced extended survival but persistent severe disability and refractory seizures; a 2023 case report showed it reduced myoclonic seizures resistant to multiple drugs.[56,57] Recent reassessment argues that reduced brain D-serine in NKH may actually under-activate NMDA receptors, and that clear added benefit of dextromethorphan/ketamine beyond glycine reduction has not been demonstrated; routine use should be critically reconsidered because of potential adverse effects.[16]

Dextromethorphan is typically administered at doses ranging from 3 to 15 mg/kg/day, with higher doses required in younger patients (approximately 10 mg/kg/day in neonates, 5 mg/kg/day in children, and 3 mg/kg/day in adolescents and adults). The total daily dose is usually divided into three to four administrations or given as a sustained-release formulation twice daily. Interindividual variability in response is significant and partly explained by pharmacogenomic differences involving CYP2D6, CYP3A4, and UGT1A1 enzymes. Concomitant use of medications such as cimetidine may inhibit metabolism and increase the risk of toxicity. Routine therapeutic drug monitoring is not recommended because a defined therapeutic range has not been established. Adverse effects are generally dose-related and include sedation and movement disorders at higher doses. Additionally, suppression of the cough reflex may increase the risk of aspiration and pneumonia in this group. Long-term high-dose use may lead to tolerance, and abrupt discontinuation can result in withdrawal symptoms; therefore, gradual tapering over several months is recommended when discontinuation is required.[58,59]

Ketamine is available as R-, S-, and racemic formulations and has low oral bioavailability (8-24%) due to extensive first-pass metabolism via CYP3A and CYP2B6, producing norketamine, a less active metabolite. It exerts its primary effect via non-competitive antagonism of NMDA receptors, which reduces GABAergic interneuron activity; it also interacts with opioid receptors and inhibits monoamine transporters, thereby increasing adrenergic activity. Oral dosing is approximately 15 mg/kg/day in neonates and approximately 9 mg/kg/day in infants (range, 1-32 mg/kg/day). At high doses, ketamine induces dissociative anesthesia and may be used in refractory status epilepticus, whereas lower doses provide analgesic and antidepressant effects, though they may also cause cognitive impairment and hallucinations. Its metabolite, hydroxynorketamine, acts as an AMPA receptor agonist and may contribute to antidepressant effects. However, long-term safety data are limited, and chronic use has been associated with urinary tract toxicity, including cystitis and bladder dysfunction.[16]

Evidence supporting dextromethorphan and ketamine for NKH remains limited and derives primarily from case reports, small case series, and clinical observations. Although some patients may experience improved seizure control or neurological status, controlled clinical trials have not established its efficacy.

Partial clinical improvement with strychnine (GlyR antagonist) has been reported in a few historical cases. Although it improves tone and breathing, long-term use has been abandoned due to serious side effects.

Perampanel (an AMPA receptor antagonist) was administered as an intervention in a patient who did not benefit from sodium benzoate and dextromethorphan treatment. Gradually increasing the dose to 0.65 mg/kg/day resulted in a 50% reduction in epileptic spasms.[55]

A dose-dependent decrease in Gly levels induced by sodium cinnamate was detected in the animal model (GLDC def) and in the research phase in humans.

Epilepsy Management:

Seizures are frequently drug-resistant, especially in severe NKH.[28]

Benzodiazepines (clonazepam, clobazam) - most effective in myoclonic seizures in early infancy.

Levetiracetam, topiramate, and phenobarbital - are frequently used in older children.

Felbamate (NMDA blocker) - may be considered for resistant seizures, but its use is limited by serious side effects.

ACTH - is ineffective in West syndrome; it can even cause worsening, including coma.

Valproate and vigabatrin are contraindicated. Valproate inhibits the GCS and has precipitated worsening; vigabatrin has been reported to aggravate West syndrome in NKH.60

Vagal nerve stimulator - useful in some severe cases.

Supportive and Palliative Care:

Because current treatments do not halt neurodegeneration, long-term management is supportive: Respiratory, feeding (including gastrostomy), orthopedic and gastrointestinal care; monitoring for cardiopulmonary complications. Early, integrated pediatric palliative care can prolong survival and improve quality of life, even in severe NKH with profound disability. All recent series emphasize multidisciplinary follow-up.[3,61]

Emerging and Experimental Approaches:

Excitotoxicity, oxidative stress, and impaired brain bioenergetics are increasingly recognized as key mechanisms, suggesting future adjuvant therapies aimed at providing antioxidant and mitochondrial support, although these remain experimental.[18]

One major area of interest is gene-based therapy. A novel recombinant adeno-associated viral vector 9 expressing *GLDC* (rAAV9-GLDC) conferred 100% protection against disease progression and fatality in humanized NKH mice over a ten-month follow-up. A single intraperitoneal dose boosted astrogenesis without triggering an inflammatory response, which indicates a durable neuroprotective mechanism.[62] In a separate GLDC-deficient mouse model, neonatal administration of AAV9-GLDC restored GLDC mRNA and protein expression, leading to a significant reduction in plasma and brain glycine levels.[12] This vector also normalized the folate profile, restoring the glycine-derived one-carbon supply for folate metabolism. Unlike current treatments, gene therapy that restores GCS function simultaneously lowers glycine levels and supplies glycine-derived one-carbon groups to folate one-carbon metabolism. Preclinical studies in animal models have shown partial biochemical correction and improved survival, although translation to humans remains at an early stage.[12]

Methyl group donors have not been proven effective in humans, although they are effective in mice. Folinic acid is among the treatments that have not demonstrated benefit in humans. In addition, cofactor therapy has not been tried for rare mutations: GLDC mutations—pyridoxal phosphate; AMT mutations—folinic acid.

D-serine substitution has been proposed as a metabolite-based approach to address the under- activation hypothesis, but no clinical studies have been conducted.[26]

The evidence base for all NKH therapies is limited to case reports and small retrospective series and is therefore insufficient to support formal treatment recommendations.[54] The ketogenic diet shows the most reproducible benefit for seizure control and glycine reduction, though it does not normalize brain glycine or consistently improve neurodevelopment.[48] NMDA receptor antagonists have an increasingly contested mechanistic rationale, variable clinical responses, and emerging safety concerns.[26] Prospective controlled studies are needed across all interventions.

PROGNOSIS

The prognosis for NKH depends strongly on disease severity (severe versus attenuated), age of onset, brain imaging findings, EEG findings, and genotype. As summarized in Table 5, neonatal onset, severe neurological involvement, markedly elevated CSF-to-plasma glycine ratios, profound reduction in GCS activity, severe/null variants, burst-suppression EEG patterns, and extensive MRI abnormalities are generally associated with poorer outcomes, whereas residual enzyme activity, later onset, lower CSF-to-plasma glycine ratios, milder genetic variants, and less severe neurological abnormalities are more commonly associated with attenuated disease and a relatively better prognosis. However, these factors should not be interpreted as independent or absolute predictors because substantial clinical variability exists among patients. The prognosis is poor in the severe classical neonatal form and highly variable in attenuated cases.[12,63]

Table 5. Prognostic factors related to NKH.

Factor	Good prognosis	Poor prognosis
Clinic onset	Infant/childhood onset (attenuated NKH)	Neonatal onset (classical NKH)
Neurological findings	Mild hypotonia, controllable seizures	Refractory seizures, coma, severe encephalopathy
Developmental course	Partial motor and cognitive gains	Serious global developmental delay
CSF to plasma glycine ratio	Lower rate (close to <0.08)	High rate (0.08>)
GCS activity	Residual enzyme activity	Profound enzyme deficiency
Genetics	Mild/partial loss of function mutations	Null or severe mutations (*GLDC*, *AMT*)
EEG	Mild slowing, controllable epileptiform activity	Burst-suppression, severe epileptic encephalopathy
MRI findings	Minimal or non-specific changes	Hypoplasia of the corpus callosum, widespread brain damage
Response to treatment	Improvement in seizure control	Treatment-resistant epilepsy
Systemic status	Stable clinical course	Need for respiratory support, frequent intensive care

NKH, non-ketotic hyperglycinemia; CSF, cerebrospinal fluid; GCS, glycine cleavage system; EEG, electroencephalogram; MRI, magnetic resonance imaging.

Classical (neonatal severe) NKH is associated with a poor prognosis, characterized by early-onset encephalopathy, refractory seizures, and profound developmental impairment. Many patients require long-term ventilatory and nutritional support, and mortality is highest in the neonatal period or early infancy. Survivors typically exhibit severe intellectual disability, spasticity, and intractable epilepsy.[22,64]

In contrast, attenuated NKH demonstrates a more heterogeneous clinical course. Patients may achieve limited developmental milestones, including partial motor and cognitive skills; seizure control is often more achievable. However, long-term outcomes remain variable, and many individuals still experience persistent neurodevelopmental deficits.[3]

In a natural-history study of 65 NKH patients, one-third of patients died; a significant proportion of deaths occurred during the neonatal period. Most of the survivors had severe developmental delays, epilepsy, and spasticity; however, 10 of the 25 children (all boys) who were alive and reached the age of ≥3 were able to walk and communicate with words/signs.[63] In the Chinese cohort, 17 of the 20 patients were of the neonatal type; of the 18 patients, 9 died (8 during the neonatal period). All 9 surviving patients had varying degrees of neurodevelopmental impairment.[3] In the Turkish series, overall mortality was 30% among 10 classic NKH patients and 50% among those in the neonatal group; severe sequelae were reported in most survivors.[25]

Neuroimaging and electrophysiological findings also carry prognostic value. Severe EEG abnormalities, such as burst-suppression patterns, and structural brain anomalies on MRI are typically associated with worse outcomes. Overall, despite advances in supportive care, NKH remains a condition with significant morbidity, and current treatments are largely unable to alter the natural course of severe disease.[36] In a cohort of 124 patients, CSF glycine levels and the CSF-to-plasma glycine ratio were inversely correlated with the developmental quotient. A CSF glycine level >230 µM and a CSF/plasma ratio ≥0.15 were identified as strong predictors of severe disease, whereas ratios ≤0.08-0.09 were associated with attenuated phenotypes.[22] These findings were further supported by a smaller study of 25 patients, in which disease onset at ≥3 months and a CSF/plasma ratio ≤0.09 were confirmed as sensitive indicators of attenuated NKH.[36] At the molecular level, an attenuated phenotype requires at least one allele with residual enzyme activity, whereas biallelic mild mutations are associated with the most favorable developmental outcomes.[22] Additionally, clinical outcome scores and mutation severity scores derived from large-scale GLDC variant analyses demonstrate high specificity in distinguishing severe from attenuated forms.[40] Although most patients with severe NKH die in early childhood, some survive to age 7 with severe neurodevelopmental disorders, and reports indicate that long-term palliative care can prolong life.[61] In attenuated NKH, individuals can survive into adulthood. Individuals who have reached their 50s, who were diagnosed in childhood, and who have lifelong mild-to-moderate disabilities are reported.[65]

Taken together, the prognostic factors summarized in Table 5 may assist in early clinical stratification and counseling, particularly when age at onset, neurological severity, biochemical findings, neuroimaging, EEG, and genotype are considered. Nevertheless, they should be used cautiously because reliably predicting individual long-term outcomes remains challenging.

CONCLUSION

Despite advances in symptomatic management, NKH remains a devastating disorder with limited disease-modifying treatment options, highlighting significant unmet clinical needs. Emerging therapeutic strategies for NKH, including gene replacement targeting GLDC and *AMT,* read-through inducers for premature

stop codons, chemical chaperones for unstable missense variants, antisense oligonucleotides for selected splicing defects, and genotype-guided approaches, offer promising mutation-specific therapeutic options to restore or improve GCS activity. However, major limitations such as effective central nervous system delivery, durable expression, safety, and the need for robust functional validation remain unresolved, making genetic testing increasingly important for prognosis and individualized therapeutic planning.[19,38,66]

Targeting downstream neurotoxicity represents another critical area of development. Beyond glycine-mediated NMDA receptor overactivation, growing evidence suggests that excitotoxicity, oxidative stress, and bioenergetic dysfunction contribute to NKH neuropathology. These mechanisms support future therapeutic strategies, including antioxidants, mitochondria-targeted interventions, and modulators of non-NMDA receptor pathways.[18]

Demonstration of restructuring of serine-glycine-one-carbon metabolism and of a shift in glial differentiation in the iPSC-derived NKH astrocyte model provides a powerful platform for human cell-based drug screening.[14]

Severity-estimation models developed using multiparameter approaches (age at onset, CSF/plasma glycine ratio, CSF glycine threshold, genotype) accurately distinguish severe from attenuated cases.[22] This can help determine more rationally to whom we will give intensive treatment and to whom we will provide palliative care.[28,36] The Weighted Multiparametric Mutation Score, which combines *in silico* predictions of stability, conservation, and interaction, distinguished severe from attenuated disease with high statistical significance.[37] Brain MRI adds prognostic value: as corpus callosum growth is more pronounced in severe cases, with 71% of severe patients having brain malformations versus 7.5% of attenuated patients.[22,29] Small sample sizes constrain all models, and no external validation exists for any NKH severity model; the multiparametric approach is described as promising but unvalidated in independent cohorts. Clinical utility evaluation is equally absent, as no study has assessed whether these models improve clinical decision-making or patient outcomes. Without external validation and utility data, these models remain promising developmental tools rather than validated clinical instruments ready for implementation.

A major unresolved gap is the lack of human clinical trials evaluating gene-based and one-carbon metabolic therapies, despite encouraging preclinical findings in mouse models. NMDA receptor-targeted treatments also lack prospective controlled evidence, and their efficacy is increasingly questioned in light of biochemical data indicating D-serine deficiency.[41] Current prognostic models are constrained by small cohorts of rare-disease patients and require validation in larger retrospective datasets. Similarly, iPSC-derived models remain at an early stage and have not yet been used for therapeutic screening. Overall, disease rarity, insufficient biomarkers, and lack of controlled trials continue to limit therapeutic progress. Future studies should therefore focus on earlier diagnosis, biomarker discovery, and multicenter collaborative trials that can translate experimental approaches into clinically meaningful outcomes.

FOOTNOTES

Financial Disclosure: The author declare that this study received no financial support.

References

1. Selvanathan A, Hertzog A, Coughlin CR 2nd, Swanson MA, Van Hove JLK. The history and nosology of the glycine disorders: a framework for clinicians. J Inherit Metab Dis. 2026;49(1):e70138. doi:10.1002/jimd.70138
2. Reeves S, Wahle A, Goldstein LB, Toupin DN. Nonketotic hyperglycinemia in central and appalachian Kentucky. J Child Neurol. 2026;41(8):1163-1169. doi: 10.1177/08830738251409555
3. Zhou Z, Cai Y, Li X, et al. Natural history and outcome of nonketotic hyperglycinemia in China. Front Neurol. 2024;15:1440883. doi: 10.3389/fneur.2024.1440883
4. Applegarth DA, Toone JR, Lowry RB. Incidence of inborn errors of metabolism in British Columbia, 1969-1996. Pediatrics. 2000;105(1):e10. doi: 10.1542/peds.105.1.e10
5. von Wendt L, Hirvasniemi A, Similä S. Nonketotic hyperglycinemia. A genetic study of 13 Finnish families. Clin Genet. 1979;15(5):411-417. doi: 10.1111/j.1399-0004.1979.tb01773.x
6. Wang W, Wu Z, Dai Z, Yang Y, Wang J, Wu G. Glycine metabolism in animals and humans: implications for nutrition and health. Amino Acids. 2013;45(3):463-477. doi: 10.1007/s00726-013-1493-1
7. Cavalcante DP, Carvalho GA, Nunes AÍS, et al. GlyT1 (SLC6A9) inhibition in neurological and psychiatric disorders. Naunyn Schmiedebergs Arch Pharmacol. 2026;399(9):12983-13008. doi: 10.1007/s00210-026-05276-y
8. Raiteri L. Interactions involving glycine and other amino acid neurotransmitters: focus on transporter-mediated regulation of release and glycine-glutamate crosstalk. Biomedicines. 2024;12(7):1518. doi: 10.3390/biomedicines12071518
9. Swanson MA, Jiang H, Busquet N, et al. Deep postnatal phenotyping of a new mouse model of nonketotic hyperglycinemia. J Inherit Metab Dis. 2024;47(5):971-990. doi: 10.1002/jimd.12755
10. Swanson MA, Jiang H, Kolora LD, et al. A nonketotic hyperglycinemia mouse shows wide-ranging biochemical consequences of elevated glycine, reduced folate one-carbon charging, and serine deficiency. J Inherit Metab Dis. 2026;49(1):e70137. doi: 10.1002/jimd.70137
11. Lopez-Ramirez A, Worth AJ, Wang ZL, et al. Variants in glycine decarboxylase activate catabolic mechanisms of mitochondrial energy metabolism in the brain. J Biol Chem. 2026;302(6):113098. doi: 10.1016/j.jbc.2026.113098
12. Leung KY, Santos C, De Castro SCP, et al. AAV-mediated expression of mouse or human GLDC normalises metabolic biomarkers in a GLDC-deficient mouse model of non-ketotic hyperglycinemia. Mol Genet Metab. 2024;142(3):108496. doi: 10.1016/j.ymgme.2024.108496
13. Weaver NE, Healy A, Wingert RA. gldc Is Essential for renal progenitor patterning during kidney development. Biomedicines. 2022;10(12):3220. doi: 10.3390/biomedicines10123220

14. Arribas-Carreira L, Castro M, García F, Navarrete R, et al. Metabolic rewiring and altered glial differentiation in an iPSC-derived astrocyte model derived from a nonketotic hyperglycinemia patient. Int J Mol Sci. 2024;25(5):2814. doi: 10.3390/ijms25052814

15. Arribas-Carreira L, Dallabona C, Swanson MA, et al. Pathogenic variants in GCSH encoding the moonlighting H-protein cause combined nonketotic hyperglycinemia and lipoate deficiency. Hum Mol Genet. 2023;32(6):917-933. doi: 10.1093/hmg/ddac246.

16. Van Hove JLK. The role of NMDA-receptor type glutamatergic antagonists dextromethorphan or ketamine in the treatment of nonketotic hyperglycinemia: a critical reassessment. Mol Genet Metab. 2024;143(3):108594. doi: 10.1016/j.ymgme.2024.108594

17. UCL. Non-ketotic hyperglycinemia and the glycine cleavage system. Available from: https://www.ucl.ac.uk/population-health-sciences/non-ketotic-hyperglycinemia-and-glycine-cleavage-system.

18. Leipnitz G, da Rosa JS, Wajner M. The role of excitotoxicity, oxidative stress and bioenergetics disruption in the neuropathology of nonketotic hyperglycinemia. Neurotox Res. 2024;42(4):32. doi: 10.1007/s12640-024-00711-5

19. Coughlin CR 2nd, Swanson MA, Kronquist K, et al. The genetic basis of classic nonketotic hyperglycinemia due to mutations in GLDC and AMT. Genet Med. 2017;19(1):104-111. doi: 10.1038/gim.2016.74

20. Hove JL Van, Curtis Coughlin I, Swanson M, Hennermann JB. Nonketotic hyperglycinemia. GeneReviews®. Published online May 23, 2019. Accessed: March 11, 2026. https://www.ncbi.nlm.nih.gov/books/NBK1357/

21. Drackley A, Peter M, Rathbun P, Ing A, Prada CE, Yap KL. Homozygosity for disease-causing variants in AMT and GLDC in a patient with severe nonketotic hyperglycinemia. Am J Med Genet A. 2024;194(8):e63622. doi: 10.1002/ajmg.a.63622

22. Swanson MA, Coughlin CR Jr, Scharer GH, et al. Biochemical and molecular predictors for prognosis in nonketotic hyperglycinemia. Ann Neurol. 2015;78(4):606-618. doi: 10.1002/ana.24485. Erratum in: Ann Neurol. 2016;79(3):505. doi: 10.1002/ana.24600.

23. Johan LK, Hove V, Coster RV. Nonketotic hyperglycinaemia and lipoate deficiency disorders. In: Saudubray, Jean-Marie, Baumgartner Matthias R., Cazorla ÁG, Walter JH, eds. Inborn Metabolic Diseases Diagnosis and Treatment. 7th ed. Springer; 2022:459-470. doi:-3-662-63123-2 (eBook) https://doi.org/10.1007/978-3-662-63123-2

24. Genç Sel Ç, Kılıç M, Yüksel D, et al. Nonketotic hyperglycinemia: clinical range and outcome of a rare neurometabolic disease in a single-center. Brain Dev. 2018;40(10):865-875. doi: 10.1016/j.braindev.2018.06.007

25. Bayrak H, Yıldız Y, Olgaç A, et al. Genotypic and phenotypic features in Turkish patients with classic nonketotic hyperglycinemia. Metab Brain Dis. 2021;36(6):1213-1222. doi: 10.1007/s11011-021-00718-3

26. Van Hirtum LDFM, Van Damme T, Van Hove JLK, Steyaert JG. The behavioral phenotype of children and adolescents with attenuated non-ketotic hyperglycinemia, intermediate to good subtype. Orphanet J Rare Dis. 2024;19(1):150. doi: 10.1186/s13023-024-03172-3

27. Baker PR 2nd, Friederich MW, Swanson MA, et al. Variant non ketotic hyperglycinemia is caused by mutations in LIAS, BOLA3 and the novel gene GLRX5. Brain. 2014;137(Pt 2):366-379. doi: 10.1093/brain/awt328

28. Nowak M, Chuchra P, Paprocka J. Nonketotic hyperglycinemia: insight into current therapies. J Clin Med. 2022;11(11):3027. doi: 10.3390/jcm11113027

29. Stence NV, Fenton LZ, Levek C, et al. Brain imaging in classic nonketotic hyperglycinemia: quantitative analysis and relation to phenotype. J Inherit Metab Dis. 2019;42(3):438-450. doi: 10.1002/jimd.12072

30. Viola A, Chabrol B, Nicoli F, Confort-Gouny S, Viout P, Cozzone PJ. Magnetic resonance spectroscopy study of glycine pathways in nonketotic hyperglycinemia. Pediatr Res. 2002;52(2):292-300. doi: 10.1203/00006450-200208000-00024

31. Hennermann JB, Berger JM, Grieben U, Scharer G, Van Hove JL. Prediction of long-term outcome in glycine encephalopathy: a clinical survey. J Inherit Metab Dis. 2012;35(2):253-261. doi: 10.1007/s10545-011-9398-1

32. Terek D, Koroglu OA, Gunes S, et al. Diagnostic tools of metabolic and structural brain disturbances in neonatal non-ketotic hyperglycinemia. Pediatr Int. 2012;54(5):717-720. doi: 10.1111/j.1442-200X.2012.03591.x

33. Bhumika S, Basalingappa KM, Gopenath TS, Basavaraju S. Glycine encephalopathy. Egypt J Neurol Psychiatr Neurosurg. 2022;58(1):132. doi: 10.1186/s41983-022-00567-6

34. Yuan F, Song X, Yin R, et al. The precise molecular diagnosis of novel GLDC compound heterozygous variants highlights the benefits for a Chinese family with nonketotic hyperglycinemia. Mol Genet Metab Rep. 2025;43:101209. doi: 10.1016/j.ymgmr.2025.101209

35. Pang P, Wan L, Liang Y, Zhao X, Yang G. Severe nonketotic hyperglycinaemia due to a synonymous variant. Mol Genet Metab Rep. 2025;45:101268. doi: 10.1016/j.ymgmr.2025.101268

36. Kuseyri Hübschmann O, Juliá-Palacios NA, Olivella M, et al. Integrative approach to predict severity in nonketotic hyperglycinemia. Ann Neurol. 2022;92(2):292-303. doi: 10.1002/ana.26423

37. Farris J, Calhoun B, Alam MS, Lee S, Haldar K. Large scale analyses of genotype-phenotype relationships of glycine decarboxylase mutations and neurological disease severity. PLoS Comput Biol. 2020;16(5):e1007871. doi: 10.1371/journal.pcbi.1007871

38. Bravo-Alonso I, Navarrete R, Arribas-Carreira L, et al. Nonketotic hyperglycinemia: functional assessment of missense variants in GLDC to understand phenotypes of the disease. Hum Mutat. 2017;38(6):678-691. doi: 10.1002/humu.23208

39. Bayrak H, Sharafi P, Özketen AÇ, Kılıç M. Comparative small RNA sequencing reveals candidate functional miRNAs in nonketotic hyperglycinemia. Mol Syndromol. 2026;17(4):347-362. doi: 10.1159/000548948

40. Farris J, Alam MS, Rajashekara AM, Haldar K. Genomic analyses of glycine decarboxylase neurogenic mutations yield a large-scale prediction model for prenatal disease. PLoS Genet. 2021;17(2):e1009307. doi: 10.1371/journal.pgen.1009307

41. Swanson MA, Miller K, Young SP, et al. Cerebrospinal fluid amino acids glycine, serine, and threonine in nonketotic hyperglycinemia. J Inherit Metab Dis. 2022;45(4):734-747. doi: 10.1002/jimd.12500

42. Parviz S, Hooshyar D. Neonatal nonketotic hyperglycinemia: a severe case with prenatal indicators and comprehensive review of recognition and management. Clin Case Rep. 2025;13(1):e70035. doi: 10.1002/ccr3.70035

43. Alcicek S, Oeltzschner G, Lin DDM, Barker PB. Distinguishing myo-inositol from glycine in brain MRS at 3T: a pitfall using intermediate echo times. J Neuroimaging. 2025;35(6). doi:10.1111/jon.70107

44. Ning JJ, Li F, Li SQ. Clinical and genetic analysis of nonketotic hyperglycinemia: a case report. World J Clin Cases. 2022;10(22):7982-7988. doi: 10.12998/wjcc.v10.i22.7982

45. Dinopoulos A, Matsubara Y, Kure S. Atypical variants of nonketotic hyperglycinemia. Mol Genet Metab. 2005;86(1-2):61-69. doi: 10.1016/j.ymgme.2005.07.016

46. Wang JH, Gessler DJ, Zhan W, Gallagher TL, Gao G. Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduct Target Ther. 2024;9(1):78. doi: 10.1038/s41392-024-01780-w

47. Van Hove JL, Vande Kerckhove K, Hennermann JB, et al. Benzoate treatment and the glycine index in nonketotic hyperglycinaemia. J Inherit Metab Dis. 2005;28(5):651-663. doi: 10.1007/s10545-005-0033-x

48. Shelkowitz E, Saneto RP, Al-Hertani W, et al. Ketogenic diet as a glycine lowering therapy in nonketotic hyperglycinemia and impact on brain glycine levels. Orphanet J Rare Dis. 2022;17(1):423. doi: 10.1186/s13023-022-02581-6. Erratum in: Orphanet J Rare Dis. 2023;18(1):54. doi: 10.1186/s13023-023-02646-0.

49. Kök San C, Muslu M, Gökçay G. Ketogenic diet interventions in inborn errors of metabolism: a review article. Clin Exp Health Sci 2024;14:283-95. DOI: 10.33808/clinexphealthsci.1310203

50. Bozacı AE, Erdal İ, Öner G, Clark ÖA, Köken ÖY, Soyuçen E. Clinical and laboratory outcomes of ketogenic versus glycine-restricted diet in nonketotic hyperglycinemia: a comparative study. Mol Genet Metab. 2026;148(2):110118. doi: 10.1016/j.ymgme.2026.110118

51. Kava MP, Robertson A, Greed L, Balasubramaniam S. Ketogenic diet, a potentially valuable therapeutic option for the management of refractory epilepsy in classical neonatal nonketotic hyperglycinemia: a case report. Eur J Clin Nutr. 2019;73(6):961-965. doi: 10.1038/s41430-018-0286-8.

52. Cusmai R, Martinelli D, Moavero R, et al. Ketogenic diet in early myoclonic encephalopathy due to non ketotic hyperglycinemia. Eur J Paediatr Neurol. 2012;16(5):509-513. doi: 10.1016/j.ejpn.2011.12.015.

53. Pizzo F, Collotta AD, Di Nora A, Costanza G, Ruggieri M, Falsaperla R. Ketogenic diet in pediatric seizures: a randomized controlled trial review and meta-analysis. Expert Rev Neurother. 2022;22(2):169-177. doi: 10.1080/14737175.2022.2030220

54. Shbarou RM, Boustany RM, Daher RT, Pakdel P, Noureddine A, Karam PE. Outcome of nonketotic hyperglycinemia in Lebanon: 14-year retrospective review. Neuropediatrics. 2019;50(4):235-243. doi: 10.1055/s-0039-1692207

55. Daida A, Hamano SI, Ikemoto S, et al. Use of perampanel and a ketogenic diet in nonketotic hyperglycinemia: a case report. Neuropediatrics. 2020;51(6):417-420. doi: 10.1055/s-0040-1708536

56. Ichikawa K, Inami Y, Kaneko K. Seventeen-year long-term survival of a case of neonatal nonketotic hyperglycinemia. Pediatr Int. 2020;62(9):1111-1113. doi: 10.1111/ped.14254

57. Vila-Bedmar S, Talbott MLV. Neonatal status epilepticus secondary to nonketotic hyperglycinemia: efficacy of low-dose dextromethorphan. J Pediatr Epilepsy. 2023;12(04):140-143. doi:10.1055/S-0043-1770052

58. Bjoraker KJ, Swanson MA, Coughlin CR 2nd, et al. Neurodevelopmental outcome and treatment efficacy of benzoate and dextromethorphan in siblings with attenuated nonketotic hyperglycinemia. J Pediatr. 2016;170:234-239. doi: 10.1016/j.jpeds.2015.12.027.

59. Mutschler J, Koopmann A, Grosshans M, Hermann D, Mann K, Kiefer F. Dextromethorphan withdrawal and dependence syndrome. Dtsch Arztebl Int. 2010;107(30):537-540. doi:10.3238/arztebl.2010.0537

60. Tekgul H, Serdaroğlu G, Karapinar B, et al. Vigabatrin caused rapidly progressive deterioration in two cases with early myoclonic encephalopathy associated with nonketotic hyperglycinemia. J Child Neurol. 2006;21(1):82-84. doi: 10.1177/08830738060210011801

61. Pereira D, Santos T, Janeiro P, Palaré MJ. Palliative care approach in a case of severe nonketotic hyperglycinemia with long survival: a case report. Cureus. 2025;17(9):e91636. doi: 10.7759/cureus.91636

62. Lopez-Ramirez A, Bali A, Alam MS, et al. Gene therapy prevents disease and death from non-ketotic hyperglycinemia. bioRxiv. 2025:2025.03.26.645560. doi:10.1101/2025.03.26.645560

63. Hoover-Fong JE, Shah S, Van Hove JL, Applegarth D, Toone J, Hamosh A. Natural history of nonketotic hyperglycinemia in 65 patients. Neurology. 2004;63(10):1847-1853. doi: 10.1212/01.wnl.0000144270.83080.29

64. Applegarth DA, Toone JR. Glycine encephalopathy (nonketotic hyperglycinemia): comments and speculations. Am J Med Genet A. 2006;140(2):186-188. doi: 10.1002/ajmg.a.31030

65. Tramontana TF, Wilson TE, Hainline BE. Consideration of a metabolic disorder in the differential of mild developmental delay: a case of nonketotic hyperglycinemia revisited 36 years later. JIMD Rep. 2021;59(1):16-19. doi: 10.1002/jmd2.12208

66. Cao Y, Meng L, Zhang Y, Jiao J, Pu W, Ma L. Novel GLDC compound heterozygous variant leading to nonketotic hyperglycinemia: case report and literature review. Front Pediatr. 2021;9:725930. doi: 10.3389/fped.2021.725930