Meta Gene 25 (2020) 100751

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

# Genotype and clinical phenotype in four patients with glutathione synthetase deficiency

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ARTICLE INFO

*Keywords:*
5-Oxoprolinuria
*GSS* gene
Glutathione synthetase
Pyroglutamic aciduria
Metabolic acidosis

ABSTRACT

*Background and aims:* Glutathione synthetase deficiency (GSSD) is an autosomal recessive disorder described in the literature in roughly 80 patients. Currently, there is little known about genotype-phenotype correlations in GSSD though severity can be predicted to a limited extent via mutation analysis. Here, we describe four patients with GSSD and assess their genotypes and phenotypes. Additionally, we have provided an updated review of the literature.

*Methods:* We retrospectively reviewed all medical charts for patients with GSSD in the last ten years at Al-Makassed Hospital in Jerusalem, Palestine. We reviewed the literature for medical management and latest research in treatment and discussed the phenotype-genotype correlations.

*Results:* We describe four patients with confirmed GSSD. Clinical presentation varied in severity, but patients typically presented with hemolytic anemia and lactic acidosis. Urine organic acid analysis demonstrated massive excretion of lactic acid and pyroglutamic acid. All patients were treated with *N*-acetylcysteine, vitamin E, vitamin C, and sodium hydrogen carbonate. All improved significantly following treatment, apart from one patient who died at two months of age.

*Conclusion:* GSSD presents similarly to many other diseases, at times causing a delay in diagnosis. Early initiation of treatment can improve clinical outcomes and overall development. If there is a high suspicion for GSSD, it is important to consider mRNA sequencing in an effort to prevent a delay in diagnosis when a splice site mutation is present.

## 1. Introduction

Glutathione synthetase deficiency (GSSD) is an autosomal recessive disorder caused by mutations in the gene encoding glutathione synthetase (GS), *GSS* (OMIM #601002) on chromosome 20 at locus 20q11.2. Thirty-two unique mutations have been reported in over 80 patients around the world. GS is one of six enzymes involved in glutathione synthesis via the γ-glutamyl cycle. Glutathione plays a role in both DNA and protein synthesis and is an excellent biologic antioxidant. When glutathione is decreased, however, there is an increase in formation of γ-glutamylcysteine, which is readily converted to 5-oxoproline. Due to the strongly acidic nature of 5-oxoproline, its accumulation in plasma and in urine lead to metabolic acidosis (Al-Jishi et al., 1999; Njålsson et al., 2005; Shi et al., 1996).

Patients with GSSD can be divided into three groups based on clinical presentation: mild, moderate, or severe. Mildly affected patients have isolated hemolytic anemia while moderately affected patients will also have metabolic acidosis. In severe cases, patients typically present in the neonatal period with hemolytic anemia, metabolic acidosis, and neurological manifestations, consisting of intellectual and/or motor deficits (Al-Jishi et al., 1999; Njålsson et al., 2005; Signolet et al., 2016). Diagnosis is based on clinical findings, elevated urine 5-oxoproline, and confirmatory mutation analysis. Here, we discuss four patients with confirmed diagnoses of GSSD.

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https://doi.org/10.1016/j.mgene.2020.100751
Received 20 March 2020; Received in revised form 29 May 2020; Accepted 11 June 2020
Available online 13 June 2020

## 2. Patients and methods

### 2.1. Sample collection

The patients were recruited from Al-Makssed Hospital in Jerusalem, Palestine. Informed consent was obtained from the patients and/or their parents for both genetic analysis and publication of the patient's medical information. The study was approved by the Institutional Review Board of the hospital.

### 2.2. Patient 1

Patient 1 is a female born full-term via cesarean section to consanguineous parents who are first cousins. The patient is their fifth child and has one living healthy sibling; three siblings had died in the neonatal period, each due to lactic acidemia of unknown etiology. The pregnancy and delivery of the patient were uneventful. Cesarean delivery was required due to fetal distress. Her birth weight was 2460 g. Apgar scores were 8 and 9 at 1 and 5 min, respectively. The patient was admitted to the neonatal intensive care unit (NICU) for respiratory distress, poor sucking, hyperbilirubinemia (14.5 mg/dL), and metabolic acidosis (pH: 7.15, PCO2: 19.4 mmHg, $HCO3^-$: 6 mEq/L) in the first 24 h of life. Chest X-ray was normal. Coombs test and blood culture were negative. Basic metabolic panel and liver function tests were within normal limits. Plasma amino acid profile was normal. Urine organic acid analysis demonstrated massive excretion of lactic acid and pyroglutamic acid. Genomic amplification and direct sequencing of the *GSS* exons were performed on DNA extracted from peripheral blood and revealed homozygosity for the mutation R283C (c.847C > T) in exon 9. The patient has been managed primarily with *N*-acetylcysteine (15 mg/kg/day), vitamins C (100 mg/kg/day) and E (10 mg/kg/day), and sodium hydrogen carbonate (0.9 mEq/kg/day). Follow-up at one year of age showed significant improvement. Now, at one year of age, her motor, social, and language development are appropriate for age, though she is small for age (3rd percentile).

### 2.3. Patient 2

Patient 2 is a female born full-term via normal vaginal delivery to consanguineous parents who are first cousins. She is their first child. The pregnancy was complicated by intrauterine growth restriction. Her birth weight was 2250 g. Apgar scores were 8 and 9 at 1 and 5 min, respectively. The patient initially presented with respiratory distress, jaundice, and anemia. Shortly after feeding, her condition rapidly deteriorated and she developed metabolic acidosis (pH: 7.06, PCO2: 15 mmHg, $HCO3^-$: 7 mEq/L), elevated lactate levels to 6.6 mmol/L, and hyperammonia to 225 μg/dL. Chest X-ray was normal and infectious work-up negative. Hemolysis was appreciated on blood smear and Coombs test was negative (Table 2). Liver function tests were within normal limits. Plasma amino acid profile was normal. Urine organic acid analysis was significant for massive excretion of lactic acid and pyroglutamic acid. DNA was extracted from peripheral blood and genomic amplification with direct sequencing of the *GSS* exons revealed homozygosity for the mutation R125C (c.373C > T) in exon 4. The patient was managed with *N*-acetylcysteine, vitamins E, C and B complex, sodium hydrogen carbonate, L-carnitine, and ResQ-10. Despite these interventions, her hemoglobin remained low, with a nadir of 5.2 g/dL. Two months later, she developed severe bradycardia and electrolyte derangements with a peak sodium level of 179 mEq/L and chloride level of 148 mEq/L. The patient passed away thereafter at two months of age.

### 2.4. Patient 3

Patient 3 is a male born full term via normal vaginal delivery to consanguineous parents who are first cousins. The patient is their second child and he has one healthy living sibling. The pregnancy and delivery were unremarkable. His birth weight was 2900 g. Apgar scores were 7 and 9 at 1 and 5 min, respectively. The patient developed tachypnea on the second day of life that progressed to respiratory distress in the setting of fever and cough. He was found to have lactic acidosis resistant to treatment. Initial work-up revealed metabolic acidosis (pH: 7.29, PCO2: 12 mmHg, and $HCO3^-$: 8 mEq/L) and hemolytic anemia (hemoglobin: 11.5 g/dL and reticulocytes: 2.6%). Chest X-ray, Coombs test, and blood cultures were negative. Basic metabolic panel and liver function tests were within normal limits. Plasma amino acid profile was normal. Urine organic acid analysis demonstrated massive excretion of lactic acid and pyroglutamic acid. Genomic amplification and direct sequencing of the *GSS* exons were performed on DNA extracted from peripheral blood. No pathogenic mutations were found using PCR-mediated sequence analysis. RT-PCR, however, led to the discovery of his homozygous c.129 + 1663A > G mutation in intron 2 of *GSS*. The patient has been managed with *N*-acetylcysteine, vitamins E and C, and sodium hydrogen carbonate. At five years of age he was admitted due to recurrent seizure activity and he was started on carbamazepine 8 mg/kg/day. Currently, he is six years old and his motor, social, and language development are appropriate for age. His weight and height are each at the 50th percentile. Additionally, he has not had additional seizures since starting carbamazepine.

### 2.5. Patient 4

Patient 4 is a female born full term via cesarean section to consanguineous parents who are first cousins. She is their fourth child. Her three siblings are alive and healthy. The pregnancy and delivery were unremarkable. Her birth weight was 3600 g. Apgar scores were 7 and 9 at 1 and 5 min, respectively. The patient developed tachypnea on the fifth day of life that was associated with poor feeding and hyperactivity. She was found to have lactic acidosis. Initial work-up revealed metabolic acidosis (pH: 6.96, PCO2: 11 mmHg, and $HCO3^-$: 6 mEq/L) with hemoglobin of 14.6 g/dL and hyperammonemia to 182 μmol/L. Liver function tests were within normal limits. Plasma amino acid profile was normal. Urine organic acid analysis demonstrated massive excretion of lactic acid and pyroglutamic acid. Genomic amplification and direct sequencing of the *GSS* exons were performed on DNA extracted from peripheral blood. No pathogenic mutations were found using PCR-mediated sequence analysis. RT-PCR, however, led to the discovery of her homozygous c.129 + 1663A > G mutation in intron 2 of *GSS*. The patient has been managed with *N*-acetylcysteine, vitamins E and C, and sodium hydrogen carbonate. Currently, she is 10 months old. Her weight is at 35th percentile and her length is at the 3rd percentile. Her motor, social, and language development are appropriate for age.

## 3. Discussion

Here, we describe four patients with confirmed homozygous mutations in *GSS* (Tables 1 and 2). In each case, the patient presented with metabolic acidosis, often with anemia and/or hyperbilirubinemia as well. Though little is currently known about genotype-phenotype correlations in GSSD, severity can be predicted to a limited extent via mutation analysis (Njålsson et al., 2005).

In a study by Njålsson et al. (2005) where 41 patients with GSSD were considered, two were described with homozygous R283C mutations (c.847C < T) in exon 9. Compound heterozygous mutations in R283C were also discovered in a patient by Shi et al. (1996). R283C is thought to be associated with a moderate to severe clinical phenotype, which is in agreement with Patient 1 described here. She too carries homozygous R283C mutations and developed severe GSSD.

R125C is another mutation that was identified in a patient by Shi et al. (1996). The patient had 3 sequence changes in *GSS*: 2 missense mutations (373C > T, leading to R125C, and 941C > T, leading to P314L, and a 6 base pair in-frame deletion (1137del6, resulting in the

**Table 1**
laboratory findings of the studied patients at presentation.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|---|---|---|---|
| WBC [mm3] | 17,900 | 12.4 | 9500 | 13.2 |
| Hb [g/dL] | 7.27 | 9 | 11.5 | 14.6 |
| Hct [%] | 21 | 26 | 33 | 46 |
| RC [%] | 3.5 | 20 | 2.6 | 2 |
| PLT [mm3] | 576 | 127 | 250 | 325 |
| T. Bil [mg/dL] | 14.5 | 11.4 | 6 | 11.7 |
| D.Bil [mg/dL] | 0.66 | 0.6 | 1 | 0.73 |
| pH | 7.15 | 7.06 | 7.29 | 6.96 |
| pCO2 [mmHg] | 19.4 | 15 | 12 | 11 |
| HCO3–[mEq/L] | 6 | 5 | 8 | 6 |
| Lactic acid [mmol/L][a] | 2.33 | 6.6 | 5.3 | 23.3 |
| Ammonia [μmol/L][a] | 41.5 | 225 | 56.9 | 182 |
| Urine lactic acid | Elevated | Elevated | Elevated | Elevated |
| Urine pyroglutamic acid | Elevated | Elevated | Elevated | Elevated |

White blood cell [WBC], Hemoglobin [Hb], Hematocrit [Hct], Reticulocyte [RC], Platelet [PLT], Total Bilirubin [T. Bil], Direct Bilirubin [D.Bil], Bicarbonate [HCO3–].

[a] Normal Ammonia levels: < 100 μmol/L, Lactate:1.1–2.3 mmol/L.

deletion of val380 and gln381) in exons 4, 9, and 11, respectively. R125C was associated with severe GSSD and no detectable GS enzyme activity. Patient 2 also presented with severe disease, resulting in infantile death, and she was homozygous for R125C. Patients previously reported with severe disease had hemolytic anemia at birth and those who survived the neonatal period developed neurological manifestations, including seizure and psychomotor retardation (Ristoff et al., 2001).

A considerable number of patients with GSSD have been found to have splice site mutations that are not readily detected by PCR-mediated sequence analysis of genomic DNA. Instead, RT-PCR is used to uncover abnormal splice patterns, as was the case with Patients 3 and 4 above. RT-PCR led to the discovery of Patient 3's homozygous c.129 + 1663A > G mutations in intron 2 of *GSS*. This mutation leads to the insertion of a pseudoexon between exons 2 and 3, resulting in a frame shift and the introduction of a premature stop codon (Njålsson et al., 2003). This particular mutation is associated with severe disease (Njålsson et al., 2003). In the care of our patients, however, they had moderate severity at presentation. Their condition significantly improved upon initiation of medication and supplementation and they did not suffer from developmental delay. Patient 3 did progress to develop seizures, consistent with a more severe disease phenotype. His seizures, however, were mild and well-controlled on antiepileptic medication. Atwal et al. (2016) also report a case of mild disease, complicated by seizure development in childhood. Ristoff et al. (2001) emphasize the importance of vitamins C and E supplementation for all patients, regardless of disease severity, to prevent the development of neurological symptoms at a later age.

While genotype-phenotype correlations are becoming apparent, clinical correlations are also being identified. CNS involvement, for example, has only been observed in cases with untreated metabolic acidosis in the neonatal period (Soylu Ustkoyuncu et al., 2018). CNS involvement is therefore a component of the natural progression of the disorder that can seemingly be prevented with early intervention. Neurological manifestations in patients with GSSD is thought to be due to the accumulation of free radicals and peroxides, causing damage to nerves and brain tissue. Additionally, the loss of glutathione may alter neuromodulation and weaken protection against glutamatergic excitotoxicity. Of note, however, there has been no correlation between GS levels and presence or severity of neurological symptoms (Ristoff et al., 2001).

Management of GSSD is dependent on the severity of the disease. The most important factors in management are early diagnosis, correction of the acidosis, and early supplementation with vitamins C and E (Ristoff et al., 2001). Prenatal diagnosis of GSSD is possible through analysis of 5-oxoproline in amniotic fluid (Erasmus et al., 1993; Manning et al., 1994). The metabolic acidosis that develops should be managed with bicarbonate, citrate or tris-hydroxymethyl aminomethane (THAM). A study by Gündüz et al. (2016) showed that sodium hydrogen carbonate is effective for treatment of chronic metabolic acidosis in GSSD. *N*-acetylcysteine is also recommended as it has been shown to increase the low intracellular glutathione concentrations and cysteine availability (Mårtensson et al., 1989). Vitamin E has been shown to protect leukocytes against oxidative damage and, as a result, prevents the neutropenia that occurs in this patient population. It also increases the survival of erythrocytes and prevents the onset of hemolytic anemia (Boxer et al., 1979). Oral administration of glutathione analogues also seem to increase glutathione concentration in both leukocytes and plasma (Jain et al., 1994). Blood transfusions are also often required for management of anemia, in addition to blood exchange in cases of neonatal hyperbilirubinemia.

In summary, we present four cases of GSSD. Clinical presentation varies in severity, but patients typically present with lactic acidosis and hemolytic anemia. Difficulty identifying the cause of hemolytic anemia should raise suspicion for GSSD. Early diagnosis of the disease can prevent its progression and eventual CNS involvement. Given the delay in diagnosis that can occur when GSSD is the result of a splice site mutation, starting the investigation with mRNA sequencing is recommended. Future studies considering the optimization of disease management and prevention of its progression will be important as we continue to identify and care for patients with GSSD.

**Table 2**
Characteristics of the patients.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|---|---|---|---|
| Age of onset | First day of life | First day of life | Second day of life | Fifth day of life |
| Gender | Female | Female | Male | Female |
| Consanguinity | First cousins | First cousins | First cousins | First cousins |
| Affected siblings | Yes, three | No | No | No |
| Clinical presentation | Metabolic acidosis, jaundice and anemia | Metabolic acidosis, jaundice and anemia | Metabolic acidosis and anemia | Metabolic acidosis and jaundice |
| PRBC transfusion | Twice | Six times | Never | Never |
| Phototherapy | Yes | yes | Never | Never |
| Intubation and MV | Yes | Never | Never | Never |
| Current age | One year | Died at 2 months of age | Six years | Ten months |
| Treatment | NAC, Vit.E, Vit.C,NaHCO3 | NAC, Vit.E, Vit.C, NaHCO3, L-carnitine, B-100 complex, ResQ-10. | NAC, Vit.E, Vit.C,NaHCO3 | NAC, Vit.E, Vit.C,NaHCO3 |
| Molecular genetic analysis | R283C [C > T] in exon 9; homozygous | R125C [C > T] in exon 4; homozygous | c.129 + 1663[A > G] mutations in intron 2; homozygous | c.129 + 1663[A > G] mutations in intron 2; homozygous |

Packed Red Blood Cell [PRBC]. Mechanical ventilation [MV], *N*-acetylcystein [NAC], Vitamin E [Vit.E], Vitamin C [Vit.C], Sodium hydrogen carbonate [NaHCO3].

## Authors contribution

**Imad Dweikat:** Conceptualization, Methodology, Investigation, Supervision. **Firas Alqarajeh**: Data curation, Writing- Original draft preparation. **Sadi Abukhalaf**: Writing - Review & Editing, Data curation **Bara' Hmeidat**: Writing - Review & Editing, Data curation. **Jacklyn Omorodion**: Conceptualization, Writing - Review & Editing.

## Data availability statement

Research data are not shared. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

## Acknowledgements

We are grateful to the patients and families for their participation in this study.

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