Clinical and genetic analysis of nonketotic hyperglycinemia: A case report

nonketotic hyperglycinemia

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Abstract

BACKGROUND

Nonketotic hyperglycinemia (NKH), a rare disease, is an autosomal recessive genetic disorder of abnormal glycine metabolism caused by insufficient activity of the glycine cleavage enzyme system. Glycine is believed to function mainly as an inhibitory neurotransmitter, but it can also act as a co-agonist of the N-methyl-D-aspartate (NMDA) receptor. The accumulation of a large amount of glycine in the brain leads to neuronal and axonal injury via overactivation of NMDA receptors located in the hippocampus, cerebral cortex, olfactory bulb and cerebellum and to stimulation of the inhibitory function of glycine receptors located in the spinal cord and brain stem, resulting in central apnea, hiccups and hypotonia in the early stage of the disease.

CASE SUMMARY

The child described in this report had typical clinical manifestations of NKH, such as hiccups, disturbance of consciousness, hypotonia and convulsions, within the first week after birth. Whole-exome genetic testing revealed that the child had a compound heterozygous mutation, namely, c.395C>A (p.S132X) and c.2182G>A (p.G728R), in the GLDC gene through, and he was diagnosed with NKH. For treatment, we administered an oral levetiracetam solution and added topiramate and prednisone for epilepsy control, but the epilepsy remained uncontrollable. Ketogenic diet therapy was started at 6 mo of age, his seizures were significantly reduced, and there were no obvious adverse reactions during ketogenic treatment. Furthermore, we found that with the development of the disease, high levels of serum glycine decreased or even disappeared without intervention, and as the disease progressed, the corpus callosum became dysplastic.

CONCLUSION
This case shows that plasma glycine levels cannot be used to evaluate the prognosis of NKH, that the development of the corpus callosum can be affected by NKH, and that a ketogenic diet may be effective for seizure control in NKH patients.

**Key Words:** Nonketotic hyperglycinemia; Compound heterozygosity; GLDC gene; Case report


**Core Tip:** Herein, we present the case of a child who had typical clinical manifestations of NKH, such as hiccups, disturbance of consciousness, hypotonia and convulsions within the first week after birth. These symptoms combined with the results of gene testing led to a diagnosis of classical nonketotic hyperglycinemia caused by compound heterozygous variants in the GLDC gene. Plasma glycine levels cannot be used to evaluate the prognosis of NKH, and the corpus callosum can be affected by NKH. A ketogenic diet may be effective for seizure control in NKH patients.

**INTRODUCTION**

Nonketotic hyperglycinemia (NKH), also known as glycine encephalopathy, is an autosomal recessive genetic disease with abnormal glycine metabolism caused by insufficient activity of the glycine cleavage enzyme system (GCS), and NKH is clinically characterized by the abnormal accumulation of glycine in all tissues of the human body, especially in the serum and cerebrospinal fluid. According to an epidemiological survey of 55,000 newborns, the incidence of NKH is approximately 1/63,000. The GCS is composed of glycine decarboxylase (P protein), aminomethyl transferase (T protein), hydrogen carrier protein (H protein) and dihydroamide dehydrogenase (L protein). The P, T and H proteins are encoded by the GLDC (OMIM 238300), AMT (OMIM 238310) and GCSH (OMIM 238330) genes, respectively. Variations in these genes can cause a
decrease in GCS activity and lead to glycine accumulation, and 70%-75% of NKH patients carry GLDC variations [3]. Here, we present a case of a young boy who presented with clinical features of NKH and was ultimately diagnosed by whole-exome genetic testing.

CASE PRESENTATION

Chief complaints
A child, male, aged 7 days, was admitted to the neonatology department of our hospital on December 30, 2020, due to "eating less, crying less and moving less for 7 days".

History of present illness
The patient was the firstborn child and was delivered vaginally at full term, with a birth weight of 3.75 kg. The Apgar scores at 1, 5, and 10 minutes after birth were all 10 points. He was provided a reasonable amount of food after birth but had low sucking power, hiccups and occasional apnea. The mother denied a history of exposure to poisons, chemicals or radiation and had regular prenatal examinations during pregnancy; no abnormality was found. The parents did not have blood relations.

History of past illness
No history of past illness.

Personal and family history
There was no history of family hereditary diseases.

Physical examination
Upon admission examination, the following was observed: body temperature, 36.8°C; heart rate, 128 beats/min; respiratory rate, 34 times/min; arterial blood pressure, 83/46 mmHg; SpO₂, 95%; slightly dry skin; poor elasticity; no rash or ecchymosis on the skin; irregular breathing; no obvious three concave sign; trachea in the middle; rough
tracheal sounds in both lungs; and no wet rales. Examination of the heart and abdomen did not reveal any abnormalities. Neurological examination: no response after stimulation; the anterior fontanelle measuring 1.0 cm × 1.0 cm that was flat and soft; hypotonia; and an inability to elicit primitive reflexes. A few hours after admission, the child was observed to have frequent apnea neonatorum.

**Laboratory examinations**

Arterial blood gas analysis showed the following: pH, 7.16 (reference range: 7.35–7.45); PCO₂, 96 mmHg (reference range: 35–45 mmHg); PO₂, 276 mmHg (reference range: 80–100 mmHg); HCO₃⁻, 34.2 mmol/L (reference range: 21.4–27.3 mmol/L); extracellular fluid base excess, 5.5 mmol/L (reference range: -3–3 mmol/L); lactic acid, 0.9 mmol/L (reference range: 0.5–2.2 mmol/L); and blood ammonia, 100 µmol/L (reference range: 18–72 µmol/L). An electroencephalogram showed that diffuse low-amplitude irregular 1–6 Hz δ, θ waves and low-amplitude β waves were mixed in the quiet state, and the external stimulation background did not change. The electroencephalogram (EEG) activity voltage was low, which represented a moderately abnormal neonatal EEG. Serum tandem mass spectrometry showed that the glycine concentration was 850.05 µmol/L (reference range: 130–650 µmol/L), and urine organic acid analysis showed no obvious abnormality. CSF glycine levels were not measured. A routine blood test, routine blood coagulation test, myocardial enzyme, C-reactive protein, procalcitonin, liver and kidney function tests, electrolyte assessment, and cerebrospinal fluid and biochemistry tests did not show obvious abnormalities.

**Imaging examinations**

A head magnetic resonance imaging (MRI) in the neonatal period (aged 7 days old) showed that a myelinated T1 hypersignal was not found in the hind limbs of the bilateral internal capsules or cerebellar dentate nucleus, and no abnormal corpus callosum was found. When the child was 2 mo old, re-examination of the head MRI showed that the corpus callosum was smaller than it was on earlier imaging, the
bilateral ventricles were full and irregular (more pronounced on the left side); the corticospinal tract, the white matter of bilateral ventricles and the parietal lobe showed symmetrical high signal intensity on diffusion-weighted imaging (DWI); the apparent diffusion coefficient (ADC) map showed slightly low signal intensity (see Figure 2).

**WHOLE-EXOME SEQUENCING**

The proband has a variant on exon 8, position chr9:6620259G>T, NM_000170.3:c.395C>A, p.(Ser132*) and a variant on exon 18, position chr9:6556173C>T, NM_000170.3:c.2182G>A, p.(Gly728Arg). The p.(Ser132*) variant has been described in one individual in the gnomAD database v3.1.1 (entry: 9-6620259-G-T). Its allele frequency is 0.000006573. It is reported on dbSNP (rs386833576). According to ACMG guidelines, this variation was judged to be a pathogenic variation based on the supporting evidence (PVS1+PM2+PM3). In the pedigree analysis, the father of the proband had no mutation at this site, while the mother of the proband had a heterozygous mutation at this site. The variant p.(Gly728Arg) has been reported on ClinVar as likely pathogenic (accession number VCV000580932.2), and it has been described on dbSNP (rs386833542). Its allele frequency on gnomAD database v2.1.1 is 0.000003977. According to ACMG guidelines, this variation was judged to be a pathogenic variation based on the supporting evidence (PS1+PM1+PM2+PM5+PP3). By pedigree analysis, the father of the proband had heterozygous variation at this site, while the mother had no variation at this site. The parents of the child were heterozygous, with a normal phenotype, which is consistent with the pathogenesis of autosomal recessive compound heterozygous genetic diseases.

**FINAL DIAGNOSIS**

The final diagnosis was classical nonketotic hyperglycinemia and epilepsy syndrome.

**TREATMENT**
After admission, invasive ventilation, aggressive anti-infective therapy and symptomatic treatments were given. After one week, the child could obviously breathe spontaneously, the arterial blood gas analysis was basically normal after re-examination, and the ventilator was successfully withdrawn. However, during hospitalization, the child developed convulsions, characterized by loss of consciousness, staring in both eyes, clenching of fists with both hands, and chewing movements of the lips. After demonstrating rigidity of the limbs, the child quickly developed atonic seizures that lasted for 15-220 s each time and occurred 3-5 times a day. Re-examination by EEG still showed abnormalities. During the attack, persistent multifocal or extensive irregular sharp waves or sharp slow waves were observed, most of which were in a burst-suppression state, and the inhibition segment lasted for 2-66 s (see Figure 1). Levetiracetam was added to relieve convulsive treatment.

OUTCOME AND FOLLOW-UP

When the child was 2 mo old, he had good suckling and swallowing but still had repeated convulsions, with hypotonia in the extremities. Re-examination by EEG showed a burst-inhibition state. The blood glycine level became normal. For treatment, we continued to administer an oral levetiracetam solution (60 mg/kg/d) and added topiramate (5 mg/kg/d) and prednisone (2 mg/kg/d) for epilepsy control. Unfortunately, the epilepsy of the child remained uncontrollable. Ketogenic diet therapy with a 4:1 (lipid:nonlipid) ketogenic milk formula was started at 6 mo of age, ensured daily calorie and protein requirements, and monitored the child's urinary ketones daily. The number of attacks and adverse reactions were recorded during ketogenic diet treatment. On the 25th day after ketogenic treatment, the child's seizures were completely controlled, and the EEG improved after review. No serious adverse reactions occurred during the ketogenic treatment (see Figure 3).

DISCUSSION
Nonketotic hyperglycinemia is a rare inherited genetic metabolic disease with variable clinical manifestations. Three types of glycine encephalopathy have been identified according to the clinical phenotype and the presence or absence of genetic variation: classic, atypical and transient. Most neonatal NKH cases are classified into the classic type, showing a normal phenotype at birth. In most cases, drowsiness, coma, hiccups, hypotension and myoclonic seizures gradually appear in the first week after birth and develop into central apnea requiring ventilator-assisted breathing, with a mortality rate as high as 50% at this time[4]. These symptoms subside on their own after 1 to 3 wk, but surviving infants experience serious nervous system sequelae within 6 mo, such as epileptic encephalopathy, developmental delay, and growth retardation[5-6]. Atypical NKH is rare and has heterogeneous and nonspecific disease courses, which make the diagnosis more difficult. If hypotonia, developmental delay and epilepsy occur in infancy and the symptoms are milder than those of classic NKH, it is necessary to pay attention to this type of possibility. Transient NKH is even rarer; although it develops after birth, as the activity of glycine lyase increases, it may heal itself within a few months.

In NKH, serum and cerebrospinal fluid glycine levels are elevated, and the ratio of cerebrospinal fluid to plasma glycine is greater than 0.08. Absence of ketoacidosis and urine organic acid abnormalities indicate the diagnosis of nonketotic hyperglycinemia. However, perinatal medication (especially the use of sodium valproate), congenital intrauterine infection and neonatal asphyxia can cause the level of neonatal glycine to rise; thus, the diagnosis ultimately depends on the pathogenic variant of the GCS genes. Notably, transient NKH has a good prognosis, but its onset is similar to that of the classic type; therefore, it is necessary to distinguish between the two. Our case showed that if the child’s symptoms gradually improve, the serum fluid glycine levels have been repeatedly tested and decrease or become normal, and the genetic test does not show any genetic mutations related to the disease, then it may be a temporary type of NKH, but additional data from more cases are needed for further verification.
The child described in this report had typical clinical manifestations of NKH, such as hiccups, disturbance of consciousness, hypotonia and convulsions, within the first week after birth. Although cerebrospinal fluid glycine was not measured, no organic acid abnormality was found by blood and urine tandem mass spectrometry and gas chromatography. Combined with the gene detection results for the child, a diagnosis of classic NKH caused by compound heterozygous variations in the GLDC gene was made. To date, only 4 NKH patients with compound heterozygote variations in the GLDC gene have been reported in China[7-9]. In this study, a nonsense mutation c.395C > A was found in the GLDC gene, which led to the replacement of serine at position 132 of the coding region by a termination codon (p.S132X), which may lead to the loss of gene function. This is the first time that this mutation has been found in the Chinese population. Compound heterozygosity with another pathogenic mutation may be the basis of the pathogenesis of NKH in this child, which enriches the variation spectrum of the GLDC gene.

A retrospective cross-sectional study showed that a small corpus callosum is the most common structural abnormality of NKH and that this structural abnormality is directly related to the severity of the clinical phenotype[10]. In this case, no abnormal corpus callosum was found on neonatal magnetic resonance imaging, but with the development of the disease, the corpus callosum became dysplastic, suggesting that the corpus callosum could be affected by glycine metabolism. Other studies have found that EEG can evaluate the therapeutic effect of each stage and provide a clinical basis for adjusting the administration scheme and its dosage. Plasma glycine levels cannot be used to evaluate the prognosis of NKH, as this study found that a high serum glycine level can decrease or even disappear by itself, but the EEG will still show a burst-inhibition state, which further indicates that NKH is an irreversible brain injury. At present, there is no effective treatment for this rare disease, and the focus of treatment is to rationally use antiepileptic drugs to control epileptic seizures, reduce the plasma concentration of glycine by injecting sodium benzoate, and antagonize NMDA receptors by injecting ketamine or oral dextromethorphan. The ketogenic diet is
a high-fat, low-carbohydrate and moderate protein diet that is used mainly for the adjuvant treatment of drug-resistant epilepsy and epileptic encephalopathy. It has been reported that a ketogenic diet has a good effect on infantile spasm, Dravet syndrome, Lennox-Gastaut syndrome, and epileptic encephalopathy caused by gene mutations such as SCN1A, KCNQ2, STXB1 and SCN2A. This case showed that a ketogenic diet may be a valuable treatment modality for refractory seizure control in classical NKH.

CONCLUSION
This study found that a high serum glycine level can decrease or even disappear on its own, indicating that plasma glycine levels cannot be used to evaluate the prognosis of NKH. With the development of the disease, the corpus callosum can be affected by glycine metabolism. A ketogenic diet may be effective for seizure control in classical NKH patients.
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