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Clinical and molecular characterization of 6 children with glutamate-cysteine ligase deficiency causing hemolytic anemia

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ABSTRACT

Glutathione (gamma-glutamylcysteinylglycine) has diverse functions including free radicals scavenging and modulating many critical cellular processes. Glutathione is synthesized by the consecutive action of the enzymes glutamate-cysteine ligase (GCL) and glutathione synthetase. GCL is composed of a catalytic subunit encoded by the *GCLC* gene and a regulatory subunit encoded by the *GCLM* gene. GCL deficiency due to homozygous mutations in *GCLC* has been reported in 6 individuals from 4 independent families. All presented with hemolytic anemia and 4 had additional neurological manifestations including cognitive impairment, neuropathy, ataxia, and myopathy. In this report, we present additional 6 children from 2 independent consanguineous families with GCL deficiency. All the children presented with neonatal hemolytic anemia. Beyond the neonatal period, they did not have jaundice or hemolysis, but continued to have mild anemia. They all had normal development and neurological examination. The affected children from the first family had the homozygous mutation c.1772G > A (p.S591N) and the second family had the homozygous mutation c.514T > A (p.S172T) in *GCLC*. GCL deficiency can have a mild non-neurological phenotype or a more severe phenotype with neurological manifestations. GCL deficiency can be an underdiagnosed cause of hemolytic anemia, thus awareness may aid in early diagnosis, appropriate genetic counseling, and management.

1. Introduction

Glutathione (gamma-glutamylcysteinylglycine), a tripeptide found in all mammalian tissues, is a multifunctional molecule with diverse functions. It plays essential roles in free radicals scavenging, xenobiotic detoxification, maintenance of protein thiol status, cysteine storage, and modulating other critical cellular processes. Glutathione exists in the thiol-reduced (GSH) and disulfide-oxidized (GSSG) forms. GSH is the predominant form. Most of the cellular glutathione presents in the cytosol [1]. Glutathione is an essential antioxidant. Free radicals (superoxide and hydrogen peroxide) are generated as side product of mitochondrial respiration and can cause lipid peroxidation and cell injury. GSH can reduce hydrogen peroxide in the presence of glutathione peroxidase that converts hydrogen peroxide to water and oxidizes GSH to GSSH. The later can be reduced back to GSH via the action of glutathione reductase which requires NADPH. GSH can also conjugate with xenobiotics either spontaneously or enzymatically by GSH-S-transferase. The conjugates are then excreted from the cell or into the bile as in the case of hepatocytes. Therefore, glutathione plays essential role in the elimination of these compounds [1,2]. GSH is the dominant non-protein thiol in mammalian cells and it is essential in maintaining the thiol status of proteins. Through the action of thiol-transferase, GSH undergoes thiol-disulfide exchange with protein residues. The thiol-disulfide equilibrium regulates a diverse number of metabolic processes including enzyme activity, transport activity, signal transduction, and gene expression [3]. Cysteine storage is another essential function for glutathione because extracellular cysteine is unstable and rapidly auto-oxidized to cystine. Gamma-glutamyltranspeptidase (GGT), present on the external surfaces of certain cell types, transfers the glutamyl moiety from extracellular glutathione resulting in cysteinyl-glycine. Dipeptidase then breaks cysteinyl-glycine into cysteine and glycine. The generated cysteine is taken by cells and used in protein or glutathione synthesis [1]. Glutathione also modulates other essential cellular functions including DNA synthesis, cell growth and proliferation, apoptosis, nitric oxide homeostasis, protein post-translational modifications, and neurotransmitter receptor activity

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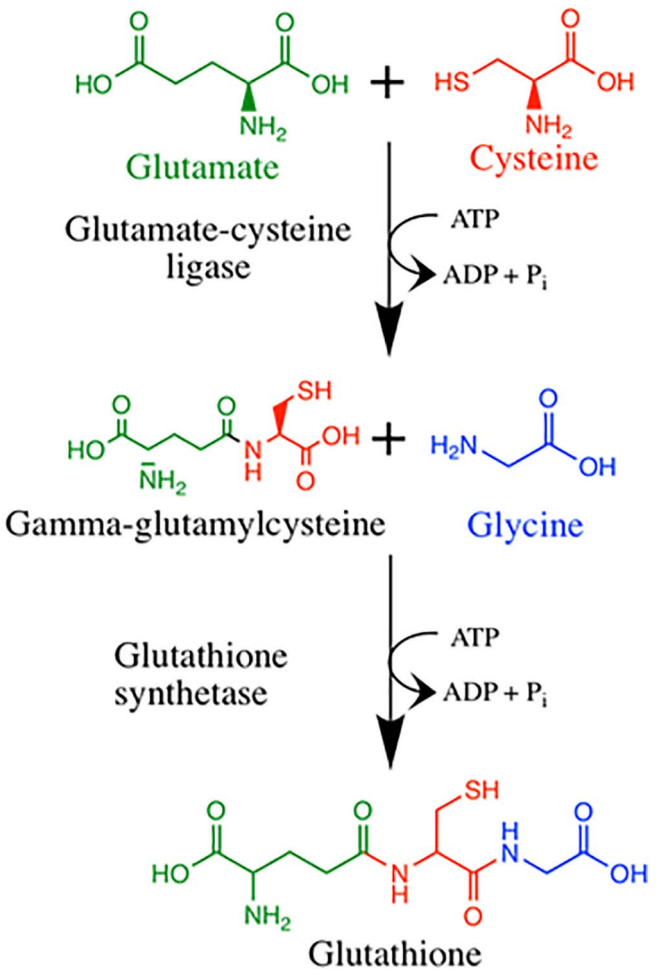

Fig. 1. Biosynthesis of glutathione.

[1,2].

Glutathione is synthesized by the consecutive action of the enzymes glutamate-cysteine ligase (GCL; also known as gamma-glutamylcysteine synthetase (GCS)), and glutathione synthetase (GS). The first step, which is the rate limiting and is catalyzed by GCL, involves peptide bond formation between glutamate and cysteine. Next, GS catalyzes the addition of the third amino acid, glycine (Fig. 1). GCL is composed of a catalytic subunit encoded by the *GCLC* gene and a regulatory subunit encoded by the *GCLM* gene [4,5].

GCL deficiency causing hemolytic anemia was first reported in 1972 [6]. The genes encoding the two GCL subunits, *GCLC* and *GCLM*, were cloned in 1992 and 1995, respectively [4,5]. In 1999, homozygous mutations in *GCLC* were first described to be the molecular basis for GCL deficiency [7]. Since then, molecularly confirmed GCL deficiency has been reported in 6 individuals from 4 independent family [7–10]. In this report, we present additional 6 children from two unrelated consanguineous families with GCL deficiency. We describe their clinical, biochemical, and molecular data. In addition, we review the previously reported cases.

2. Clinical description

Herein we describe two consanguineous families, each with three affected sisters (Fig. 2). In the first family, the older sister was 14 years old born at term after an uncomplicated pregnancy. During the newborn period, she developed hyperbilirubinemia that required phototherapy and anemia that required packed red blood cell (RBC) transfusion. Initial work up showed reticulocytosis, negative Coombs test, and normal hemoglobin electrophoresis. After the neonatal period,

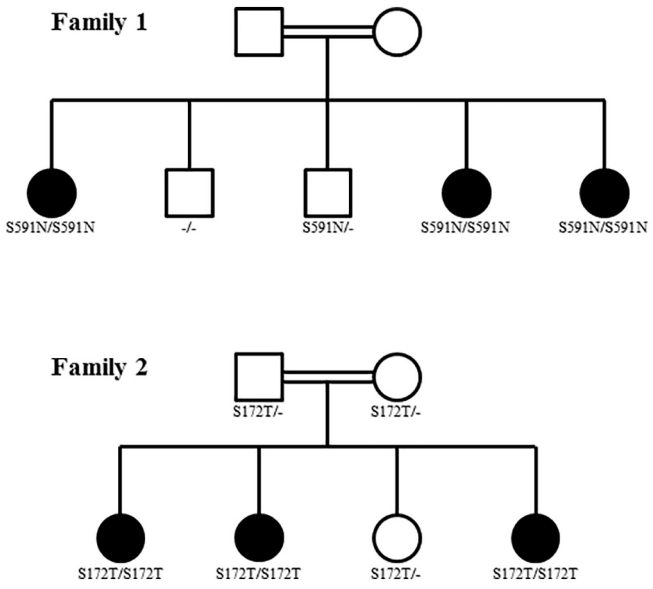

Fig. 2. Pedigrees for the two reported families.

Table 1
Latest investigations for the three affected sisters from the first family.

	Older sister (14 years)	Middle sister (3 years)	Younger sister (14 months)
Hemoglobin (g/dL)	10.0 (12.0–16.0)	11.8 (11.5–13.5)	10.2 (10.5–13.5)
MCV (fL)	92 (78–98)	87 (73–85)	76 (70–84)
Reticulocytes (%)	6.5 (0.5–1.6)	5.5 (0.5–1.6)	5.1 (0.5–1.6)
LDH (IU/L)	121 (98–192)	203 (110–295)	196 (180–430)
Haptoglobin (g/L)	0.57 (0.40–2.00)	< 0.15 (0.40–2.00)	< 0.15 (0.40–2.00)
Total bilirubin (μmol/L)	16 (5–22)	19 (5–22)	7 (5–22)
Iron (μmol/L)	13 (5–30)	24 (5–30)	12 (5–30)
Transferrin (g/L)	2.3 (1.9–2.8)	2.9 (1.9–2.8)	2.5 (1.9–2.8)
Transferrin saturation (%)	22 (15–50)	33 (15–50)	19 (15–50)
Ferritin (μg/L)	115 (11–307)	110 (11–307)	31 (11–307)
TIBC (μmol/L)	55 (46–85)	68 (46–85)	59 (46–85)
Folate (nmol/L)	25 (8–50)	33 (8–50)	52 (8–50)
Vitamin B12 (pmol/L)	133 (133–675)	158 (133–675)	488 (133–675)
Blood film	–	Polychromasia	Microcytes and hypochromasia

she did not develop any jaundice or hemolysis but she continued to have mild anemia (Table 1). She had normal development and school performance. Her physical examination revealed normal growth parameters, normal neurological examination, and absence of hepatosplenomegaly.

The middle sister was 3 years old who was born at term. During pregnancy, her mother had diabetes mellitus that was controlled with insulin treatment. Similar to her older sister, she developed neonatal jaundice that required phototherapy and anemia. However, no blood transfusion was required. Initial evaluation revealed reticulocytosis, negative Coombs test, and normal hemoglobin electrophoresis. Erythrocyte enzyme evaluation did not show any significant reduction in G6PD, pyruvate kinase, glucose phosphate isomerase, hexokinase, adenylate kinase, phosphofructokinase, phosphoglycerate kinase, and triosephosphate isomerase. Erythrocyte glutathione, measured by a kinetic spectrophotometry method (http://www.mayomedicallaboratories.com), was reduced at 23 mg/dL RBC (reference 47–90). Beyond the neonatal period, she did not have any jaundice or hemolysis. But she continued to have mild anemia (Table 1). Her