Child Neurology Open
Volume 6: 1-5

DOI: 10.1177/2329048X19831486
journals.sagepub.com/home/cno
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Glycine Transporter 1 Encephalopathy From Biochemical Pathway to Clinical Disease: Review

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Abstract

Glycine transporter 1 encephalopathy (OMIM# 617301; glycine encephalopathy with normal serum glycine, GLYT1 transporter dysfunction, and nonketotic hyperglycinemia) is caused by mutations in the *SLC6A9* gene. To date, 6 cases have been reported in the literature, characterized as having neonatal onset, respiratory failure that required mechanical ventilation, severe hypotonia at birth that progressed to limb hypertonicity, and startle-like responses provoked by sudden loud noises and tactile stimulation. Additional characteristics included dysmorphic features, musculoskeletal abnormalities, and abnormal antenatal findings. Initial diagnosis include elevated levels of glycine in cerebrospinal fluid and an elevated cerebrospinal fluid to plasma glycine ratio. Abnormal magnetic resonance imaging findings included white matter abnormalities, thin corpus callosum, dilatation of the lateral and third ventricles, caudate atrophy, and tiny cysts. Patients reported so far showed normal electroencephalogram results. Treatment was supportive and appeared severe as 50% of the patients died between 2 days and 7 months of age, while surviving children had global developmental delay. In this report, we reviewed the published cases having glycine transporter 1 encephalopathy and retrospectively characterizing the disease phenotypes, affected biochemical pathways, neuroradiological abnormalities, diagnosis, genetic issues, and treatment; additionally, key discussion points are also presented.

Keywords

GLYT1 encephalopathy, GLYT1 transporter dysfunction, *SLC6A9* gene, autosomal recessive nonketotic hyperglycinemia

Received January 23, 2019. Accepted for publication January 24, 2019.

Autosomal recessive nonketotic hyperglycinemia (alternatively known as glycine encephalopathy) is caused by mutations in the genes encoding components of the glycine cleavage system: the glycine decarboxylase gene, which encodes the P protein component of the glycine cleavage system; the aminomethyltransferase gene, which encodes the T protein component; and the glycine cleavage system H gene, which encodes the H protein component.[1] In 2016, Alfadhel et al presented the first published case of nonketotic hyperglycinemia resulting from mutation of the *SLC6A9* gene, which encodes glycine transporter 1 (OMIM# 617301). In the same year, Kurolap et al described 5 more cases of nonketotic hyperglycinemia resulting from *SLC6A9* mutation.[2,3] This disorder has several names, including glycine encephalopathy with normal serum glycine, glycine transporter 1 encephalopathy, and nonketotic hyperglycinemia caused by *SLC6A9* gene defect.[2–4]

Epidemiology

To date, only 6 cases linked to glycine transporter 1 encephalopathy have been described in the literature. The first case described from Saudi Arabia and the other 5 from Israel. All

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the 3 families were consanguineous. However, all cases were of Arab ethnicity, and due to the recent discovery of glycine transporter 1 encephalopathy, the exact incidence and prevalence of this disorder is still unknown.

Metabolic Pathway

Glycine is the smallest amino acid and has a vital role in the central nervous system as an inhibitory and excitatory neurotransmitter.[5,6] Glycine mediates its inhibitory effect by hyperpolarizing the postsynaptic glycine receptors in the caudal area of the central nervous system, while its excitatory effect results from functioning as a coagonist of glutamate receptors of the *N*-methyl-D-aspartate (NMDA), which are predominant throughout the central nervous system.[5,6] Glycine levels are regulated by 2 key pathways: the glycine cleavage system pathway, which degrades glycine and, to a lesser extent, forms serine,[7] and the glycine transport system pathway, which is controlled by 2 sodium-dependent glycine transporters, that is, the glycine transporter 1 and the glycine transporter 2. *SLC6A9* (OMIM# 601019) encodes the glycine transporter 1 and SLC6A5 (OMIM# 604159) encodes the glycine transporter 2. Both glycine transporter 2 and glycine transporter 1 are located predominantly in the caudal regions of the brain (spinal cord, brain stem, and cerebellum). In contrast, the glycine cleavage enzyme system is only present in the rostral region.[8] Glycine transporter 2 is exclusively found at presynaptic glycinergic receptors and required for the maintenance of presynaptic glycine pools, whereas glycine transporter 1 is located mainly on astrocytes and is essential for the transport of glycine from the extracellular space and termination of glycinergic transmission.[9,10] Of note, defects in the glycine transporter 2 cause hyperekplexia 3 (OMIM# 614618). This disorder is characterized by hypertonia, an exaggerated startle response to external stimuli, and apneic attacks during the neonatalperiod.[11]

Clinical Phenotype

Detail clinical features of patients with glycine transporter 1 encephalopathy reported to date has been summarized in Table 1. All the reported patients experienced neonatal onset, respiratory failure that required mechanical ventilation, severe hypotonia at birth which progressed to limb hypertonicity, and startle-like responses provoked by sudden loud sounds and tactile stimulation. Additional characteristics included dysmorphic features, musculoskeletal abnormalities, abnormal antenatal findings, abnormal brain magnetic resonance imaging (MRI), high concentrations of glycine in cerebrospinal fluid, and an elevated cerebrospinal fluid to plasma glycine ratio.[2–4] Antenatal findings included cervical cysts, nuchal translucency, fourth ventriculomegaly, bilateral clubfeet, clenched fists, overriding toes, hyperextension of the knees, joint contractures, and arthrogryposis. One male was aborted at 13 weeks of gestation and had hydrops fetalis.[3] The 2 patients suffered from polyhydramnios antenatally. Four (67%) patients were delivered by caesarian due to fetal distress and 1 patient was delivered by normal spontaneous vaginal delivery. Half (3 of 6) of the patients were born prematurely.

The common dysmorphic features included dolichocephaly or trigonocephaly, broad forehead, long myopathic facies, tent-shaped mouth, depressed nasal bridge, small upturned nose, deep prominent philtrum, low set ears, and retrognathia. Eye abnormalities included esotropia optic nerve atrophy, abnormal visual evoked potential, and ptosis in 3 (50%) patients. Musculoskeletal features included joint laxity with bilateral club feet and developmental dysplasia of the hip (documented in one family).[2] Musculoskeletal features of 2 other families included club feet, hyperextension of knees, bilateral hip dislocation, contractures of elbows, wrists and hips, and overriding toes. Absent patella was found in 1 child[3]; other rare findings included renal hydronephrosis, hypertension with elevated urinary catecholamines, atrial septal defect, right cryptorchidism, right inguinal hernia, motor and sensory polyneuropathy, and right mild conductive hearing loss.[3]

Neuroradiological Findings

Brain MRI evaluation was done in 50% of the cases; however, results were not specific to glycine transporter 1 encephalopathy. One patient had bilateral scattered subcortical, periventricular white matter hyperintensities, and caudate atrophy of the right side of the brain with a tiny cyst. Another patient had dilatation of the lateral and third ventricles, and a third patient had thin corpus callosum. Additional cases of glycine transporter 1 encephalopathy in the future may provide insight into whether this disorder has specific neuroradiological imaging features.[2,3] Further, electroencephalogram results were normal in all the described patients with glycine transporter 1 encephalopathy with no epileptic discharges, unlike with nonketotic hyperglycinemia.[2,3] In both the reports, the hyperekplexia (startle-like response) was confused with seizure and antiepileptic medications were ordered.[2,3]

Biochemical Abnormalities

All cases of glycine transporter 1 encephalopathy had high levels of glycine in cerebrospinal fluid, a high cerebrospinal fluid to plasma glycine ratio, high levels of glycine in urine, while normal levels of glycine in serum. Thus, also called glycine encephalopathy with normal serum glycine. Other tests were normal, including newborn screening, ammonia, serum lactic acid, urine organic acid, liver function, and renal function.

Genetics

SLC6A9, comprised of 14 exons, encodes the glycine transporter 1 that contains 12 transmembrane domains and an intracellular N- and C-termini. Binding of 2 sodium and chloride ions and glycine promotes glycine transporter 1 activity, which transports glycine mainly to glial cells.[11,12] Three different

Table 1. Summary of Glycine Transporter 1 Encephalopathy Clinical Phenotypes Reported To Date.

Families Reported	Family I	Family II		Family III		
Number of cases	1	1	2	1	2	3
Age of presentation	15 months	33 weeks and 3 days	13 weeks	35 weeks and 4 days	41 weeks	30 weeks
M:F	F	F	M	F	F	M
Mutation detected	(c.1219 A>G; p.Ser407Gly)	(c.928_932delAAGTC; p.Lys310Phefs*31)	(c.928_932delAAGTC; p.Lys310Phefs*31)	(c.1717C>T; p.Gln573*)	(c.1717C>T; p.Gln573*)	(c.1717C>T; p.Gln573*)
Neonatal onset	Yes	Yes	Yes	Yes	Yes	Yes
Consanguinity	Yes	Yes	Yes	Yes	Yes	Yes
Respiratory failure	Yes	Yes	Yes	Yes	Yes	Yes
Encephalopathy	Yes	Yes	Yes	Yes	Yes	Yes
Severe hypotonia that progressed to limb hypertonicity	Yes	Yes	Yes	Yes	Yes	Yes
Response loud sounds and tactile stimulation	Yes	Yes	Yes	Yes	Yes	Yes
Global developmental delay	Yes	Yes	Yes	Yes	Yes	Yes
Dysmorphic features	Yes	Yes	Yes	Yes	Yes	Yes
Abnormal antenatal ultrasound	Yes	Yes	Yes	Yes	Yes	Yes
Perinatal features	Polyhydramnios	Cervical cysts, polyhydramnios, 4th ventriculomegaly	Hydrops (abdominal wall and scalp edema)	Bone defect	Bone defect	Bone defect
Delivery	NSVD	C/S	Termination at 13 weeks	C/S	C/S	C/S
Gestational age	Term	Prematurity	Termination	Prematurity	Prematurity	Prematurity
Dysmorphic features	Broad forehead, microcephaly, dolichocephaly esotropia, low set ears, retrognathia, deep prominent philtrum, and sparse eyebrows	Trigonocephaly, long myopathic facies, tent-shaped mouth, retrognathia, small upturned nose, depressed nasal bridge, low-set ears	Not available	Not available	Not available	Dolichocephaly, long myopathic facies, tent-shaped mouth, small upturned nose, depressed nasal bridge, pronounced
Ophthalmological findings	Esotropia	Long eyelashes, pronounced eyebrows, ptosis	Not available	Not available	Not available	Pronounced eyebrows, ptosis
Musculoskeletal abnormalities	Joint laxity, DDH, dislocation of the right proximal femur, and bilateral club feet	Arthrogryposis, club feet, hyperextension of knees, bilateral hip dislocation, contractures of elbows, wrists and hips, overriding toes, absent patellae	Not available	Not available	Not available	Club feet, hyperextension of knees, bilateral hip dislocation, contractures of hips, and wrists, clenched fists

(continued)

Table 1. (continued)

Families Reported	Family I	Family II		Family III		
Rare findings	None	Renal hydronephrosis, hypertension with elevated urinary catecholamines, ASD, right cryptorchidism, right inguinal hernia, motor and sensory polyneuropathy, right mild conductive hearing loss	Not available	Not available	Not available	Motor and sensory polyneuropathy, abnormal VEP, right mild conductive hearing loss
References	Alfadhel et al, 2016	Karolap et al, 2016	Karolap et al, 2016	Karolap et al, 2016	Karolap et al, 2016	Karolap et al, 2016

Abbreviations: ASD, atrial septal defect; C/S, caesarian section; DDH, developmental dysplasia of the hip; F, female; M, male; NSVD, normal spontaneous vaginal delivery; VEP, visual evoked potential.

SLC6A9 genetic mutations has been reported in 3 families including a homozygous missense mutation (c.1219 A>G (p.Ser407Gly)), a homozygous small deletion (c.928_932delAAGTC (p.Lys310Phefs*31)), and a homozygous nonsense mutation (c.1717 C>T (p.Gln573*)). An autosomal recessive inheritance pattern is suggested by the reported consanguinity in all the cases. Therefore, the recurrence risk is 25% in each *pregnancy and no clear genotype–phenotype correlation has been reported.*

Diagnosis

Diagnosis can be made based on high levels of glycine in cerebrospinal fluid, *normal plasma glycine*, a high cerebrospinal fluid to plasma glycine ratio, and genetic testing for *SLC6A9* mutations, in addition to the aforementioned clinical findings.

Treatment

To date, no curative treatment has been discovered and disease management is exclusively supportive. The management of glycine transporter 1 encephalopathy requires a multidisciplinary team approach including pediatricians, neurologists, geneticists, genetic counselors, dietitians, physiotherapists, occupational therapists, and orthopedic surgeons. One patient was treated with ketamine and sodium benzoate with no improvement.[3] Children with glycine transporter 1 encephalopathy are at risk of gastroesophageal incoordination and swallowing difficulties, which may lead to aspiration, malnutrition, and failure to thrive. In many patients, a feeding tube, such as gastrostomy tube, would be an efficient solution to ensure the patient receives enough calories and avoids recurrent aspirations. Additionally, periodic hearing and vision assessments are essential. Finally, regular follow-up visits with physiotherapists, occupational therapists, and orthopedic surgeons are mandatory.

Prognosis

Only 2 patients survived until childhood, while the other patients died early in the neonatal or infantile period. Such observations indicate that glycine transporter 1 encephalopathy is a lethal disease; however, this conclusion is based on a small sample size (6 cases).

Key Points For Discussion

1. What is the explanation for respiratory failure that requires mechanical ventilation and generalized hypotonia?
   Several publications showed that the glycine transporter 1 has an essential function in the development of basic respiratory patterns and tone, based on glycine transporter 1-deficient mice (*SLC6A9* knockout) that displayed hypotonia, respiratory failure, and demise shortly after birth.[13,14]
2. What is the mechanism of the startle-like response (hyperekplexia) in these patients?
   The postulated mechanism is based on either superactivation of NMDA receptors or inhibition of glycine receptors due to negative feedback and termination of glycinergic transmission.[14] Also, hyperekplexia is a feature of patients with *SLC6A5* gene defects but is not associated with nonketotic hyperglycinemia, suggesting hyperekplexia might be a common finding between glycine transporter 1 and glycine transporter 2 systems.[2]
3. Does GLY-T1 have a crucial importance only in the neonatal period with a possible adaptor mechanism to this defect?
   The 2 patients who survived the neonatal period and currently are ventilation-free indicate that the role of glycine transporter 1 may be of primary importance only in first few months of life; additionally, the startle-like clonus episodes and encephalopathy subsequently resolved in all cases (Table 1). Additionally, follow-up visits of neonates

with glycine encephalopathy showed that glycine concentrations in cerebrospinal fluid decreased with age, suggesting that glycine transporter 1 primarily functions in the neonatal period.[15,16] Glycine transporter 1-deficient mice survived the postnatal period, which is consistent with the idea that glycine transporter 1 is important only in the neonatal period, which may indicate that there is a possible adaptor mechanism in the central nervous system that compensates for the glycine transporter 1 defect.[3]

Other questions include: Do the high levels of glycine in cerebrospinal fluid generate a high glycine peak in magnetic resonance spectroscopy, which is a potential noninvasive biomarker for this disorder? What is the role of the glycine transporter 1 in utero, since all patients suffered from perinatal findings? Further research is needed to characterize the role of glycine transporter 1 in the human central nervous system and how the human body responds to the glycine transporter 1 genetic defects.

Conclusion

Clinicians should consider glycine transporter 1 encephalopathy for any neonatal patient with respiratory failure that requires mechanical ventilation, severe hypotonia at birth that progresses to limb hypertonicity, startle-like responses provoked by sudden loud sounds and tactile stimulation, dysmorphic features, high levels of glycine in cerebrospinal fluid, and a high cerebrospinal fluid to plasma glycine ratio. Animal models are vital to understanding the pathophysiology of glycine transporters in the brain. Additional case series with a larger cohort will add to our understanding of the clinical presentation, complications, and management of this rare genetic disease.

Author Contributions

RAF wrote the first draft of the manuscript and MAF performed all the work associated with preparing, writing, and submitting the manuscript and supervised and approved the final manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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Ethical Approval

Not applicable as this is a review article.

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