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# A Case Report of a Patient with Nonketotic Hyperglycemia Caused by a Novel Splicing Mutation in the *AMT* Gene

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**KEYWORDS** Alternative Splicing. *AMT Gene*. Autosomal Recessive Inheritance. Nonketotic Hyperglycinemia. Whole Exome Sequencing

**ABSTRACT** Nonketotic hyperglycinemia (NKH) is an inborn disease. Mutations in related genes lead to impaired glycine metabolism, allowing the accumulation of excessive glycine in body tissues. Patients usually develop severe neurological symptoms early. In this report, whole exome sequencing (WES) was applied to a female proband with hyperglycinemia and her unaffected parents. Meanwhile, cDNA sequencing was conducted to study the impact of the mutation on transcription. The results revealed that the proband exhibited a homozygous mutation of c.471+3A>G in *AMT*. The cDNA sequencing results demonstrated that this mutation resulted in the skipping of exon 4, leading to the loss of 132 nucleotides. This report enriched the study of *AMT* gene mutations. In addition, the researchers discussed the genetic and clinical heterogeneity of NKH, providing valuable insights for genetic counselling.

## INTRODUCTION

Nonketotic hyperglycinemia (NKH), or glycine encephalopathy, manifests as a disorder of glycine metabolism that allows glycine to accumulate excessively in body tissues, particularly in the brain tissue. The elevated level of glycine has been observed in both cerebrospinal fluid (CSF) and plasma of affected individuals, with an abnormally high CSF/plasma glycine ratio (Nowak et al. 2022). NKH can be classified into three types based on the timing of symptom onset, that is, neonatal, infantile, or late-onset (Kure et al. 2006). Late-onset NKH is associated with milder symptoms and diverse clinical presentations. Previous reports found that the late-onset has no or mild epilepsy and variable psychiatric development (Swanson et al. 2015). However, most newborn patients presented with severe symptoms such as deep coma and significant hypotonia. Approximately 30 percent of such patients died in this period (Kure et al. 2006). Clinical presentations of infantile type often had no apparent lethargy or coma in the neonatal period except possibly hypotonia. There might be mild developmental delays and infantile seizures. Some patients might become severe later.

*GLDC* gene and *AMT* gene encode the glycine cleavage enzyme system (GCS) P-protein component and T-protein component, respectively. Biallelic pathogenic mutations in the two genes are common genetic causes of NKH. Mutations in *GLDC* were detected in 80 percent of NKH patients while the rest of 20 percent patients were caused by mutations in *AMT* (Coughlin et al. 2017). In addition, two suspected NKH individuals were found in homozygous state of *GCSH* gene mutation (Majethia et al. 2021). However, evidence of the pathogenicity of NKH caused by the *GCSH* gene is still lacking.

In this case, the researchers reported a Chinese Yi girl diagnosed with NKH after birth. WES was performed for the girl and her parents to reveal the genetic aetiology. After analysis of the sequencing results, a novel mutation in the *AMT* gene, which could explain the symptoms, was identified for the girl. The researchers further studied the impact of this mutation on transcription by sequencing cDNA. Combined with the patient's clinical examinations and genetic test results, the patient was confirmed with NKH. The results also provided valuable information on genetic counselling for the parents who planned to have another child in the future.

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### Objectives

The present study is aimed to report a nonketotic hyperglycinemia patient caused by a novel variant in *AMT*. The researchers expect this report to shed light on the value of genetic tests in the diagnosis of this disease.

## MATERIAL AND METHODS

### Subject

In this study, the proband was a female newborn who developed the following symptoms shortly after birth, such as hypotonia, poor feeding, coma and myoclonus. She later died of respiratory failure. Cranial computerised tomography (CT) scan showed subarachnoid haemorrhage and small hemorrhagic foci in the cerebellar hemispheres bilaterally. Video-EEG recording showed that the background wave was dominated by continuous low voltage, suggesting moderate to severe abnormalities. No significant abnormalities were observed for reactive C protein or blood glucose. Metabolic screening showed increased plasma glycine level (1095.95 umol/l) and increased Glycine/Phenylalanine ratio (18.08).

The parents were close relatives. They had four other children before the patient in this case. Except for the first child who is seven years old and is unaffected, the other three had similar symptoms to the patient in this case and died in the neonatal period because of respiratory failure. The pedigree is shown in supplementary Figure S1.

### Sequencing

Informed consent to participate in this study was obtained from all subjects and the experiment was approved by the Ethical Review Board of West China Second University Hospital, Sichuan University (Tan et al. 2023). Genomic DNA (gDNA) of the individuals' (IV1, IV2, V1, V5 ) were extracted

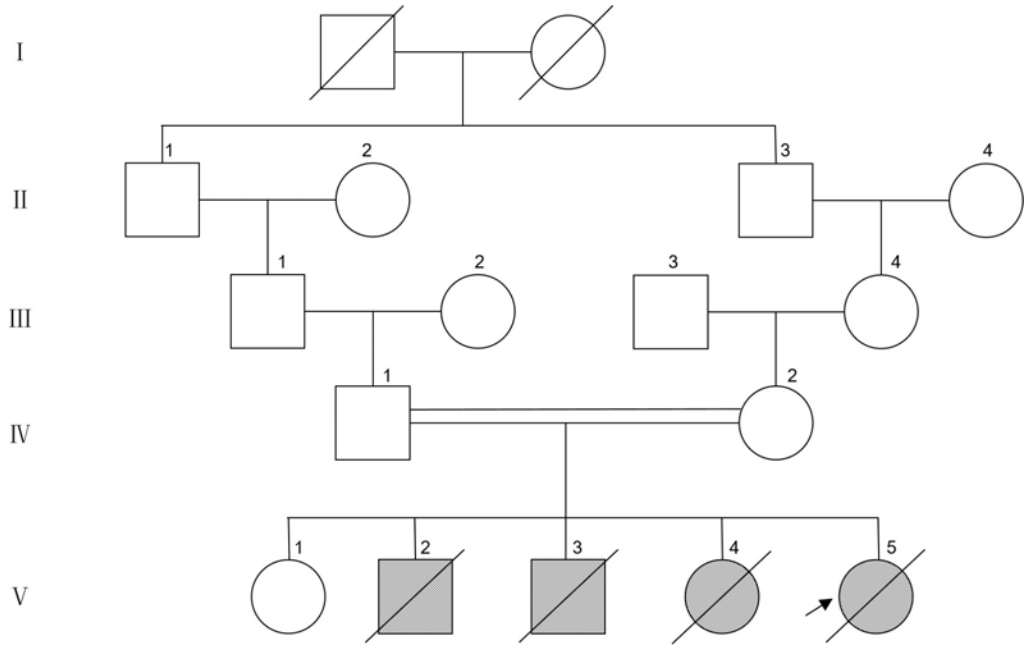


**Fig.1. Validation of the candidate mutation (AMT, c.471+3A>G) of gDNA (?1, ?2, ?1, ?5) by Sanger sequencing on gDNA: the red rectangle indicates where the mutation occurs**

by QIAamp DNA Blood Mini Kit (QIAGEN). Then whole exome sequencing was conducted on Illunima NovaSeq6000 platform (Illunima). After alignment to the reference human genome GRCh38/hg38 with Burrows-Wheeler Aligner software (BWA), single-nucleotide mutations (SNVs) and small insertions or deletions (InDels) were identified by GATK Unified Genotyper (DePristo et al. 2011). Functional annotation was performed by ANNOVAR (Wang et al. 2010).

Sanger sequencing targeting the identified mutation on gDNA and cDNA were both performed. Primers were designed and shown in Table S1. The cDNA of the proband's parents and a negative control was obtained by reversing the total RNA by a synthesis kit (Thermo Scientific, K1622). After cleaning up of PCR products, Sanger sequencing was performed on ABI 3500 Genetic Analyzer (Thermo Fisher Scientific).

## RESULTS

After analysis the sequencing results, the researchers identified a homozygous mutation (c.471+3A>G) in the *AMT* gene, inherited from her parents, respectively. This mutation has not been reported previously. The mutation was further validated through Sanger sequencing. The patient's unaffected sister was also sequenced for this variant. The results confirmed that except the proband, which was homozygous, the parents and sister were all heterozygous (Fig. 1). Unfortunately, because the siblings died young and had no specimens preserved, co-segregation could not be verified.

The mutation *c.471+3A>G* in *AMT* was predicted to impact splicing by spliceAI software (Jaganathan et al. 2019). To figure out how this mutation impacts splicing, the researchers performed cDNA sequencing of the parents because limited dried blood spots of the proband were preserved, which makes it difficult to extract enough total RNA for detection. Meanwhile, the researchers selected an unrelated individual without the mutation as a negative control. Figure 2A shows the gel electrophoresis results of target PCR products on the cDNA level of the proband's parents and a negative control. Two bands (Band 1 and Band 2) were shown on both parents' gel lanes, whereas only Band 1 was observed for the negative control. The cDNA sequencing results demonstrated that Band 1 and Band 2 corresponded to the initial transcription sequence and the alternative splicing sequence with exon 4 skipping in the *AMT* gene (Fig. 2B). Exon 4 of the *AMT* gene encodes 44 amino acids and therefore the skipping of exon 4 does not result in a reading frame shift.

Therefore, *c.471+3A>G* in *AMT* was classed as "likely pathogenic" (PVS1_strong, PM2_ moderate, PP4) based on the ACMG criteria (Richards et al. 2015).

## DISCUSSION

NKH has clinical heterogeneity. The worldwide incidence of NKH at birth was around 1:76,000 (Coughlin et al. 2017). Patients onset in the neonatal period usually developed severe NKH symptoms. Symptoms worsen within a short period after birth, with about 30 percent of children dying in the neonatal period and most dying within 1 year of age (Zhou et al. 2022). The proband in this case was a classic NKH patient who developed severe symptoms during the first four days after birth and ended up dying of respiratory failure. Previous reports indicated that female children appeared to have more severe neurologic symptoms, a poorer prognosis, and a relatively higher neonatal mortality rate (Hoover-Fong et al. 2004; Dulac 2013). In this case, the researchers reported a female patient who was in a homozygous state of *c.471+3A>G* in *AMT*. The proband had four siblings, three of whom had similar symptoms to the proband and died as a newborn. Unfortunately, they did not have specimens retained for co-segregation validation. The parents in this case are consanguineous, which greatly increases the risk of autosomal recessive disorders. Genetic testing is therefore important and valuable in such families. Once the disease-causing gene has been identified, assisted reproductive techniques such as preimplantation genetic testing (PGT) can be an option.

**Table S1: Primers used in the current study**

| *Primers* | *Forward* | *Reversed* |
|---|---|---|
| gDNA primers | GCCTGGAGGTAATGTGAGT | GGGACACTGTCGCTGTTTA |
| cDNA primers | GTGAAGCTGATGGAGAGTC | GAACGGAGAAGAGGAACTC |

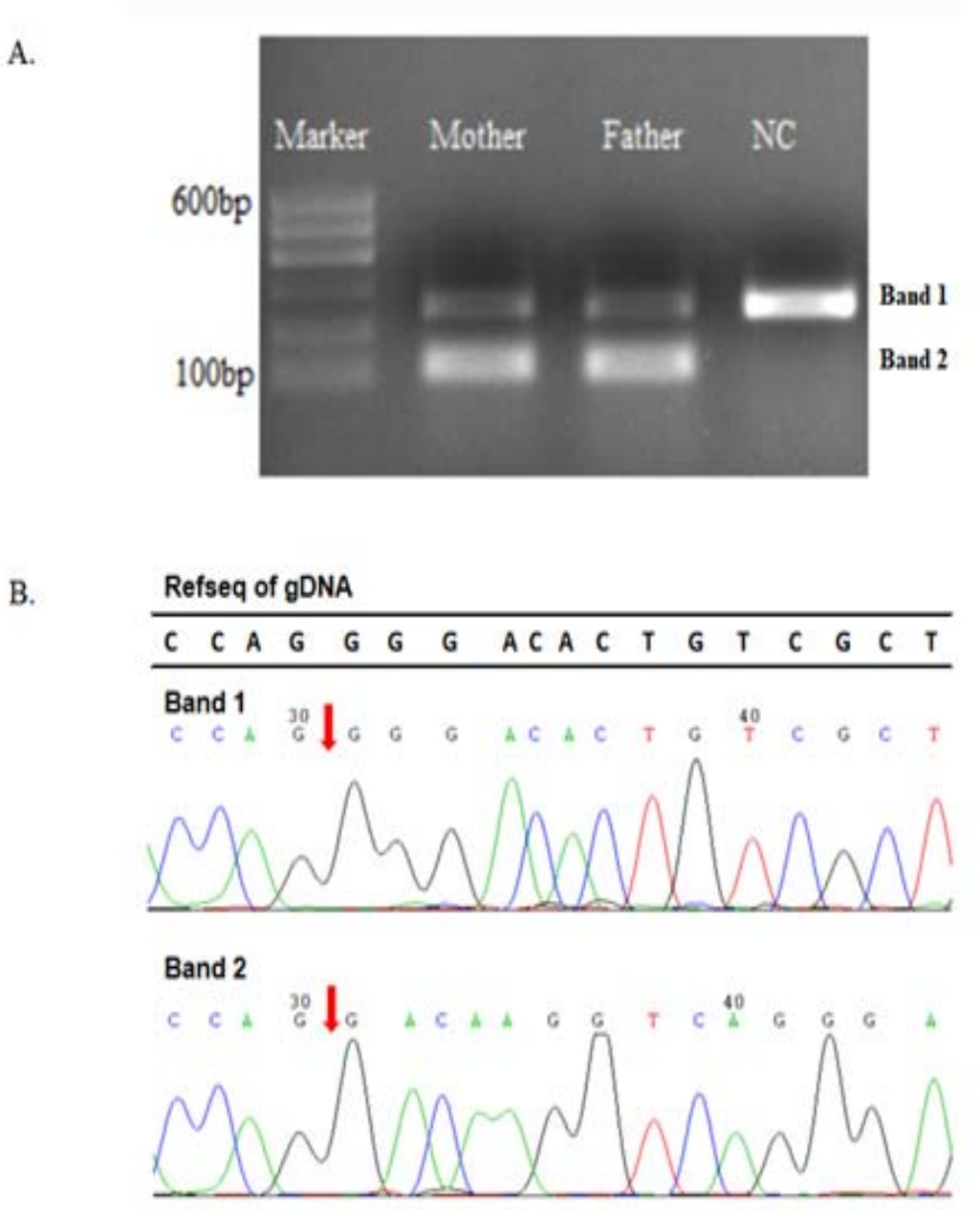


**Fig.2. (A) Gel electrophoresis of targeted cDNA fragments:Only band 1 was shown on the negative control (NC) lane, while two bands (band 1 and band 2) were displayed on both the parents' lanes. (B) Sanger sequencing results of cDNA PCR products of band 1 and band 2, respectively. The red arrow indicates where the skipping of exon 4 happens**

The glycine lyase system, made up of four protein components, is responsible for the metabolism of glycine in the body. These four proteins are P-protein, H-protein, T-protein and L-protein, which are encoded by the *GLDC, GCSH, AMT* and *DLD* genes, respectively (Bravo-Alonso et al. 2017; Kure et al. 2006; Poothrikovil et al. 2019). They form an enzyme complex binding to the inner membrane of mitochondria. T, P, and H protein defects will cause NKH. P protein defects are the most common cause of patients with NKH, followed by T protein defects and, less commonly, L protein defects. Different protein defects result in different residual activity of the GCS, which in turn affects symptom severity. Most NKH patients with undetectable GCS activity were usually caused by P-protein defects, whereas patients with T-protein defects, caused by pathogenic mutations in the *AMT* gene, had activity up to 25 percent of normal values (Toone et al. 2003). The *AMT* gene is composed of 9 exons and 403 amino acids (NM_000481.4). In this report, the proband was detected a homozygous variant in the *AMT* gene. The cDNA sequencing results demonstrated that this mutation caused an alternative splicing, resulting in the loss of 44 amino acid codons. This mutation was supposed to cause defects of T protein, which led to an increased level of glycine in the tissues. Impaired glycine metabolism caused damage to the central nervous system, explaining clinical manifestations such as lethargy and coma of the patient in the report.

Currently, the treatment of NKH is mainly focused on three parts, that is, lowering plasma glycine concentrations, use of N-methyl-D-aspartate (NMDA) receptor site antagonists, and symptomatic treatment. For NKH patients with milder symptoms, reducing plasma glycine concentration by sodium benzoate or blockade of NMDA receptors can improve neurologic development and reduce seizure frequency (Korman et al. 2004; Bjoraker et al. 2016). However, for severe NKH patients, these treatments are not effective in relieving symptoms (Korman et al. 2006). But even so, there are still some therapeutic benefits such as decreased frequency and severity of seizures, improved attentiveness, and neonatal apnea (Hennermann et al. 2012). Therefore, appropriate treatment should be given as early as possible to alleviate the symptoms in patients with clinically confirmed NKH, which will affect the prognosis of the patients. In addition, for families in need of another child, genetic testing and PGT can be suggested.

## CONCLUSION

In conclusion, this study reported a NKH patient caused by homozygous *c.471+3A>G* mutation in *AMT*. The report enriched the study of *AMT* gene mutations. In addition, the researchers discussed the genetic and clinical heterogeneity of NKH, providing valuable insights for genetic counselling.

## RECOMMENDATIONS

For patients with clinical symptoms suspicious of NKH, genetic testing can help clarify the diagnosis. Early treatment is needed after diagnosis of NKH in order to have a better prognosis.

## AUTHOR CONTRIBUTIONS

The manuscript was written by Yu Tan. The study was conducted by He Wang and Shanling Liu. Sample collection and experiment was performed by Huan Tian, Shuo Yang and Jingqun Mai. Sequencing results were analysed by Mei Yang.

## FUNDING

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## CONFLICT OF INTEREST

No conflict of interest.

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