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Original article

Clinical heterogeneity of glycine encephalopathy in three Palestinian siblings: A novel mutation in the glycine decarboxylase (*GLDC*) gene

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Abstract

Introduction: Glycine encephalopathy (GE), also known as non-ketotic hyperglycinemia (NKH), is a rare inborn error of glycine metabolism caused by a defect in glycine cleavage system, a multi-enzyme complex located in mitochondrial membrane. This defect results in elevated glycine concentration in plasma and cerebrospinal fluid (CSF). Clinical manifestations vary from severe lethargy, hypoactivity and apneic episodes in the neonatal form, mild or moderate psychomotor delay and seizures in the infantile form, and abnormal behaviors, ataxia and choreoathetoid movements in late onset form. More than 50 *GLDC* mutations were found, reflecting large heterogeneity of the gene.

Methods: We describe the clinical, biochemical and molecular characteristics of three Palestinian siblings who have distinct clinical phenotypes. Molecular study was performed utilizing standard Polymerase Chain Reaction (PCR) amplification then direct DNA sequencing for the affected family members.

Results: Their phenotypes included severe symptoms in neonatal period, infantile onset of seizure and psychomotor delay and a mild late-onset form with speech delay at age 20 months. All siblings were homozygous for a novel mutation Y164H in exon 4 of *GLDC* gene. The described novel homozygous variant in our study is predicted deleterious and pathogenic.

Conclusions: This article further expands the genetic spectrum of glycine encephalopathy and adds an evidence of the clinical heterogeneity of glycine encephalopathy even in siblings with identical mutation.

Keywords: GLDC gene; Glycine encephalopathy; Non-ketotic hyperglycinemia; Seizure; Psychomotor delay

1. Introduction

Glycine encephalopathy, also called non-Ketotic hyperglycinemia (NKH), is an inborn error of glycine metabolism caused by autosomal recessive defect in glycine cleavage system, a multi-enzyme complex found in the inner mitochondrial membrane of liver, kidney and brain [1,2]. This complex consists of four individual protein components; termed P-protein (pyridoxal glycine decarboxylase), H-protein (lipoic acid-containing hydrogen carrier protein), T-protein (a tetrahydrofolate-dependent amino methyltransferase) and L-protein (lipoamide dehydrogenase). Approximately 65–80% of children with Glycine encephalopathy

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have a defect in P-protein, encoded by glycine decarboxylase (*GLDC*) gene that consists of 25 exons on Chromosome 9p22–24 [2,3], while about 11–25% of cases, have mutations in the nine exons of amino methyltransferase (*AMT*) gene located on Chromosome 3q21.1–21.2 [3,4]. The defect leads to accumulation of glycine in body fluids, mainly central nervous system. The clinical phenotype of the disease is heterogeneous in its onset, characteristics and severity. Three clinical forms were described; classic neonatal, infantile and late onset [3]. Classic neonatal form usually presents with progressive lethargy, hiccups, feeding difficulty and apneic episodes requiring assisted ventilation. Survivors almost always display profound neurological disability and intractable seizures [1]. Infantile form may present with mild to moderate psychomotor delay and seizure that can be of any type. On the other hand, late onset form may present with behavioral abnormalities and choreoathetoid movements that are often difficult to control [1,3].

Before this report, the mutation spectrum of the disease showed 78 novel mutations in *GLDC* and 18 novel mutations in *AMT*, reflecting extensive intragenic molecular heterogeneity in classic NKH [5].

Herein, we describe three siblings with GE born to consanguineous Palestinian parents with a variable phenotype including neonatal and infantile onset forms and a late-onset mild form characterized by speech delay as the only symptom at age 20 months. The diagnosis was made by elevated CSF/plasma glycine ratio. Genetic analysis showed that all were homozygous for the novel mutation Y164H (Tyr164His) in exon 4 of *GLDC* gene.

2. Patients and methods

2.1. Patients

Clinical, biochemical and molecular characteristics of patients are illustrated in Table 1.

2.1.1. Patient 1

The 6-year-old male was born at term after uneventful pregnancy and labor. Birth weight was 3600 g (zero SD). Length and occipitofrontal circumference at birth were not known. At age 7 days, he developed fever, urine culture revealed E.coli that required treatment with IV antibiotics. During infantile period, he had hypotonia and motor delay. At age 6 months, he developed clonic convulsions that worsened after treatment with valproate. Phenytoin and clonazepam were added without response. He was hospitalized at age 10 months because of intractable seizure and global psychomotor delay. Weight was 9400 g (−2 SD), length 78 cm (+2 SD) and occipitofrontal circumference 44 cm (−2 SD). He had axial hypotonia but no dystonia or limb spasticity. Ophthalmic exam showed optic atrophy and brain MRI showed widening of CSF spaces with no major brain malformations. Electroencephalogram (EEG) showed monomorphic depressed slow background composed mainly of delta waves alternating with spiky activity consistent with multifocal epilepsy.

Investigations included the following: Plasma ammonia 41 μmol/L (controls 10–50 μmol/L), lactic acid 1.8 mmol/L (controls 1.1–2.4 mmol/L). Plasma glycine level was 786 μmol/L (controls 127–341 μmol/L) and simultaneous CSF glycine was 66.7 μmol/L (controls 0.7–14.7 μmol/L). CSF:Plasma glycine ratio was 0.08 (control < 0.04). Urine organic acid analysis was normal. Valproate was stopped immediately after obtaining CSF and plasma glycine levels to avoid worsening of symptoms and he was treated with ketamine (15 mg/kg/day), sodium benzoate 450 mg/kg/day, dextromethorphan (5 mg/kg/day) with gradual weaning of phenytoin and clonazepam. Currently aged 6 years, he can run with normal gait, says only words and still has stranger anxiety. He is seizure-free without anticonvulsant therapy.

2.1.2. Patient 2

The second child was born at term after uneventful pregnancy and labor, birth weight was 3200 g (−0.7 SD). In view of her brother's course, she was hospitalized at neonatal intensive care unit for observation. At age 2 days, she developed frequent hiccups, lethargy and poor sucking. Complete septic work up was negative. Plasma glycine was 489 μmol/L and CSF glycine was 61.6 μmol/L with elevated CSF/Plasma glycine ratio 0.12 (control < 0.04). She was treated with ketamine, sodium benzoate and dextromethorphan with dramatic improvement in sucking and activity. At age

Table 1
Clinical, biochemical and genetic characteristics of the patients.

Patient No.	Age of onset	Gender	Clinical features	Plasma glycine (μMol/L)	CSF glycine (μMol/L)	CSF/plasma ratio	Genotype
1	Infantile (6 mon)	♂	Intractable seizure Psychomotor delay	786	66.7	0.08	All are homozygous for mutation Y164H in exon 4 of *GLDC* gene
2	Neonatal (2nd day)	♀	Poor sucking Hiccups Lethargy Speech delay	489	61.6	0.12	Both parents are heterozygous for the same mutation
3	Late-onset	♀	Speech delay	410	63	0.15	

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13 months, she was only able to sit without support and had speech delay but the family did not report any seizure. Sodium benzoate, ketamine and dextromethorphan were discontinued by the parents since age 7 months. She then lost to follow up. Currently aged 5 years, she has speech delay saying two-word sentences. She is able to run and has normal social interaction. Hospitalization for clinical and biochemical re-evaluation was denied.

2.1.3. Patient 3

The third child is a female patient who was born at term after uneventful pregnancy and labor. Birth weight was 3100 g (−1.3 SD). Because of the two previously affected siblings with NKH, Prenatal genetic testing was performed on the DNA extracted from cultured amniotic fluid sample. Genomic amplification of exon 4 of the *GLDC* gene and direct sequencing confirmed that the fetus was homozygous for the same mutation. Counseling of the family for termination of the pregnancy was offered which was abandoned for social and religious reasons. At age 15 months and without treatment, she was asymptomatic and had normal neurological examination. Plasma glycine was 410 μmol/L, CSF glycine was 63 μmol/L (control < 14.7) and CSF: Plasma glycine ratio was 0.15. Urine organic acid analysis was also normal. She was treated with sodium benzoate 250 mg/kg/day and ketamine 15 mg/kg/day. Currently aged 20 months, she has speech delay saying only syllables but has normal neurological examination, can run and has normal social interactions.

2.2. Method of molecular study

DNA extraction: genomic DNA was extracted from whole blood collected in EDTA tubes and the extraction was performed by the Epicenter DNA Purification Kit (Cat No. MCD85200) according to standard technique followed by the standard Polymerase Chain Reaction (PCR) amplification [6]. Then, direct DNA sequencing of the genes known to cause NKH (*GLDC* gene of 25 exons, *AMT* gene of 10 exons and GCSH gene of 5 exons) was performed for the affected family members utilizing an automated sequencer (ABI 3130). A total 50 μl reaction for PCR containing 25 μl of ready mix (Go Taq green master mix -Promega), 0.1 μg/μl of genomic DNA, 4% primers (F/R) and water.

The PCR cycling conditions included preheating for 5 min at 95 °C, followed by 35 cycles of 95 °C for 30 s, annealing 60 °C for 30 s; 72 °C for 30 s and a final extension of 5 min at 72 °C using the primers, GLDC-4F tactgcttatccccaaacaaag and GLDC-4R accaagaaggaccctgagag.

Deletions/duplications and *GLDC* gene sequence of the coding and flanking intronic regions of genomic DNA were analyzed using Multiplex Ligation-Dependent Probe Amplification (MLPA) [SALSA MLPA P209 Glycine Encephalopathy probe mix (MRCHolland)] [7] (Fig. 1).

The functional effect of this mutation and the resulting amino acid substitution in exon 4 at codon 164 was performed using the Prediction of Disease-Related Mutations (PredictSNP) [8]. The analysis results showed deleterious effect with expected accuracy of 87%. The analysis was also performed using other function prediction softwares (MAPP, PhD-SNP, PolyPhen-1, PollyPhen-2, SIFT and SNAP) and all of them showed deleterious effect ranging from 43% to 81% on the protein function of *GLDC*.

None of the pathogenic mutations were detected in the exons and exon–intron boundaries of the AMT and GCSH genes.

Permission for publication of this article was obtained by An-Najah National University Institutional Review Board. Written informed consent was also obtained from the parents for publication of this article.

3. Results and discussion

Glycine encephalopathy is considered a severe metabolic disorder due to defect in glycine cleavage system, the majority of classical NKH patients present within hours or days of birth with severe symptoms. However, some of patients have atypical NKH, which include later onset with milder disease, early onset with milder disease, or later onset with still rapid and severe course [3,9].

Molecular heterogeneity of the *GLDC* gene was confirmed in 28 unrelated patients with neonatal onset NKH identifying 40 different gene alterations, eighteen alterations were clearly disease causing [2]. This molecular heterogeneity was in contrast to the homogeneous clinical presentation.

The described novel homozygous variant in our article is predicted deleterious and pathogenic; though no functional studies were performed, based on the following: First: The positive segregation in the family (all the affected children were homozygous and the parents were heterozygous for the mutation). Second: The mutated site is a conserved amino acid residue. Third: The result of analysis by the Prediction of Disease Related mutations (PredictSNP) software of this amino acid chain at codon 164 conserved amino acid showed deleterious effect with expected accuracy of 87% [8]. Fourth: The search for any nucleotide chain in exon 4 at codon 164 of the *GLDC* gene using the Exac browser showed a documented stop codon that was gained at this codon resulting from a nucleotide change of T to G (9:6610335 G/T). This indicates that mutation at this site has deleterious effect; though it is different from the mutation in the reported family and it is a stop

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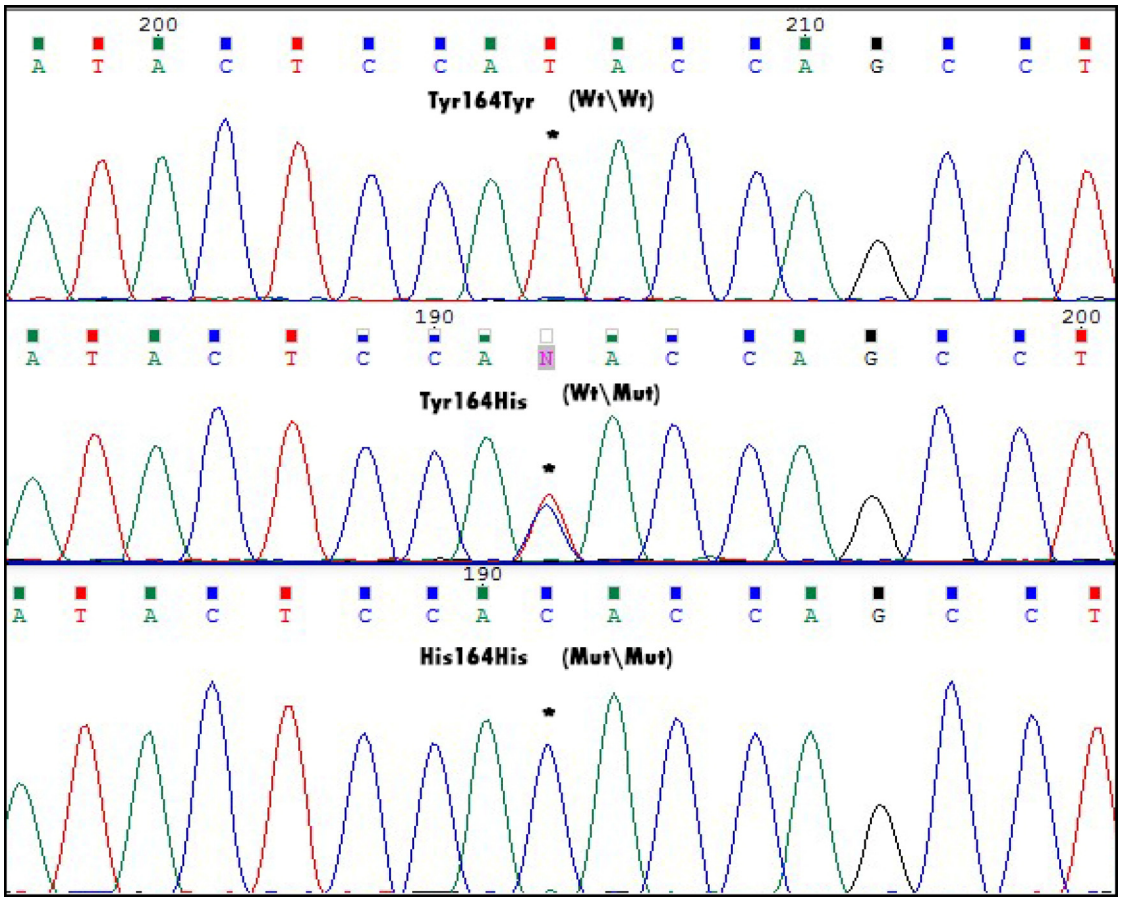

Fig. 1. Mutation analysis: Direct sequencing of exons 4 of the *GLDC* gene depicting 3 different sequencing of the mutation/polymorphism: – Normal (Tyr/Tyr), heterozygous (Tyr/His) and homozygous (His/His).

codon which usually result in degradation or tertiary structural change of the protein [7,8].

The phenotype in our patients is distinct by its heterogeneity varying from infantile onset, classic neonatal form and mild late-onset form in patients 1, 2 and 3 respectively. In addition, there was lack of correlation of the phonotype with the CSF/Plasma glycine ratio (Table 1). CSF/Plasma glycine ratio was highest in patient 3 who was completely asymptomatic during infantile period without treatment and presented only with speech delay. On the other hand, patient 2 who presented with the clinical and biochemical features of classic NKH had normal neurological examination and motor developmental milestones at age 5 years apart from speech delay. Patient 1 has the lowest CSF/Plasma glycine ratio although he was on valproate therapy. He also achieved normal motor milestones but has the most severe speech and social delay. Seizure subsided in all patients and eventually anticonvulsant medications were stopped.

Four Moslem Palestinian patients born to first-cousin parents from two unrelated families were reported with unique clinical phenotype ranging from severe neonatal form to asymptomatic status with normal development during childhood. The patients showed modest CSF-plasma glycine ratio elevation and all were homozygous for the mutation c.2405C > T in exon 20 of *GLDC* gene [10]. The authors suggested that the nature and the timing of therapeutic intervention were crucial in determining the outcome, namely assisted ventilation, sodium benzoate and ketamine. The patients in our report are born to first-cousin Moslem Palestinian parents and showed similar clinical heterogeneity with two distinctions: First: The lack of significant correlation between the outcome and therapeutic intervention especially in patient 3 who did not receive any therapeutic intervention during the first year of life. Second: The lack of correlation between CSF-plasma glycine ratio and the clinical course.

In a systematic study of clinical data on 45 patients with different forms of GE, parameters and severity scores were identified that allowed prediction of long-term outcome in infancy. The authors found that 19% of patients presenting in the newborn period and 50% of patients presenting in infancy made developmental progress. A severe outcome was associated with spasticity developing within the first six months of life, frequent hiccupping, microcephaly, cerebral malformation

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including hypoplasia of the corpus callosum and extracerebral congenital malformation [11]. CSF: plasma glycine ratio was not found to affect the long-term prognosis and specific mutations such as p. A802V have been suggested to predict an outcome of the attenuated form even in patients with a neonatal presentation [11,12]. Brain MRI of patient 1 in our report showed widening of CSF spaces but brain imaging was not performed in the other two patients. This may also be explained by the Y164H mutation but further studies are needed to prove this genotype-phenotype correlation.

Data for 65 patients (36 boys, 29 girls) collected in an international survey showed a striking gender difference in mortality and developmental progress. One-third of the patients died; 8 girls died during the neonatal period and 14 patients died thereafter (2 girls and 12 boys). Of 25 patients living $>$ or $=$ 3 years, 10 were able to walk and say/sign words; all were boys [12]. The majority of survivors were treated with sodium benzoate and dextromethorphan, with 41% showing increased alertness and decreased seizures.

Transient neonatal hyperglycinemia is clinically or biochemically indistinguishable from nonketotic hyperglycinemia at onset [13]. Three members of a consanguineous family were reported, the first child had clinical and biochemical features of classical neonatal NKH and was treated with sodium benzoate. Despite the persistence of increases in plasma and CSF glycine, at age of 10 years she had completely normal development. Two subsequent children also had increase glycine CSF/plasma levels but were asymptomatic and having normal development. In the three children, a heterozygous *GLDC* mutation was identified. This could be explained by partially enzymatically active *GLDC* mutation, which, however, cannot be proved due to lack of liver biopsy data to measure residual glycine cleavage system activity [4,13].

4. Conclusion

Our study reveals a novel mutation in *GLDC* gene further expanding the genetic spectrum of glycine encephalopathy, and adds to the increasingly recognized evidence that the clinical phenotypes of this disease is variable even in the same family. Other genetic and environmental factors may also be responsible for the clinical variability of glycine encephalopathy.

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