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19-Year Follow-up of A Patient With Severe Glutathione Synthetase Deficiency

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Abstract

Glutathione synthetase deficiency is a rare autosomal recessive disorder resulting in low levels of glutathione and an increased susceptibility to oxidative stress. Patients with glutathione synthetase deficiency typically present in the neonatal period with hemolytic anemia, metabolic acidosis and neurological impairment. Lifelong treatment with antioxidants has been recommended in an attempt to prevent morbidity and mortality associated with the disorder. Here we present a 19-year-old female who was diagnosed with glutathione synthetase deficiency shortly after birth and who has been closely followed in our metabolic clinic. Despite an initial severe presentation, she has had normal intellectual development and few complications of her disorder with a treatment regimen that includes polycitra (citric acid, potassium citrate and sodium citrate), vitamin C, vitamin E and selenium.

Keywords

5-oxoprolinuria; Glutathione Synthetase Deficiency; Glutathione

Introduction

Glutathione (GSH) is a ubiquitous tripeptide (L-y-glutamyl-L-cysteinylglycine) known to function in many aspects of cellular activity including protein and DNA synthesis, transport, detoxification of xenobiotics and carcinogens, metabolism, and defense against free radicals and oxidative stress[1]. It is synthesized in the γ-glutamyl cycle (Fig 1) by glutathione synthetase (GS). GSH is most commonly found in its reduced form with only 1–5% existing

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Disclosure
None

Contribution Statement
All authors above made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work AND drafting the work or revising it critically for important intellectual content AND gave final approval of the version to be published AND agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

in the oxidized form glutathione-disulphide (GSSG). GSSG is reduced back to GSH by glutathione-disulphide reductase requiring one equivalent of NADPH[2]. Biallelic, pathogenic variants in the *GSS* generesult in glutathione synthetase deficiency (GSSD).

GSSD (MIM:266130) is a rare autosomal recessive disorder that has only been described in approximately 70 individuals worldwide[3]. Erythrocytes have some of the highest concentrations of GSH in the body, and thus, erythrocytes from patients with GSSD are more susceptible to oxidative stress. As a result, hemolytic anemia is a common problem in GSSD[4]. Depending on their clinical manifestations, patients can be divided into mild, moderate, or severe phenotypes. Patients with the mild GSSD present with isolated hemolytic anemia as their only clinical symptom whereas patients with the moderate form of the disorder present with hemolytic anemia and metabolic acidosis in the neonatal period. In addition to these symptoms, patients with severe disease have neurologic findings including motor disturbances and developmental delay[5]. Some severely affected patients show an increased susceptibility to bacterial infections, thought to be due to defective granulocyte function. To date there is only one severely affected individual who was treated with antioxidants from birth and long-term follow-up on that individual is not reported. 5-oxoprolinuria can also be found in all patients with GSSD due to the accumulation of γ-glutamylcysteine being hydrolyzed to 5-oxoproline and cysteine by γ-glutamyl cyclotransferase (GCT). This accumulation of 5-oxoproline exceeds the capacity of 5-oxoprolinase leading to high urinary excretion of 5-oxoproline that is detectable on urine organic acid analysis[6]. In the present report, we describe long-term follow-up of a case of severe GSSD.

Case Presentation

The proband is a female born at 40 weeks gestation by Cesarean section weighing 2.970 kg (16th centile) with Apgar scores of 8 and 9 at 1 and 5 minutes respectively. At approximately 18 hours of life, she was found to be tachypneic with a metabolic acidosis. Arterial blood gas showed pH of 7.32 (normal pH 7.35–7.45), $PaCO_2$ 17 mmHg (normal 35–45 mmHg), PaO_2 94 mmHg (normal 80–100 mmHg) and bicarbonate of 8 mmol/L (normal 21–32 mmol/L). She was given 5 mg intravenous (IV) sodium bicarbonate and transferred to a tertiary hospital at which time her metabolic acidosis had worsened to pH 7.15, $PaCO_2$ 12 mmHg, PaO_2 94 mmHg and bicarbonate of 4 mmol/L. She was treated with IV fluids and IV sodium bicarbonate of 1.1 meq/kg/hr. Urine organic acid analysis performed at this time revealed high levels of 5-oxoproline, suggesting a defect in glutathione metabolism. Once stabilized, she was started on a combination of citric acid, potassium citrate, and sodium citrate (~30 meq/kg/day)for metabolic acidosis and vitamin C (250 mg per day)–and E (30 international units per day) to help prevent oxidative stress. Mother was also advised to avoid foods and medications that cause oxidative stress (from a list standardly used for individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency). She was then discharged home with follow-up in the metabolic clinic.

At her 2-month follow-up visit at the metabolic clinic, she had remained stable with no intercurrent illnesses or hospitalizations since her discharge. She was tolerating her medications and growth was satisfactory (40th centile for weight, 10th centile for height).

Physical exam was normal. Laboratory results showed mild macrocytic anemia with reticulocytosis and hyperkalemia, which became her baseline for most of her life, and a normal bicarbonate of 23 mmol/L. At 6 months her red blood cell GSH and GS activity levels were analyzed, and she had a marked decrease in both GSH level and GS activity (See Table 1), consistent with the diagnosis of GSSD. Her mother also had a decrease in GS activity, although normal GSH levels, suggesting heterozygosity.

Throughout early childhood, she had multiple episodes of otitis media which resolved with antibiotics and multiple minor viral respiratory infections. She also developed an episode of tachypnea and acidosis in the setting of 4 days of fever, watery diarrhea, congestion and spitting up. Her serum bicarbonate level was 10 mmol/L, and she was hospitalized. Her acidosis improved with IV fluids and IV bicarbonate. She was hospitalized again at 18 years of age when a viral gastroenteritis caused decreased oral intake and inability to take medications; her serum bicarbonate level at that time was 7 mmol/L and resolved quickly with hydration and IV bicarbonate. Numerous dental caries were reported, and her dental healthy improved after a switch to a sugar free combination of citric acid, potassium citrate, and sodium citrate.

At approximately 2 years 3 months of age, there was concern that increased oxidative stress might hinder her developmental progress, so she was started on selenium one crushed tablet daily. A formal developmental evaluation was performed at approximately 2 years 6 months and was reported as normal. However, by 3 years 9 months developmental delays were evident. She was unable to copy a circle or ride a tricycle and her language was unintelligible. She was also having difficulties with toilet training. Speech therapy led to significant improvement, and by 4.5 years her speech had greatly improved and her other developmental concerns resolved.

At her 33 months of age, weight loss and deceleration of head growth were noted, and these findings were attributed to chronic metabolic acidosis (bicarbonate of 15–18 mol/L), her citric acid, potassium citrate, and sodium citrate was adjusted to ~18 meq/kg/day and by three years of age, her acidosis and weight gain had improved.

Ophthalmology evaluation for pigmentary retinopathy, a known finding in patients with GSSD, was normal. At the age of 5 years, she underwent a tonsillectomy and adenoidectomy for chronic snoring and mouth breathing without any complications. She was diagnosed with attention deficit disorder at approximately 7 years of age and was successfully treated with guanfacine. At approximately 10 years, she was diagnosed with a seizure disorder and treated with the antiepileptic lamotrigine. However, by age 15 years, she had no additional seizures, and her EEG was normal so she was successfully weaned off lamotrigine. At age 16 years, her psychologist diagnosed her with Asperger syndrome. We are unaware of any association with of Asperger syndrome with GSSD.

Currently, at 19 years, she is doing well. She graduated from mainstream high school and attends community college. She is currently prescribed guanfacine for ADHD, citric acid, potassium citrate, and sodium citrate syrup (11 meq/kg/day) for metabolic acidosis, and vitamin C (14.5 mg/kg/day), vitamin E (150 international units per day) and selenium (50

mcg per day) to prevent oxidative stress. Her bicarbonate levels and reticulocyte counts are provided in Figure 2.

Discussion

GSSD is a metabolic disorder that requires lifelong treatment. Treatment depends on the signs and symptoms of the patient. However, high doses of vitamins C and E are recommended in all patients for protection against oxidative stress[3,7]. Avoidance of foods and drugs known to cause hemolytic crisis in G6PD deficiency is also important as these same triggers can cause hemolytic crisis in GSSD[8]. Vitamin E is also used to prevent granulocyte dysfunction, which could cause recurrent infections[9,10]. Patients with moderate and severe phenotypes typically require treatment for metabolic acidosis. In acute crisis, IV bicarbonate is given for immediate correction, and for long term management, citrate or trometamol (THAM) are given. Our patient has required very high doses of citric acid, potassium citrate, and sodium citrate (ranging from ~30 meq/kg/day in early infancy to approximately 10–20 meq/kg/day throughout most of her childhood and adolescent years) to maintain a normal serum bicarbonate level (Figure 2). Moreover, her persistently elevated reticulocyte count (Figure 2) reflects ongoing but compensated hemolytic anemia.

Previously, cysteine delivery compounds, specifically N-acetylcysteine (NAC), were given because they increase GSH levels in healthy patients[11]. However, NAC has been found to increase intracellular levels of cysteine, which are already elevated in GSSD[6] and these high levels of cysteine are known to be neurotoxic[12]. Selenium was another agent used in our patient to prevent oxidative stress, and to our knowledge this is the first reported use of selenium in a patient with GSSD. Selenium is found to have strong antioxidant properties through formation of selenoproteins, which are thought to protect against reactive oxygen species[13].

Early diagnosis and treatment is thought to correlate with a better long term outcome. If a neonate presents with hemolytic anemia and metabolic acidosis, it is important to consider GSSD[14], as rapidly fatal GSSD in newborns has been described[15]. Advanced diagnostic techniques such as antenatal diagnosis can be made by measuring 5-oxoproline in amniotic fluid[16,17] or a presumptive diagnosis can be made by detecting elevation of 5-oxoproline in newborn screen blood spots using tandem mass-spectrometry[18.]. Our patient's diagnosis was made within a few days of life, based on urine organic acid analysis and thus, treatment with vitamin C and E were started early. Njalsson et al (2005) demonstrated that early initiation of vitamins C and E could prevent the moderate phenotype from progressing to severe phenotype. In a study of 41 patients with GSSD, only 1/18 of the severely affected patients were started on vitamin therapy early in life as compared to 6/17 moderately affected patients. With this data and the fact that there is no significant difference between enzymatic activity in the moderate and severe phenotypes, they posit that early initiation of vitamins could prevent or slow down progression of the disease[19]. There were no specific comments on length of follow-up of this cohort however.

Our patient was diagnosed at birth with moderate disease because of her acidosis and hemolytic anemia. However, at the age of 10 years, she developed seizures, a finding which

is more consistent with the severe phenotype. Her seizures were very mild and anti-epileptics were eventually discontinued once she was seizure free for 5 years. We posit that early initiation of vitamins and combination citric acid, potassium citrate, and sodium citrate coupled with good compliance explains her relatively mild course. Another possibility is her seizures were unrelated to her underlying GSSD.

Another area of concern for GSSD patients is growth and developmental delay. Our patient had an episode of weight loss and deceleration in head growth in early childhood that was attributed to chronic metabolic acidosis and that improved with increased doses of her medications. Currently, she has short stature (<5th percentile, Z-score −2.1) with appropriate weight and head circumference. Developmentally, our patient experienced minor speech delays early in life that required speech therapy and there were concerns for mild fine motor delay that resolved without intervention. She had some mild difficulties in school and required extra support in the classroom. However, she is currently performing satisfactorily in high school with plans for attending college.

In conclusion, we describe long-term follow-up of a patient with severe GSSD and good outcome. We attribute early recognition of the disease, initiation of appropriate vitamin therapy and acidosis correction along with excellent compliance from our patient for her success to date. We also believe the use of selenium as an additional antioxidant has contributed to her relatively mild course. Finally, we recommend testing for GSSD in patients with metabolic acidosis or hemolytic anemia in the newborn period as early recognition and initiation of therapy appears to correlate with better outcomes.

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Abbreviations

GCT	γ-glutamyl cyclotransferase
GS	Glutathione Synthetase
GSH	Glutathione
GSSD	Glutathione Synthetase Deficiency
GSSG	Glutathione-Disulphide
NAC	N-acetylcysteine

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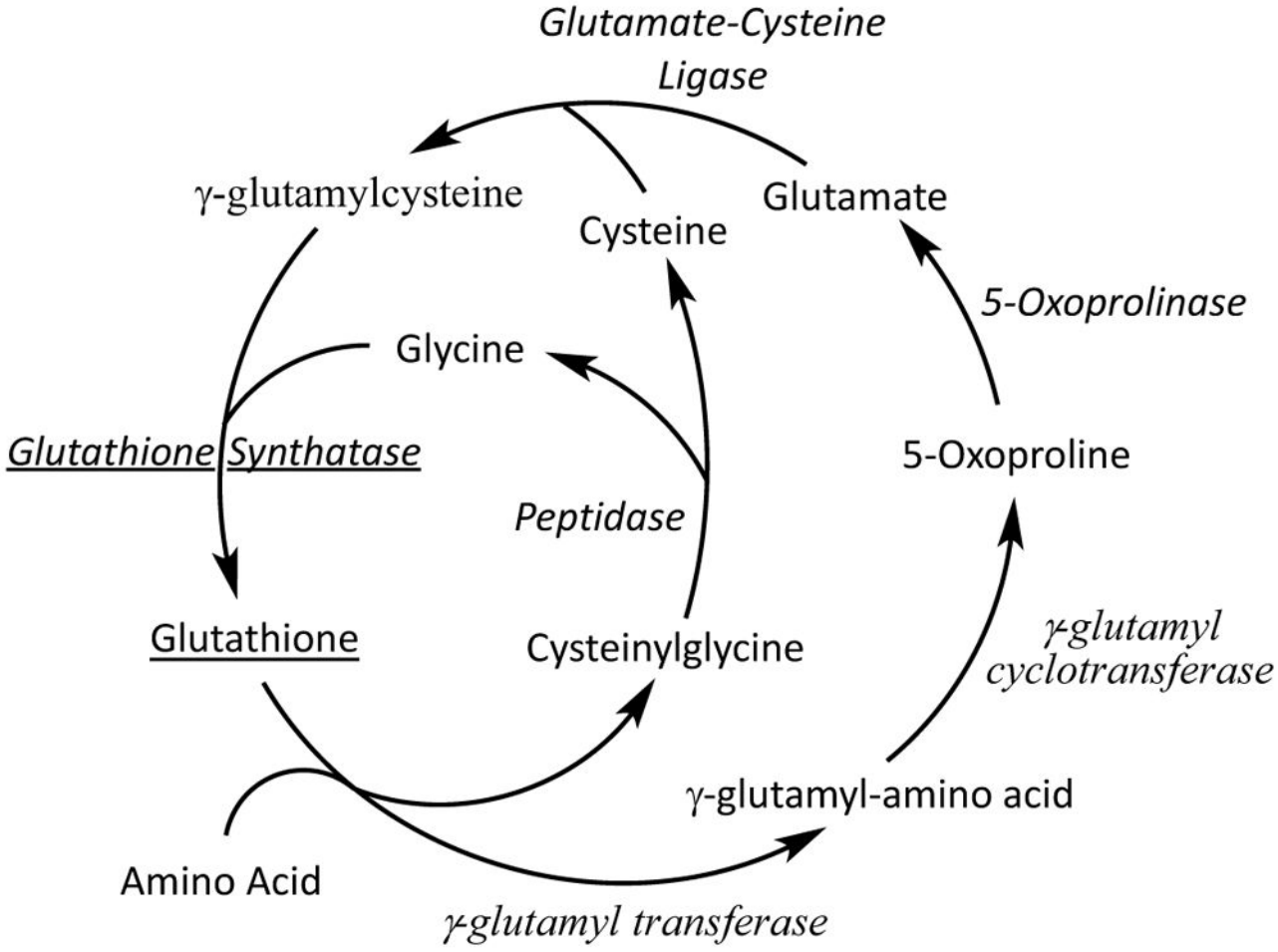
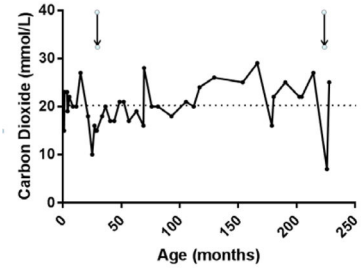


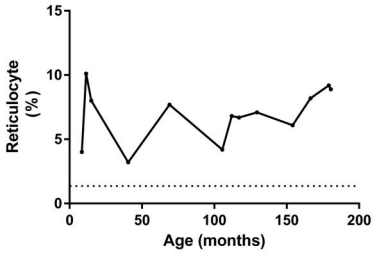
Fig 1.

γ-Glutamyl Cycle: Glutathione is synthesized by glutathione synthetase from glutamine and cysteine in a two-step process involving the enzymes *glutathione synthatase* and *glutamate-cysteine ligase*.

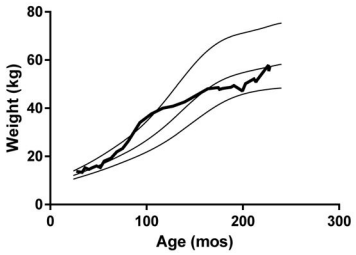
A.



B.



C.



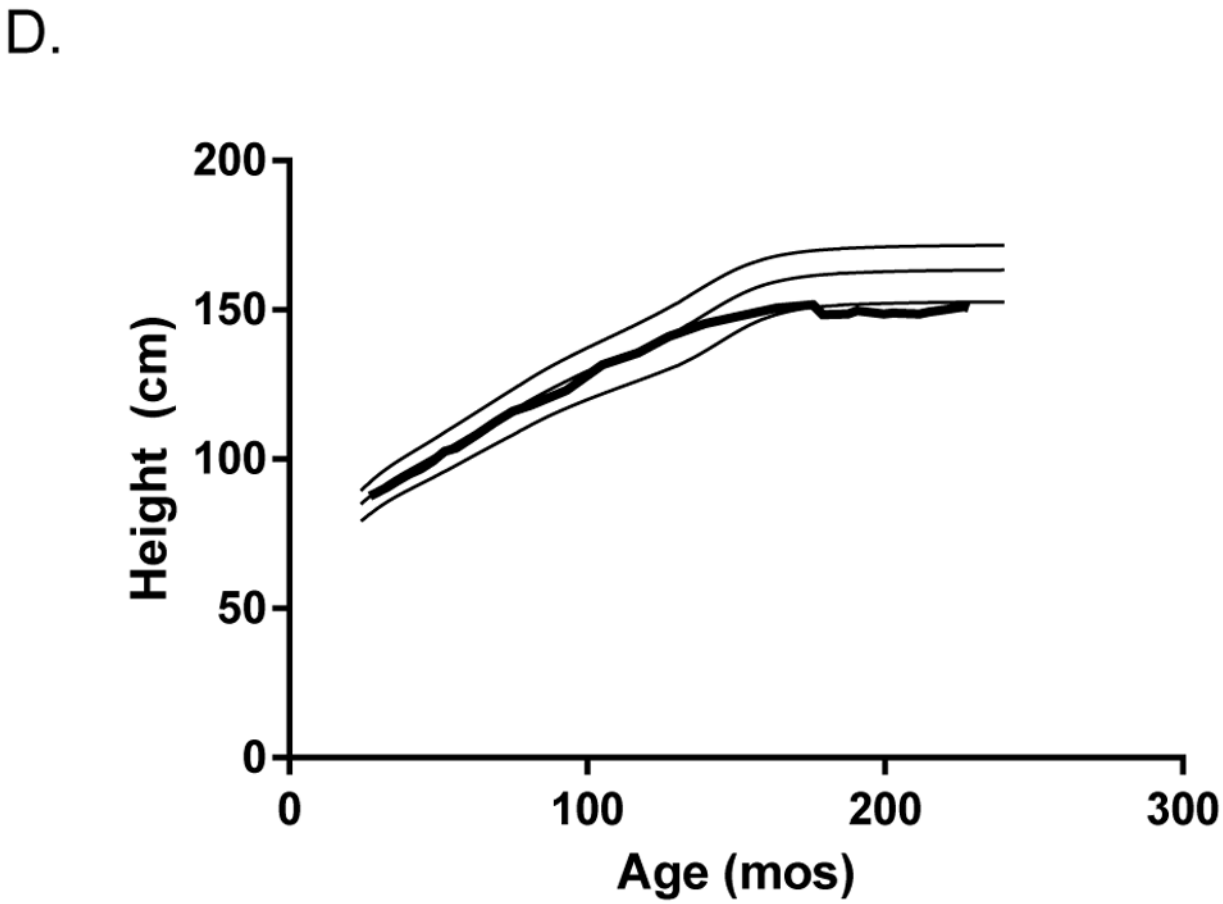


Fig 2.

Laboratory results and growth parameters. A. The proband's bicarbonate levels across her lifespan are plotted by age. Hospitalizations are marked with arrows. To maintain these levels, doses of ~30 meq/kg/day of bicarbonate were required in early infancy, but during childhood and adolescent years, she was able to maintain her bicarbonate level with doses of ~10–20 meq/kg/day. Although the normal range for bicarbonate depends on age, we labeled 20 mmol/L with a dotted line for reference. B. Reticulocyte count was persistently elevated throughout the lifespan and reflects ongoing hemolysis. The dotted line represents the upper limit of normal range (1.5%). C. The proband's weight gain (thick line) is plotted. The 10^{th}, 50^{th} and 90^{th} percentile growth curves from the CDC (2000) growth charts are provided (thin lines). D. The proband's height (thick line) is plotted. The 10^{th}, 50^{th}, and 90^{th} percentile growth curves from the CDC (2000) growth charts are provided (thin lines).

Table 1

Results of GS Synthetase Assay

	GSH level (Mol/gHb)	Glutathione synthetase activity (IU/gHb)
Patient	0.60	0.04
Mother	6.17	0.19
Control	5.44	0.45
Normal Range	4.50–8.70	0.24–0.53