Attenuated form of Glycine Encephalopathy: An Unusual Cause of Neurodevelopmental Disorder

Sir,

Nonketotic hyperglycinemia (NKH), also known as glycine encephalopathy, is a rare inborn error of metabolism characterized by a defect in the glycine cleavage system, an enzyme complex located in liver, brain, kidney, and placenta.[1] The glycine cleavage system consists of four protein components namely P-protein, T-protein, H-protein

and L-protein.[2] NKH may also result from a defect in the glycine transporter system.[3] Glycine stimulates glycinergic receptors of the brainstem and spinal cord resulting in an inhibitory effect while it acts at the glycinergic modulatory site of the N-methyl-D-aspartate (NMDA) receptor in the cerebral cortex resulting in an excitatory effect.[2] Seizures in NKH patients could be explained by the excitatory effect at the level of cerebral cortex while hypotonia and apnea result from the inhibitory effect at the level of brainstem and spinal cord.[2] In this report, we describe a four-year-old boy manifesting with developmental delay, autistic traits, seizures, and sleep disturbances. A diagnosis of glycine encephalopathy was established based on the biochemical and molecular studies.

A four-year-old boy, first born to nonconsanguineous parents, presented with a history of developmental delay since early infancy and seizures from four months of age. He was born full term by normal vaginal delivery with a birth weight of 2600 g. Hypoglycemia was documented on the third day and he was managed elsewhere with intravenous dextrose and antibiotics. He developed first episode of fever-provoked generalized tonic seizures at four months of age. Subsequently, there were multiple episodes of fever provoked and unprovoked generalized tonic seizures until 3 years of age. Seizures were well controlled after treatment with levetiracetam. By four years of age, he could walk without support, reach for toys, and recognize parents. Both expressive and receptive language delay were observed. He was dependent on his parents for most of the activities of daily living. Vision and hearing were normal. Parents reported hyperactivity and aggressive behavior, poor response to name call, and fragmented sleep at night. His family history was noncontributory.

Anthropometric assessment revealed a weight of 12.5 kg (between 1st and 3rd percentile), height of 95 cm (between 1st and 3rd percentile), and an occipitofrontal circumference of 48.5 cm. On examination, hyperactivity, poor attention span, fleeting eye contact, auditory agnosia, and poor social interaction were observed. Visual fixation and following to light were observed. Fundus examination was normal. Hypotonia, hyporeflexia, motor stereotypies, and mild choreoathetosis were observed. Symptomatic and behavioral therapy had been implemented, but without clinical improvement.

An induced sleep EEG record showed bihemispheric slow waves with absent sleep markers. Amino acid analysis performed by high-performance liquid chromatography (HPLC) revealed an increased glycine level in serum (patient value: 862 μmol/L; normal range: 127-341 μmol/L), CSF (patient value: 65 μmol/L; normal range: 4.8-8.4 μmol/L), and urine (patient value: 75126 μmol/g creatinine; normal range: 897–4500 μmol/g creatinine). CSF lactate and glucose levels were normal. While serum and CSF specimens were not collected concurrently, the CSF to serum glycine ratio is predicted to be increased. Brain MRI was normal. A prominent peak at 3.55 ppm was observed by brain MRS that corresponds to glycine at TE 135 ms. However, MRS with short TE was not acquired due to logistical difficulties with sedation. Nerve conduction parameters were normal.

A clinical exome study revealed a homozygous variant c. 1654A > G, in the exon 13 of *GLDC* gene [Transcript ID: ENST00000321612 transcribed from reverse strand; Genomic coordinate chr9:6588629T > C; GRCh37/hg19 build] resulting in a single amino acid substitution [p.Met552Val]. In silico analysis predicted this variant to be disease causing. The native amino acid is conserved across species and lies in the pyridoxal phosphate-dependent transferase domain. Furthermore, the variant in our patient has been previously reported in an individual with NKH [HGMD public database- CM107561; as likely pathogenic in ClinVar database (Variation ID: 56048)]. Taken together, all evidences indicate that this variant is pathogenic. Parents were heterozygous carriers for the same mutation; Sanger sequence showing the missense variant [c. 1654A > G] in exon 13 of the *GLDC* gene of proband and parents [Figure 1a-c].

Once the diagnosis of NKH was confirmed, treatment with sodium benzoate and pyridoxine was implemented. Levetiracetam, clonazepam and risperidone were continued for the management of seizures and behavioral symptoms. Carnitine was supplemented to avoid carnitine depletion resulting from the administration of sodium benzoate. Glycine-restricted diet was tried though only endogenous synthesis determines the blood glycine level to a larger extent. A follow-up assessment showed significant reduction in hyperactive behavior, gain in sociocognitive skills, improved attention span and quality of sleep, reduced serum glycine level, and improvement in EEG findings. The trends of serum and urine glycine levels in our patient at diagnosis and while on treatment are shown in Table 1.

The estimated incidence of NKH has been reported as 1:55,000 to 1:76,000 live births.[4,5] Based on the severity and age of onset, NKH can be categorized into severe neonatal, attenuated neonatal, severe infantile, and attenuated infantile forms.[6] Clinical features such as coma, apnea, hiccups, and spasticity were absent in our patient. The absence of these features with delayed onset of seizures at four months of age and milder phenotype in our case favors a diagnosis of attenuated infantile glycine encephalopathy. Dinopoulos *et al*. described the clinical features of children with neonatal, infantile, and late-onset atypical NKH variants.[7] Epilepsy is less frequent in late-onset atypical NKH cases while seizures, hypotonia, movement disorders with varying degrees of cognitive impairment are frequent findings in neonatal and infantile forms of atypical NKH cases.[7] Behavioral problems such as irritability, temper tantrums, aggression, anger outbursts and hyperactivity have been reported in infantile forms of atypical NKH cases.[7] While myoclonic seizures are common in patients with NKH, other seizure types reported are generalized tonic seizures, infantile spasms, tonic-clonic seizures, and complex partial seizures.[7] Generalized tonic seizures, hyperactivity, and extrapyramidal symptoms observed in our case could be attributed to glycine-mediated excitotoxicity.

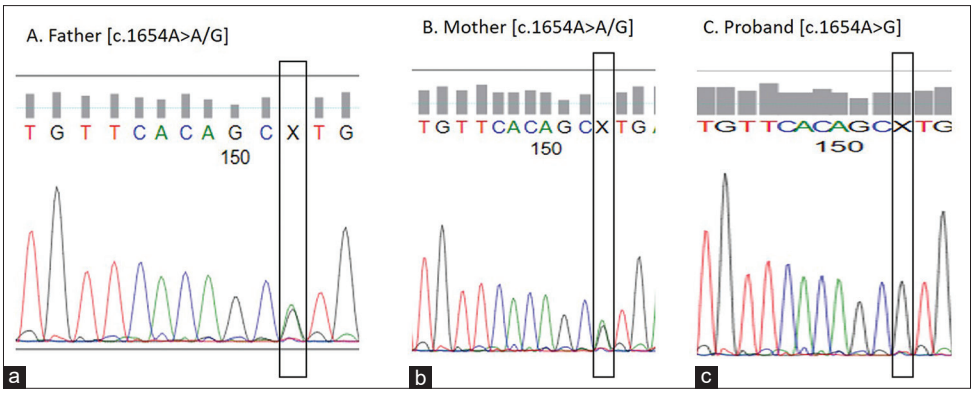

Figure 1: Sanger sequencing showing the missense variant [c.1654A > G] in the exon 13 of *GLDC* gene in the proband and family. Electropherogram (a, b) showing that the missense variant marked by the rectangular block, in heterozygous state in parents. Electropherogram (c) showing the variant in homozygous state in the proband

Table 1: Trends of serum and urine glycine levels of our patient at diagnosis and on treatment

Timeline of testing	Serum glycine (μmol/L)	Urine glycine (μmol/g creatinine)
At diagnosis	862	75,126
2 weeks after the initiation of treatment	542	21,339
3 months after the initiation of treatment	493	22,968
9 months after the initiation of treatment	373	35,707

Normal range of serum glycine: 127–341 μmol/L; Normal range of urine glycine: 897–4500 μmol/g creatinine

CSF, serum, and urine glycine levels were elevated in our patient. CSF glycine may be elevated in children with NKH, hypoxic ischemic encephalopathy, traumatic brain injury, meningoencephalitis, epilepsy, vanishing white matter disease, chronic renal failure, valproate therapy, and traumatic CSF tap.[8] The causes of elevated plasma glycine levels include propionic acidemia, methylmalonic acidemia, isovaleric acidemia, β-ketothiolase deficiency, D-glyceric aciduria, type II hyperprolinemia, undernutrition, starvation, and valproate therapy.[8] Brain MRI abnormalities described in neonates with NKH include hyperintensity of the corticospinal tract, internal capsule, and central tegmental tract.[9] Other neuroimaging findings reported in NKH patients are cortical atrophy, enlarged ventricles, myelination delay, thinning of corpus callosum, signal changes of internal capsule, and periventricular white matter, posterior fossa cyst, hypoplasia of cerebellum, gyral malformation, atrophy of basal ganglia, thalamic volume loss, and intracranial hemorrhage.[2,6,10,11] Brain MRS at long echo time in these patients show a prominent peak at 3.55 ppm depicting high glycine levels. EEG findings described are burst suppression pattern, hypsarrhythmia, and multifocal epileptiform discharges.[6,12] High-amplitude delta waves with poorly formed sleep markers were observed in our patient.

Mutations in glycine decarboxylase (*GLDC*) gene or aminomethyltransferase (AMT) gene have been reported in patients with NKH.[2] Mutations in SLC6A9, encoding a glycine transporter (GlyT1) may also result in non-ketotic hyperglycinemia.[3] Our patient harbors a homozygous variant c.1654A > G, in exon 13 of *GLDC* gene. While this variant has previously been reported in one case of NKH, the phenotypes contrast.[13] Meyer *et al*. described a severe lethal neonatal form of NKH with two missense mutations in the *GLDC* gene. It is possible that the compound heterozygous nature of this variant [p.Met552Val] along with another variant [p.Gly771Arg] that has been previously identified in neonatal onset NKH cases, determined the lethal outcome in the patient reported by Meyer *et al*.[13,14] Hence, the functional analysis of the variant [p.Met552Val] identified in our case is essential to establish the residual activity of the enzyme which thereby could explain the attenuated phenotype.

From a clinical standpoint, poor prognostic factors in patients with NKH are early infantile onset disease, early onset of spasticity, refractory seizures, hypomyelination or cortical malformation on neuroimaging, presence of microcephaly, absence of extrapyramidal involvement, and presence of burst suppression pattern or hypsarrhythmia on EEG.[2,6] The more favorable prognosis in our case might be explained by the findings of well-controlled seizures, presence of extrapyramidal signs, lack of myelination delay and cortical malformation, and absence of hypsarrhythmia.

The management of NKH includes the administration of sodium benzoate to conjugate glycine and strychnine to improve respiration. Avoidance of sodium valproate is recommended as it may inhibit any residual enzyme activity present.[6] Dextromethorphan, benzodiazepines, phenytoin, phenobarbitone, topiramate, vigabatrin, levetiracetam, carbamazepine, felbamate, and primidone have been used to control epilepsy in these patients.[6]

Neonatal screening strategies for the diagnosis of metabolic disorders might not be an effective tool in the diagnosis of atypical forms of NKH.[15] We propose that children with unexplained developmental delay, seizures, intractable behavioral abnormalities, and autistic traits be screened for

glycine encephalopathy, as early diagnosis has implications in the management and outcome.

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Conflicts of interest

There are no conflicts of interest.

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