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# Variant nonketotic hyperglycinemia caused by a novel pathogenic mutation in the *GLRX5* gene

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### *Abstract*

Nonketotic hyperglycinemia (NKH) is caused by defects in the glycine cleavage system. Hyperglycinemia without biallelic mutations in one of the 4 genes that encode the constituents of the glycine cleavage system is classified as 'variant NKH'. The defects in these cases are in the iron-sulphur cluster biogenesis and lipoate synthesis pathways. The *GLRX5* gene is one of the genes in these new pathways. We report here an 8.5-year-old male patient presented with spasticity, ataxia and optic atrophy. He lost his ability to walk after a febrile infection at the age of 1.5 year. The patient's cognitive functions were preserved. His plasma glycine level and cerebrospinal fluid/plasma glycine ratio were high. A novel homozygous mutation p.Gly116Asp (c.347G>A) in the *GLRX5* gene was identified by whole exome sequencing. In conclusion, in a child, who have neurological regression, spasticity, ataxia, and whom cognitive functions are partially preserved, if plasma glycine level is high, variant NKH should be considered in the differential diagnosis.

*Keywords:* Nonketotic hyperglycinemia, iron-sulphur cluster, GLRX5 gene

## INTRODUCTION

Nonketotic hyperglycinemia (NKH) is a disorder of glycine metabolism caused by deficient enzyme activity of the glycine cleavage enzyme system (GCS). It has three clinical forms, neonatal (classical, 0-4 weeks), infantile (5 weeks-2 years) and late-onset form (>2 years). Typical presentating features are hypotonia, apnea, hiccups and myoclonic jerks in the newborn or infantile period.[1] Other clinical features include intractable seizures, spasticity and intellectual disability. Patients typically have high plasma and cerebrospinal fluid (CSF) glycine levels and CSF/plasma glycine ratio (> 0.08).

Glycine cleavage system is a complex of four components, P-(encoded by the *GLDC* gene), H-(encoded by the *GCSH* gene), T-(encoded by the *AMT* gene) and L-protein(encoded by the *GCLS* gene).[2] It is reported that approximately 72% of NKH patients have *GLDC* gene mutation and 24% have *AMT* gene mutation.[3] In 4% of cases, no mutation was found in the genes encoding GCS despite high glycine levels in plasma and CSF. In these patients, pathology was thought to be in the cofactor lipoate synthesis and iron-sulphur cluster biogenesis genes. This group of patients was classified as "variant NKH".[3] In recent years, iron-sulfur cluster biogenesis genes; *LYRM4, NFS1, NFU1, ISCU, BOLA3, GRPEL1, HSPA9, HSCB, ISCA1, ISCA2, IBA57, IND1, GLRX5* and lipoate synthesis genes; *LIAS, LIPT1, LIPT2, ACSM1* have been identified.[4]

*GLRX5* is a 156-amino-acid mitochondrial protein that plays an essential role in the synthesis of iron-sulphur clusters. Cases of sideroblastic anemia and variant NKH due to *GLRX5* gene mutation have been reported.[5,6] In the literature, normal cognitive development with spasticity, ataxia, and optic atrophy has been reported in variant NKH cases due to *GLRX5* gene mutations.[3] Brain magnetic resonance imaging (MRI) may also have signal changes in the white matter, as well as spinal cord lesions.

With an informed consent taken from the legal guardian and approval received from the institutional review board, we present an 8.5-year-old male with novel pathogenic mutations in the *GLRX5* gene.

## CASE REPORT

Our patient was born to a parents who are first degree cousin. His two brothers are healthy. He

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was born at 39 weeks gestation after an uneventful pregnancy. His birth weight was 3200 gm, his height was 49 cm, his head circumference was 35 cm, all were within normal ranges. He had no neonatal complication. His motor development remained appropriate for his age during infancy.

He lost his ability to walk after a febrile infection at 1.5 years old. He then made a slow recovery. At the age of 2.5 years, he again lost his ability to walk following a febrile illness. He continued to have recurrent attacks resulted in an ataxic gait.

On physical examination at 8 age, his height, weight and head circumference were in the 50th percentile. He has horizontal nystagmus but there was no limitation in eye movements. He could walk with support, had spasticity in the lower extremities and deep tendon reflexes in the lower extremities were brisk. Dysdiadochokinesia was present. He was unable to perform the heel-to-shin test and tandem gait. He was attending the regular school program. He has a good speech, learned to read and write at the same pace as his peers.

Extensive investigations were performed at 5 years old. His complete blood counts, serum ferritin, serum transferrin saturation, routine blood biochemistry, blood gas, blood lactate, and blood ammonia were all normal.

Electroencephalogram (EEG), electromyogram (EMG), brain and spinal MRI, brain magnetic resonance spectroscopic (MRS) were all normal. Echocardiography and eye examination were normal. Cerebrospinal fluid neurotransmitters were normal. In the first metabolic examination at 5 age, plasma glycine level was 473 μmol/l (149-417 μmol/l) and CSF/plasma glycine ratio was 0.057 (≤0.02). In the repeated metabolic tests at 8 years old; blood acyl-carnitine analysis was normal, plasma glycine level was 1234 μmol/l (127-341 μmol/l), urine organic acid analysis was normal. A marked increase in blood glycine level was noticeable. Optic atrophy was detected in the repeated eye examination. In the visual evoked potentials and electroretinography (VEP-ERG) analysis, there was latency and amplitude reduction on the right, and lack of conduction in the optic nerve and visual pathways. Brain MRI was normal.

Genetic analysis; whole exome sequencing was applied to the patient (IV-3) on the Illumina NextSeq. Homozygous novel variant (c.347G>A, p.Gly116Asp) was detected in *GLRX5* (NM_016417.3) gene (Figure 1). This was confirmed by Sanger sequencing. His parents are carrier for this variant. Bioinformatics analysis indicate this variant is pathogenic (Table 1).

It was observed that his gait improved after botulinum toxin injection to the lower extremities. He was also treated with sodium benzoate (5.5 g/m2), alpha-lipoic acid (300 mg/day). He was followed-up for approximately 1.5 years. He had abnormal gait again during infection. Sodium benzoate and alpha-lipoic acid treatment decreased his blood glycine level, but no clinical response was observed.

## DISCUSSION

Nonketotic hyperglycinemia or glycine

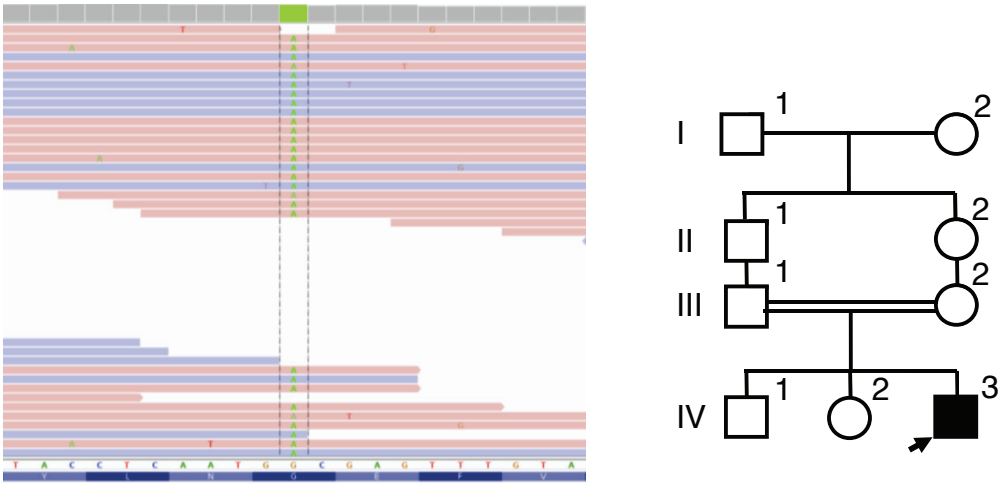


Figure 1. Integrative Genomics Viewer (IVG) representation (chr14:96010335:G:A) of the BAM files that obtained from patient NGS data; we see that guanine and adenine are displaced in the marked region and pedigree.

**Table 1: Summary of the identified variant in this study**

| Gene (NM#) | Variant | Zygosity | gnomAD | DANN | Mutation Taster | Provean | GERP RS | Reference |
|---|---|---|---|---|---|---|---|---|
| *GLRX5* (NM_016417.3) | c.347G>A p.Gly116Asp | HM | N/A | 0.9985 | DC | DM | 5.0199 | This study |

DC: Disease causing, DM: Damaging, HM: Homozygous, N/A: Not Available.

encephalopathy is an autosomal recessive glycine metabolism disorder. The biochemical defect in glycine encephalopathy is in the glycine cleavage system, which consists of a mitochondrial enzyme complex. According to the age of onset, NKH can be divided into three phenotypic subgroups, including newborn (classical), infantile and late onset.[2] Neonatal and infantile form is manifested by encephalopathy, hypotonia, myoclonic jerks, hiccups and seizures in the first days/months of life. In the following periods, severe developmental delay and refractory seizures are seen. There is a glycine level increase in all body tissues, including the central nervous system. A high glycine index (CSF/plasma glycine ratio>0.08) with increased CSF glycine level suggests classic NKH diagnosis, but in late onset forms it has been found to be lower (0.03).[7] In these patients, mutations in the *GLDC*, *AMT*, and *GCSH* genes are detected.

An increasing number of late-onset NKH cases have been reported in the literature in recent years. Brunel-Guitton et al. reported the first case of late-onset NKH. This case; a 9-year-old male patient with learning disability and choreoathetosis attacks during febrile illnesses, CSF/plasma glycine ratio (0.044) was found to be high and homozygous mutation in the GLDC gene was identified.[2] Brenton *et al*. in 2014, reported a 5-year-old child with late-onset NKH, presented with hypotonia, chorea and ataxia after febrile infection. In this case, plasma amino acid analysis indicated that the glycine level was slightly high, and the CSF/plasma glycine ratio was lower than the classical NKH (0.03). In this late-onset NKH case, mutation was detected in the *GLDC* gene.[7]

In addition, there are cases defined as "variant NKH" with mutations other than NKH genes known in the literature. The defects in these cases are thought to be in iron-sulphur cluster biogenesis and lipoate synthesis pathways. *GLRX5* gene mutation is also included in this newly defined group. It has been reported that this mutation results in sideroblastic anemia and "variant NKH" in humans.

The first *GLRX5* gene mutation was identified in a 60-year-old patient with sideroblastic anemia but no neurological symptoms.[5] Recently, a case of congenital sideroblastic anemia with two compound heterozygous missense mutations (c.301A>C and c.443T>C) in the *GLRX5* gene has been described.[8] Finally, in 2018, a 14-year-old case with compound heterozygous mutation (p.Cys67Tyr and p.Met128Lys) in the *GLRX5* gene was reported. They also emphasized, that hem biosynthesis was changed due to mutation in GLRX5 gene.[9]

Baker *et al*. identified genetic etiology in 8 of 11 cases in the "variant NKH" case series. Mutation in the *GLRX5* gene was detected in 3 of these cases. The common features of patients are that they are normal at birth, unlike the typical neonatal presentation. In advanced ages, progressive spasticity, ataxia and optic atrophy as well as partial preservation of cognitive functions. In some cases, borderline cognitive abilities, mild learning difficulties and poor concentration are reported. In brain MRI, signal changes in white matter, progression to leukodystrophy and spinal cord lesions can be seen. Common laboratory findings are high levels of glycine in plasma amino acid analysis. In these patients, deletion c.151_153delAAG was detected in the *GLRX5* gene.[3]

Our patient is similar to the variant NKH cases which Baker *et al*. reported. Our 8.5-years-old patient without sideroblastic anemia had optic atrophy, spasticity, ataxia and lost the ability of walking after infection. In our case, p.Gly116Asp (c.347G>A) homozygous novel pathogenic mutation was detected in the *GLRX5* gene, which was not previously described. Cases with the *GLRX5* gene mutation identified as "variant NKH" are limited in the literature. In some cases, with *GLRX5* gene mutation, sideroblastic anemia is seen, while in some cases variant NKH symptoms occur.

As a conclusion, in a child who have function lost (during infection), spasticity and ataxia, and whom cognitive functions are partially preserved, metabolic studies should be done to help differential diagnosis. In the presence of high glycine level in plasma amino acid

analysis, variant NKH due to iron-sulphur cluster biogenesis and lipoate synthesis pathway defects should be considered in the differential diagnosis.

## DISCLOSURE

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Conflict of Interest: None

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