

9.1 Introduction

Glutathione participates in free radical scavenging, defense against oxidative stress, redox reactions, formation of deoxyribonucleotides, xenobiotic metabolism and amino acid transport. Patients with genetic defects in four of the six γ -glutamyl cycle enzymes have been reported and they are all inherited as autosomal recessive traits [1].

The biosynthesis of the tripeptide glutathione (γ -glutamylcysteinylglycine) is catalyzed by γ -glutamylcysteine synthetase and glutathione synthetase. The initial degradative step is catalyzed by γ -glutamyl transpeptidase, which transfers the γ -glutamyl group to an acceptor, for example an amino acid, to form γ -glutamyl amino acids. The latter are typically substrates of γ -glutamyl cyclo-transferase which catalyzes release of the γ -glutamyl residue as 5-oxoproline (pyroglutamic acid) which is converted to glutamate by 5-oxoprolinase. Glutathione acts as a feedback inhibitor of γ -glutamylcysteine synthetase.

γ -Glutamylcysteine synthetase deficiency has been described in more than 10 patients in more than 6 families. All had hemolytic anemia and in addition two siblings also had cerebellar involvement, neuropathy, myopathy and aminoaciduria.

Glutathione synthetase deficiency has been detected in more than 50 patients in more than 40 families. According to clinical symptoms glutathione synthetase deficiency can be classified as mild, moderate or severe [2]. Patients with mild glutathione synthetase deficiency show hemolytic anemia as their only clinical symptom. Patients with moderate glutathione synthetase deficiency usually present in the neonatal period with metabolic acidosis, 5-oxoprolinuria and hemolytic anemia. Patients with severe glutathione synthetase deficiency also develop progressive neurological symptoms (e.g. mental retardation, seizures, spasticity), and may also develop recurrent bacterial infections, due to defective granulocyte function. Several patients have died in early life due to acidosis and electrolyte imbalance. The acidosis is due to overproduction of 5-oxoproline as a consequence of defective feedback regulation of the γ -glutamyl cycle. Patients with moderate and severe glutathione synthetase deficiency usually excrete gram quantities of 5-oxoproline in urine. Patients with mild glutathione synthetase deficiency

maintain cellular levels of glutathione which usually, but not always, is sufficient to prevent accumulation of 5-oxoproline in body fluids. Treatment of patients with glutathione synthetase deficiency includes acidosis correction and supplementation with the anti-oxidants vitamin E, vitamin C and N-acetylcysteine, as well as avoidance of drugs known to precipitate hemolytic crises in patients with glucos-6-phosphate dehydrogenase deficiency.

Deficiency of γ -glutamylcysteine synthetase or glutathione synthetase results in low intracellular levels of glutathione. This can be demonstrated in erythrocytes, leukocytes and cultured fibroblasts. Increased 5-oxoproline can only be determined via analysis of organic acids by gas chromatography-mass spectrometry (GC-MS). Analysis of the γ -glutamyl cycle enzymes in erythrocytes or nucleated cells is required for the diagnosis. The human genes for γ -glutamylcysteine synthetase and glutathione synthetase have been mapped and cloned and mutations in the genes have been characterized [1, 3–5]. Prenatal diagnostic evaluation has been practiced for severe glutathione synthetase deficiency using analysis of enzyme activity in chorionic villi and cultured amniocytes, and by analysis of 5-oxoproline levels in amniotic fluid. If the mutant alleles in the family have been identified, prenatal diagnosis can be made by mutation analysis [1].

γ -Glutamyl transpeptidase deficiency has been identified in five patients, who excrete glutathione in their urine and have elevated plasma glutathione. Three of the five patients had CNS symptoms. Increased levels of urinary glutathione can be demonstrated by various chromatographic techniques. The human γ -glutamyl transpeptidase gene is a multigenetic family with several of its loci located on chromosome 22 [1].

5-Oxoprolinase deficiency has been identified in eight patients, who lack a consistent clinical syndrome. Urinary excretion of 5-oxoproline is elevated but less than in glutathione synthetase deficiency. Erythrocytes contain an incomplete γ -glutamyl cycle; they lack both γ -glutamyl transpeptidase and 5-oxoprolinase.

9.2 Nomenclature

No.	Disorder	Tissue distribution	Chromosomal location	MIM
9.1	γ -Glutamylcysteine synthetase deficiency	Universal	Heavy subunit 6p12 Light subunit 1p21	230450
9.2	Glutathione synthetase deficiency	Universal	20q11.2	266130 231900
9.3	γ -Glutamyl transpeptidase deficiency	Nucleated cells	Multigenic family. Five loci 22	231950
9.4	5-Oxoprolinase deficiency	Nucleated cells	Unknown	260005

9.3 Metabolic Pathway

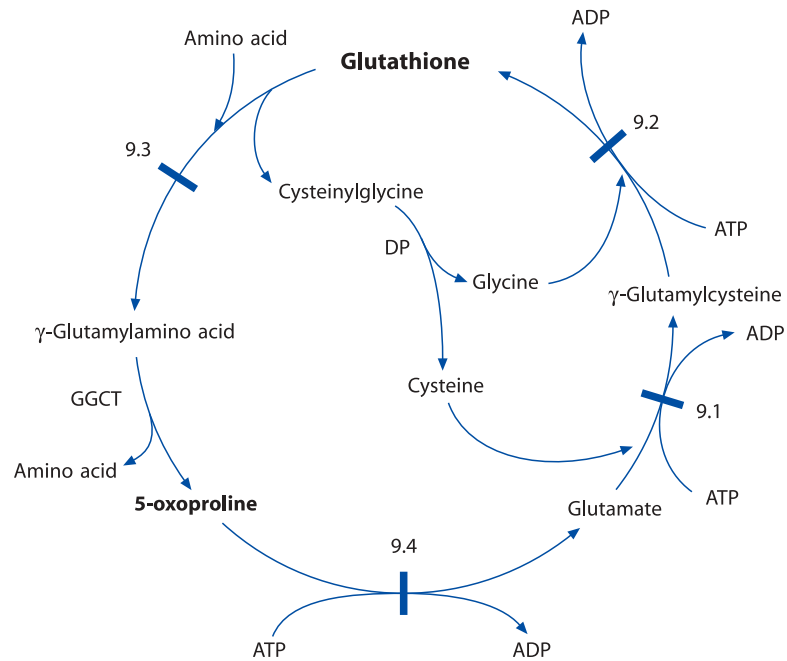


Fig. 9.1. The γ -glutamyl cycle for the biosynthesis and degradation of glutathione including known metabolic defects: 9.1, γ -glutamylcysteine synthetase; 9.2, glutathione synthetase; 9.3, γ -glutamyl transpeptidase; 9.4, 5-oxoprolinase. DP, dipeptidase (cysteinyl glycine); GGCT, γ -glutamyl cyclotransferase. Metabolites that show pathological levels in the various enzymatic defects are marked in bold. Note the role of excess 5-oxoproline (pyroglutamic acid) as a marker for two of the four disorders.

9.4 Signs and Symptoms

Table 9.1. γ -Glutamylcysteine synthetase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Jaundice	\pm	\pm	\pm	\pm	\pm
	Hemolytic anemia	+	+	+	+	+
Routine laboratory	Hemoglobin (B)	\downarrow	\downarrow	\downarrow	\downarrow	\downarrow
	Reticulocytes (B)	\uparrow	\uparrow	\uparrow	\uparrow	\uparrow
Special laboratory	Amino aciduria (U)	\pm	\pm	\pm	\pm	\pm
	Glutathione (RBC)	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$
	γ -Glutamylcysteine synthetase (RBC, FB)	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$
Hematological	Jaundice	\pm	\pm	\pm	\pm	\pm
	Hemolytic anemia	+	+	+	+	+
Musculoskeletal	Myopathy/weakness	\pm	\pm	\pm	\pm	\pm
CNS	Psychosis	\pm	\pm	\pm	\pm	\pm
	Ataxia	\pm	\pm	\pm	\pm	\pm
Peripheral nervous system	Neuropathy	\pm	\pm	\pm	\pm	\pm

Table 9.2.1. Glutathione synthetase deficiency (mild)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Hemolytic anemia	+	+	+	+	+
Routine laboratory	Hemoglobin (B)	\downarrow	\downarrow	\downarrow	\downarrow	\downarrow
	Reticulocytes (B)	\uparrow	\uparrow	\uparrow	\uparrow	\uparrow
Special laboratory	5-Oxoproline (U)	N/ \uparrow	N/ \uparrow	N/ \uparrow	N/ \uparrow	N/ \uparrow
	Glutathione (RBC, FB)	$\downarrow\downarrow$	$\downarrow\downarrow$	$\downarrow\downarrow$	$\downarrow\downarrow$	$\downarrow\downarrow$
	γ -Glutathione synthetase (RBC, FB)	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$	$\downarrow\downarrow\downarrow$
Hematological	Jaundice	\pm	\pm	\pm	\pm	\pm
	Hemolytic anemia	+	+	+	+	+

Table 9.2.2. Glutathione synthetase deficiency (moderate, severe)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Hemolytic anemia	+	+	+	+	+
	Metabolic acidosis	+	+	+	+	+
	Neurological symptoms	±	±	±	±	±
	Recurrent bacterial infections	±	±	±	±	±
Routine laboratory	Acidosis (B)	+	+	+	+	+
	Hemoglobin (B)	↓	↓	↓	↓	↓
	Reticulocytes (B)	↑	↑	↑	↑	↑
Special laboratory	5-Oxoproline (U)	↑/↑↑↑	↑/↑↑↑	↑/↑↑↑	↑/↑↑↑	↑/↑↑↑
	Glutathione (RBC, FB)	↓↓	↓↓	↓↓	↓↓	↓↓
	γ-Glutathione synthetase (RBC, FB)	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
CNS	Psychomotor or mental retardation	±	±	±	±	±
	Seizures	±	±	±	±	±
	Hyper-/hypotonia	±	±	±	±	±
	Ataxia		±	±	±	±
Musculoskeletal	Myopathy/weakness	±	±	±	±	±
Hematological	Jaundice	±	±	±	±	±
	Hemolytic anemia	+	+	+	+	+
Immune system	Recurrent bacterial infections	±	±	±	±	±
Eye	Retinal pigmentations			±	±	±
	Crystalline opacities in the lens			±	±	±
	Impaired dark adaptation			±	±	±

Table 9.3. γ-Glutamyl transpeptidase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	No consistent clinical picture					
Special laboratory	Glutathione (U)	↑	↑	↑	↑	↑
	Glutathione (RBC, FB)	N	N	N	N	N
	Glutathione (P)	↑	↑	↑	↑	↑
	γ-Glutamyl transpeptidase (WBC, FB)	↓	↓	↓	↓	↓
CNS	Mental retardation			±	±	±
	Psychosis			±	±	±

Table 9.4. 5-Oxoprolinase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	No consistent clinical picture					
Routine laboratory	Acicosis (B)	N	N	N	N	N
Special laboratory	5-Oxoproline (U)	↑	↑	↑	↑	↑
	5-Oxoprolinase (LYM, FB)	↓	↓	↓	↓	↓
CNS	Psychomotor or mental retardation	±	±	±	±	±
	Microcephaly		±	±		
Renal	Urolithiasis			±	±	±
	Renal colic			±	±	±
GI	Colitis				±	±
	Diarrhea				±	±

9.5 Reference Values

5-Oxoproline (U)

<10 mmol/mol creat

Glutathione (RBC)

4.6–10.9 nmol/mg of hemoglobin

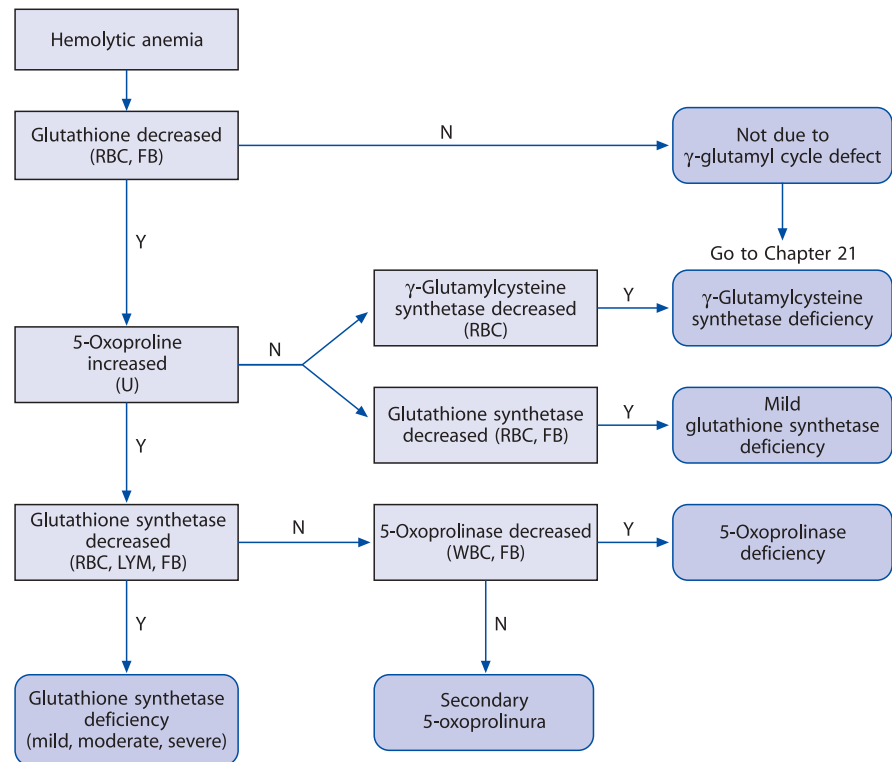
9.6 Pathological Values/Differential Diagnosis

Variant	Glutathione			5-Oxo-proline (U)	Acid-base balance (B)	Reticulo-cytes (B)
	(RBC, B)	(U)	(P)			
9.1 γ -Glutamylcysteine synthetase deficiency	↓↓	N		N	N	↑
9.2.1 Glutathione synthetase deficiency; mild	↓↓	N		N/↑	N	↑
9.2.2 Glutathione synthetase deficiency; moderate/severe	↓↓	N		↑↑↑	Acidosis	↑
9.3 γ -Glutamyltranspeptidase deficiency	N	↑	↑	N	N	N
9.4 5-Oxoprolinase deficiency	N	N	N	↑	N	N

9.7 Loading Tests

Not applicable.

9.8 Diagnostic Flow Chart

Fig. 9.2. Diagnostic flow chart for disorders of the γ -glutamyl cycle.

9.9 Specimen Collection

Test	Pre-conditions	Material	Handling	Pitfalls
Glutathione	–	RBC, B, FB	Frozen (-20°C)	Assays that do not detect oxidized glutathione tend to underestimate glutathione in stored samples
γ -Glutamylcysteine synthetase	–	RBC, LYM, FB	Frozen (-20°C)	
Glutathione synthetase	–	RBC, LYM, FB	Frozen (-20°C)	
γ -Glutamyl transpeptidase	–	WBC, FB, (P)	Frozen (-20°C)	
γ -Glutamyl cyclotransferase	–	RBC, WBC, FB	Frozen (-20°C)	
5-Oxoprolinase	–	WBC, FB	Frozen (-20°C)	
5-Oxoproline	–	U	Frozen (-20°C)	Excretion of 5-oxoproline has been found in patients with inborn errors of metabolism outside the γ -glutamyl cycle (e.g. homocystinuria OCT deficiency) and in patients receiving certain drugs (vigabatrin, paracetamol) and specific diets (acid hydrolyzed protein formula)
Mutation analysis (DNA sequencing)	–	FB, WBC, CV, AFC	Cells in culture (room temperature)	Prenatal diagnosis is greatly facilitated if the mutant allele/s in the specific family are known

9.10 Prenatal Diagnosis

Prenatal diagnosis is greatly facilitated if the mutant allele/s in the specific family are known.

Disorder	Tissue	Timing, trimester
9.2.2	CV	I
	AF	II
	AFC	II

9.11 DNA Analysis

Disorder	Tissue	Methodology
9.1	FB, WBC, LYM	DNA sequencing
9.2.1, 9.2.2	FB, WBC, LYM, CV, AFC	DNA sequencing

9.12 Initial Treatment

Defects that lead to decreased levels of glutathione can be treated according to two complementary strategies: avoidance of drugs that lead to oxidative stress, and supplementation with compounds that may act as free-radical scavengers (e.g. vitamin C, vitamin E and N-acetylcysteine).

The only disorder of the γ -glutamyl cycle for which treatment principles have been developed is glutathione synthetase deficiency (9.2) [1]. The initial symptoms in the neonatal period may be metabolic acidosis and jaundice. Acidosis usually needs to be corrected with sodium bicarbonate, THAM or sodium citrate. Patients may benefit from oral administration of vitamin E (10 mg/kg/day) and vitamin C (100 mg/kg/day). Trials have also been made with N-acetylcysteine and glutathione esters which increased glutathione in leukocytes and plasma. Both these compounds lead to increased intracellular levels of glutathione. However, no decrease in the excretion of 5-oxoprolinuria has been reported.

Patients who are deficient in γ -glutamylcysteine synthetase or glutathione synthetase should avoid drugs that can induce hemolytic crises in patients with glucose-6-phosphate dehydrogenase deficiency, e.g. phenobarbital, acetylsalicylic acid, sulfonamides.

9.13 Summary/Comments

In the γ -glutamyl cycle, hereditary defects have been detected in four of the six enzymes: γ -glutamylcysteine synthetase, glutathione synthetase, γ -glutamyl transpeptidase and 5-oxoprolinase. A single patient with tentative deficiency of dipeptidase (cysteinylglycinase) has recently been reported [6]. Deficiency of either of the two synthetases results in decreased levels of glutathione and thus increased sensitivity to oxidative stress which results in hemolytic anemia. Glutathione synthetase deficiency occurs with different severity; the mild form is only associated with hemolytic anemia, whereas moderate and severe glutathione synthetase deficiency is associated also with metabolic acidosis, progressive neurological symptoms and recurrent bacterial infections. 5-Oxoproline (pyroglutamic acid) is overproduced in glutathione synthetase deficiency due to lack of feedback inhibi-

tion. Treatment involves acidosis correction, administration of vitamin E, vitamin C, N-acetylcysteine, and avoidance of drugs inducing hemolysis. γ -Glutamyl transpeptidase deficiency is associated with glutathionuria and 5-oxoprolinase deficiency with 5-oxoprolinuria.

References

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