Novel GLDC Compound Heterozygous Variant Leading to Nonketotic Hyperglycinemia: Case Report and Literature Review

Yanyan Cao1†, Lingzhi Meng2†, Yudong Zhang2, Jiancheng Jiao2, Weicong Pu2 and Li Ma2*

1 Institute of Pediatric Research, Children’s Hospital of Hebei Province, Shijiazhuang, China, 2 Department of Neonatology, Children’s Hospital of Hebei Province, Shijiazhuang, China

Nonketotic hyperglycinemia (NKH) is a lethal autosomal recessive disease resulting from alterations in glycine metabolism, commonly caused by mutations in glycine decarboxylase (GLDC). The symptoms of NKH usually manifest in the neonatal period, and can be categorized into severe NKH and attenuated NKH based on the clinical outcome. To date, only a few NKH cases have been reported in China. We here report a case of a neonate with severe NKH carrying a novel compound heterozygous variant in GLDC. The patient was a 68-h-old girl who had progressive lethargy, no crying, and poor sucking ability from birth, and was therefore transferred to our department. On admission, the patient was supported by intubation and ventilation and presented with profound coma. Metabolic investigation indicated a markedly increased glycine concentration both in the plasma and cerebrospinal fluid (CSF). Symptomatic treatments were administered, but the patient’s condition did not improve substantially. Whole-exome sequencing identified compound heterozygous mutations (c.1261G>C, p.G421R and c.450 C>G, p.N150K) in GLDC, which were inherited from the mother and the father, respectively. The patient was hospitalized for 8 days in our department and died 2 days after discharge. We further summarize the clinical features, genetic characteristics, administered treatment, and prognosis of previously reported Chinese NKH patients for context. Our results highlight that due to the non-specific clinical phenotypes of NKH and difficulty in obtaining CSF samples, genetic testing is a crucial tool, not only for a diagnosis but also for predicting the clinical outcome and can potentially help to determine the optimal therapeutic strategy.

Keywords: nonketotic hyperglycinemia, GLDC variation, compound heterozygous variant, glycine cleavage enzyme system, inherited metabolic disease

INTRODUCTION

Nonketotic hyperglycinemia (NKH) is an autosomal recessive inherited metabolic disease characterized by deficient activity of the glycine cleavage enzyme system (GCS), leading to the accumulation of glycine in almost all body tissues. The GCS consists of four components: glycine decarboxylase (also known as P protein), aminomethyl transferase (T protein), hydrogen carrier
GLDC Nonketotic Hyperglycinemia

protein (H protein), and dihydrolipoamide dehydrogenase (L protein), encoded by \textit{GLDC}, \textit{AMT}, \textit{GCSH}, and \textit{DLD}, respectively. \textit{GLDC} mutations account for \(~80\%\) of NKH cases, whereas \textit{AMT} variants account for \(~20\%\) of cases (1). In the majority of NKH cases, symptoms first appear in the neonatal period or during early infancy. According to the clinical outcomes, NKH is classified into severe NKH and attenuated NKH. Approximately 85\% of cases with neonate onset are classified as severe NKH (2), mainly presenting with progressive lethargy and marked hypotonia, as well as severe apnea that requires ventilation. We here report the case of a neonate with NKH carrying a novel compound heterozygous variant in \textit{GLDC}, and summarize the clinical and genetic features of children with NKH reported in China to date.

**CASE DESCRIPTION**

A 68-h-old girl presenting with lethargy and a poor nutritional state among other symptoms was transferred from a local hospital to our department. She was born at 37 + 3 weeks of gestation by cesarean section due to cephalopelvic disproportion from a gravida 1, parity 1 (G1P1) mother. She had a birth weight of 2,600 g, an Apgar score of 10, and did not present asphyxia. Her parents were healthy and non-consanguine. After birth, the child manifested with progressive lethargy, no crying, and poor sucking ability. She was admitted to a neonatal department in the local hospital at 40 h of life, where she showed low spirit, decreased spontaneous breathing, poor terminal circulation, and blood pressure of 39/22 mmHg. She was intubated and ventilated, and administered 0.9\% sodium chloride and dopamine (12 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\)) intravenously, as well as cefoperazone. However, the patient's condition did not substantially improve, and she was therefore transferred to our department.

On admission, physical examination showed profound coma with intubation and ventilation, yellowish skin, no autonomic activity, no autonomic breathing, no response to orbital pressure stimulation, slow pupil response to light, soft limbs, and poor peripheral blood circulation. Blood gas analysis showed respiratory acidosis (pH = 7.197, P\(_{CO_2}\) = 65.7 mmHg, P\(_{O_2}\) = 93.4 mmHg, [HCO\(_3\)] = 24.9 mM, [Be] = 4.1 mM, and O\(_2\) saturation of 97.4\%). Electrolytes and lactic acid levels were normal, and blood glucose was slightly high (7.8 mM).

**DIAGNOSTIC ASSESSMENT**

After admission, the patient continued to be supported by intubation and ventilation, indwelling catherization, and administration of cefoperazone and sulbactam sodium to combat any potential infection. Because a diagnosis of congenital metabolic diseases could not be excluded, a regimen including

![Figure 1](./image1.jpg)

**FIGURE 1** | Cranial magnetic resonance imaging of the patient. (A) Focal cerebral hemorrhage of the left paraventricular brain. There was no high T1 signal in the posterior limb of bilateral internal capsule. (B) In the diffusion-weighted image, high signals can be seen for the posterior limb of the internal capsule. (C) The thin and short of corpus callosum.

![Figure 2](./image2.png)

**FIGURE 2** | Pedigree and GLDC variations confirmed by Sanger sequencing of the family. The patient (II) had a compound heterozygous variation c.450C>G (p.N150K) and c.1261G>C (p.G421R), which were inherited from her father (I-1) and mother (I-2), respectively.
TABLE 1 | Clinical characteristics and GLDC variants in Chinese NKH children.

| Case | Sex | Age of onset | Clinical presentation | Exons | Mutation | Inherited | Plasma glycine (normal range) (µmol/L) | CSF glycine (normal range) (µmol/L) | CSF/plasma ratio (normal ratio) | Treatment | Outcome | References |
|------|-----|--------------|-----------------------|-------|----------|-----------|----------------------------------------|--------------------------------------|-----------------------------------|-----------|---------|------------|
| 1    | Male | 1 d          | poor feeding and decreased activity, lethargy, hypotonia, absent deep tendon reflexes, developmental delay, myoclonic seizures | 21/20 | c.2516A>G (p.Y839C)/c.2457 + 2T>A | Mother/father | 947.8 (115–600) | 226.4 (3–20) | 0.24 (< 0.02) | Sodium benzoate, pyridoxine, dextromethorphan, dietary restriction of natural protein intake | Alive at 1 year 7 months: improved deep tendon reflexes and muscular hypotonia; but still poor feeding and intellectual disability | Liu S, et al. (5) |
| 2    | Male | 11 h         | early metabolic encephalopathy and Ohtahara syndrome | 15/4–15 | c.1786 C>T (p.R596X)/Exon 4–15 deletion | Mother/father | Normal | NA | NA | Adreno-corticotropin-hormone, topiramate and dextromethorphan | Died at 4 m | Gao Z, et al. (6) |
| 3    | Male | after birth  | progressively poor reaction and weak crying | 21/21 | c.2198 C>T (p.A733V) | Mother/father | 1304.32 (130–650) | NA | NA | Antibiotics, mechanical ventilation and nutritional support | Died after 4 d | Dai H, et al. (7) |
| 4    | Male | 5 d          | poor reaction, feeding difficulty and limb tremor | 13/9p24.3p22.3 | c.1607G>A(p.R536Q)/9p24.3p22.3 deletion | Mother/NA | 1409.16 (125–750) | NA | NA | Cardiopulmonary resuscitation and trachea intubation and mechanical ventilation | Died at 6 d | Cheng L, et al. (8) |
| 5    | Male | 9 m          | intractable seizure | 25/9 | c.3006C>G (p.C1002W)/c.1256C>G (p.S419X) | Mother/father | 75 (0–276) | 45.3 (1.6–19.5) | 0.06 (≤ 0.02) | NA | Alive at 6 year 8 months: intractable seizure, severe bilateral spastic paralysis and intellectual disability | Jiang T, et al. (9) |
| 6    | Female | 2 year | ataxia, chorea and behavioral abnormality | 25/9 | c.3006C>G (p.C1002W)/c.1256C>G (p.S419X) | Mother/father | Normal | 36.7 (1.6–19.5) | 0.13 (≤0.02) | NA | Alive at 3 year 5 months: language retardation, ataxia, chorea and behavioral problem | |
| 7    | Male | 2 d          | Lethargy, hypotonia, seizures, apnea | 23/3 | c.2680A>G(p.T894A)/Exon 3 deletion | Mother/father | NA | NA | NA | NA | Died at 11 d | Lin Y, et al. (10) |
| 8    | Male | 2 d          | Lethargy, hypotonia, seizures, apnea, hiccup | 23/3 | c.2680A>G(p.T894A)/Exon 3 deletion | Mother/father | 1587.87 (232–740) | 260.2 (2.2–14.2) | 0.164 (<0.08) | NA | Died at 13 d | |

(Continued)
TABLE 1 | Continued

| Case | Sex | Age of onset | Clinical presentation | Treatment | Outcome | References |
|------|-----|-------------|----------------------|-----------|---------|------------|
| 9    | Female | 3d         | Lethargy, hypotonia, seizures, hiccup | Sodium benzoate, dextromethorphan, antibiotics, mechanical ventilation, vitamin B12, B6, and C, folic acid, coenzyme Q10, and levocarnitine | Alive at 7 months: severe intellectual disability, frequent seizures | This study |
| 10   | Female | after birth | Lethargy, no crying, poor feeding and hypotonia | Antibiotics, mechanical ventilation, vitamin B12 and B6, folic acid, coenzyme Q10 and levocarnitine | Died at 13 days | This study |

The patient's sample was not obtained, and his parents carried a heterozygous mutation in GLDC.

Copy number variation was not analyzed in the parents.

NA, Not available.

The metabolic spectrum of the cerebrospinal fluid (CSF) also showed a markedly elevated glycine level, reaching a value of 3,460 times the control. The ratio of CSF to plasma glycine was 0.12 (normal < 0.08). A comprehensive panel of urine organic acids was normal.

Whole-exome sequencing (Beijing Fulgent Technologies Inc., Beijing, China) indicated that the patient had compound heterozygous variants in GLDC (reference genome hg 19, NM_000170.2): c.1261G>C (p.G421R) in exon 9 and c.450 C>G (p.N150K) in exon 3, which have not been previously reported. Sanger sequencing also confirmed that the two mutations were inherited from her mother and father, respectively (Figure 2). According to American College of Medical Genetics and Genomics guidelines, these two missense mutations are classified as likely pathogenic. The two mutations were inherited from the mother and father, respectively. Mutations were absent from controls in the GnomAD, 1000 Genomes Project, Exome Aggregation Consortium, and dbSNP databases. Bioinformatic prediction analyses using Mutation_Taster, PolyPhen2, and SIFT indicated that they were deleterious. Two other two missense mutations, p.G421V and p.N150T, at the same amino acid position had been identified as pathogenic. A diagnosis of NKH was finally made based on the clinical symptoms, elevated glycine concentration in the plasma and CSF, and increased glycine CSF/plasma ratio.

After 8 days of comprehensive anti-infection treatment, mechanical ventilation, and other symptomatic care, the patient’s conscious reaction, limb movement, and peripheral circulation improved, although she still had no obvious spontaneous breathing. Considering the poor prognosis, the parents ceased treatment, and the patient died 2 days after discharge.

DISCUSSION

Approximately 80% of the NKH-causing GLDC mutations are sequence variations, whereas the remainder are exonic copy number variations (CNVs) (3). Among the sequence variations, missense mutations are the most frequent, followed by nonsense mutations, splice-site mutations, and small insertions/deletions.
In addition, some recurrent mutations have been identified in the United Kingdom and Finland, because of a founder effect, almost all of them unique (3, 4).

To date, 13 GLDC variants, including seven missense mutations (53.8%), three CNVs (23.1%), two nonsense mutations (15.4%), and one splice-site mutation (7.7%), have been identified in 10 Chinese children with NKH from 7 different families (Table 1) (5–10). Among 11 alleles identified with single-nucleotide variants, three (27.3%) were in exon 21 and two (18.2%) were in exon 9. No mutations in AMT have been identified in Chinese children suffering from NKH to date. In this study, we detected two heterozygous missense mutations in a neonate with severe NKH, c.1261G>C (p.G421R) and c.450 C>G (p.N150K), which were inherited from the mother and the father, respectively.

Different types of genetic variants can lead to a complete absence or different degrees of residual GCS enzyme activity, thus leading to distinct clinical phenotypes. For example, loss-of-function mutations such as a copy number (11), frameshift, nonsense, or splice site variation (12) causing complete depletion of GCS activity can lead to a severe phenotype (3, 13–15). However, it is difficult to assess the residual GSC activity in the case of missense mutations (4, 16). In addition to the present report of a severe case of NKH, there have been seven severe cases of NKH and two cases of attenuated NKH reported in China. Among the 12 alleles associated with severe NKH (eight cases from six unique families), seven (58.3%) were missense mutations, whereas in the two alleles associated with the attenuated cases (two cases from one family), only one was a missense mutation. Although the effect of the novel mutations identified in this study, p.G421R and p.N150K, on GCS activity is not clear, this patient was diagnosed with severe NKH according to the age of onset and clinical characteristics, suggesting that both of them were null mutations. Furthermore, the same amino acid positions of GLDC, p.G421V and p.N150T have been previously reported in three different patients with NKH. A patient carrying the p.N150T and p.R790W mutations presented with hypotonia apnea, coma intraventricular hemorrhage since the 3 days of life; her electroencephalogram showed a suppression burst pattern and brain MRI was normal; the concentration of glycine in CSF and in serum, and the CSF/serum ratio were 270 umol/L, 880umol/L and 0.31, respectively. At 16 days of age, sodium benzoate and dextromethorphan were administered. Imipramine was initiated at 7 years of age. In vitro expression analyses showed that the mutant glycine decarboxylases with p.N150T had 1% normal enzyme activity. However, the patient had far better psychomotor development because the other mutation, p.R790W, retained 14% of the enzyme activity (16). Kure et al. reported another Asian patient with p.N150T and c.1926 + 1G>A mutations (14). Her glycine level in CSF and the CSF/serum ratio were 148 mM and 0.12, respectively. No other phenotype was described. For residue 421 of glycine decarboxylase, variations of p.G421V and p.A729Efs*3 have already been described as associated with NKH (3). No other clinical data are available.

Currently, there are no formal management guidelines for NKH. The current treatment strategy focuses on reducing the plasma glycine concentration, blocking N-methyl-D-aspartate receptors, and symptomatic care (17, 18). Treatment information is available for six patients with severe NKH previously described in the literature (Table 1). Among three cases treated with dextromethorphan and/or sodium benzoate, one died at 4 months (case 2 in Table 1) and two were alive with severe intellectual disability when followed up at 1 year and 7 months (case 1 in Table 1) and 7 months (case 9 in Table 1), respectively. The remaining three cases treated only with symptomatic care died from 4 to 13 days of life. Although information about the treatment provided was not available for the two siblings with attenuated NKH (case 5 and case 6 in Table 1), valproate administration leading to aggravation of the condition in the early stage of the disease was reported for case 5. Based on this, valproate is contraindicated for controlling epileptic seizures in NKH patients (19). In addition, based on previous case reports, vigabatrin should be avoided to treat West syndrome in NKH (20).

Since the genetic basis of NKH is well-defined and increased residual GCS activity has been associated with an improved clinical outcome, therapeutic strategies that can enhance the residual activity associated with a mutation should be pursued. For example, a translational read-through inducer has been used to promote translation through a premature stop codon (21), chemical chaperones have been used for unstable missense mutations to restore protein folding (22), and antisense oligonucleotides have been used to correct a splicing variation (23). Considering that CSF samples of neonatal children are not easily obtained, genetic testing is a powerful tool for diagnosing NKH, especially for patients with a normal plasma glycine level (case 2 in Table 1). Early use of genetic testing to diagnose NKH can avoid unnecessary drug-induced damage, as well as provide guidance to the family for future pregnancies.

**CONCLUSION**

We reported a patient with severe NKH who carried a novel compound heterozygous mutation and summarized the genetic and phenotypic characteristics, as well as the treatment strategy followed and prognosis of other NKH cases reported in China to date. This case report highlights the importance of genetic testing not only as a tool for NKH diagnosis but also for clinical outcome prediction, and the potential development of novel therapeutic strategies in the future.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and Hebei Province. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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