

Review

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Inborn errors in the metabolism of glutathione

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Abstract

Glutathione is a tripeptide composed of glutamate, cysteine and glycine. Glutathione is present in millimolar concentrations in most mammalian cells and it is involved in several fundamental biological functions, including free radical scavenging, detoxification of xenobiotics and carcinogens, redox reactions, biosynthesis of DNA, proteins and leukotrienes, as well as neurotransmission/neuromodulation. Glutathione is metabolised *via* the gamma-glutamyl cycle, which is catalyzed by six enzymes. In man, hereditary deficiencies have been found in five of the six enzymes. Glutathione synthetase deficiency is the most frequently recognized disorder and, in its severe form, it is associated with hemolytic anemia, metabolic acidosis, 5-oxoprolinuria, central nervous system (CNS) damage and recurrent bacterial infections. Gamma-glutamylcysteine synthetase deficiency is also associated with hemolytic anemia, and some patients with this disorder show defects of neuromuscular function and generalized aminoaciduria. Gamma-glutamyl transpeptidase deficiency has been found in patients with CNS involvement and glutathionuria. 5-Oxoprolinase deficiency is associated with 5-oxoprolinuria but without a clear association with other symptoms. Dipeptidase deficiency has been described in one patient. All disorders are very rare and inherited in an autosomal recessive manner. Most of the mutations are leaky so that many patients have residual enzyme activity. Diagnosis is made by measuring the concentration of different metabolites in the gamma-glutamyl cycle, enzyme activity and in glutathione synthetase and gamma-glutamylcysteine synthetase deficiency, also by mutation analysis. Prenatal diagnosis has been preformed in glutathione synthetase deficiency. The prognosis is difficult to predict, as few patients are known, but seems to vary significantly between different patients. The aims of the treatment of glutathione synthesis defects are to avoid hemolytic crises and to increase the defense against reactive oxygen species. No treatment has been recommended for gamma-glutamyl transpeptidase, 5-oxoprolinase and dipeptidase deficiency.

A. Gamma-glutamylcysteine synthetase deficiency

Disease name and synonyms

Gamma-glutamylcysteine synthetase deficiency (OMIM #230450)

Glutamate-cysteine ligase deficiency

Definition and diagnostic criteria

Gamma-glutamylcysteine synthetase deficiency is a very rare autosomal recessive disease characterized by hemolytic anemia, and, in some cases, by neurological symptoms. The diagnosis is established by:

- Low activity of gamma-glutamylcysteine synthetase in red blood cells, leukocytes and/or cultured skin fibroblasts.
- Low levels of glutathione and gamma-glutamylcysteine in red blood cells and/or cultured skin fibroblasts.
- Presence of mutation(s) in the gamma-glutamylcysteine synthetase genes. The patients whose results have been published have had homozygous mutations in the gene encoding the heavy subunit of the enzyme.

In red blood cells heterozygous carriers have an enzyme activity of about 50% of the normal mean and normal levels of glutathione [1].

Epidemiology

Gamma-glutamylcysteine synthetase deficiency is very rare disease. Nine patients in seven families have been reported worldwide (USA, Germany, Japan, The Netherlands, Poland, and Spain).

Clinical description

All patients with gamma-glutamylcysteine synthetase deficiency have had hemolytic anemia, usually rather mild [1-8]. In addition, two siblings also had spinocerebellar degeneration, peripheral neuropathy, myopathy and aminoaciduria [3,8]. Treatment with sulfonamide precipitated psychosis and pronounced hemolytic anemia in one of these siblings. One patient was reported to have learning disability with dyslexia and was also thought to be mentally retarded [5], and another had delayed psychomotor development and progressive sensory neuropathy of lower extremities, ataxia, hyperreflexia, dysarthria, and a peculiar gait suggestive of spinocerebellar degeneration [2]. Other symptoms found in patients with γ -glutamylcysteine synthetase deficiency are transient jaundice, reticulocytosis, and hepatosplenomegaly.

Etiology

Gamma-glutamylcysteine synthetase catalyzes the first and rate-limiting step in the synthesis of glutathione (GSH) (Figure 1) [9]. Hereditary gamma-glutamylcysteine synthetase deficiency is transmitted as an autosomal recessive trait. The human gamma-glutamylcysteine synthetase enzyme is a dimer consisting of a heavy (catalytic) and a light (regulatory) subunit. The human gene for the heavy subunit has been localized to chromosome 6p12 [10] and the gene for the light subunit to chromosome 1p21 [11]. The heavy subunit (molecular weight about 73 kDa) exhibits the catalytic activity of the native enzyme and is also responsible for the feedback inhibition by GSH. The light subunit (molecular weight about 28 kDa) is catalytically inactive but plays an important regulatory role [12].

Four different mutations in the heavy subunit have been identified in four families affected by gamma-glutamylcysteine synthetase deficiency [1,2,4,5].

Gammaglutamylcysteine synthetase knock-out mice have been developed for both the heavy and the light subunits [13-15]. Homozygous mice embryos with knockout of the heavy subunit fail to gastrulate and die before day 8.5 of gestation [13]. The knockout mice of the light subunit are viable and fertile and have no overt phenotype [15].

Diagnostic methods

Glutathione and sulphhydryl compounds in erythrocytes can be measured using 5,5'-dithiobis (2-nitrobenzoic acid), while glutathione in fibroblasts can be measured with the 5,5'-dithiobis (2-nitrobenzoic acid) glutathione recycling assay [16], or using high performance liquid chromatography (HPLC) [17].

Gamma-glutamylcysteine synthetase in erythrocytes and/or cultured fibroblasts can be measured as described elsewhere [1].

Sequence analysis can be made by polymerase chain reaction (PCR) and sequence analysis of the γ -glutamylcysteine synthetase genes (*GLCLC*, *GLCLR*) [1].

Differential diagnosis

Low levels of GSH can also be due to glutathione synthetase deficiency.

Genetic counseling

Families with gamma-glutamylcysteine synthetase deficiency can be tested in order to analyze the enzyme activity as well as their mutations. Limited mutation analysis data are available but it is essential to determine the underlying mutations to learn more about the functional properties of the mutant enzyme. Families should also be referred for genetic counseling.

Antenatal diagnosis

Antenatal diagnosis has not been reported. However, it should be possible if needed by measurement of gamma-glutamylcysteine synthetase activity or mutational analysis (if the mutation in the family is known) of chorionic villi or cultured amniocytes.

Management including treatment

Patients with gamma-glutamylcysteine synthetase deficiency should avoid drugs known to precipitate hemolytic crises in patients with glucose-6-phosphate dehydrogenase deficiency, e.g. phenobarbital, acetylsalicylic acid, sulfonamides. It is possible that patients would benefit from treatment with anti-oxidants but no studies have been made.

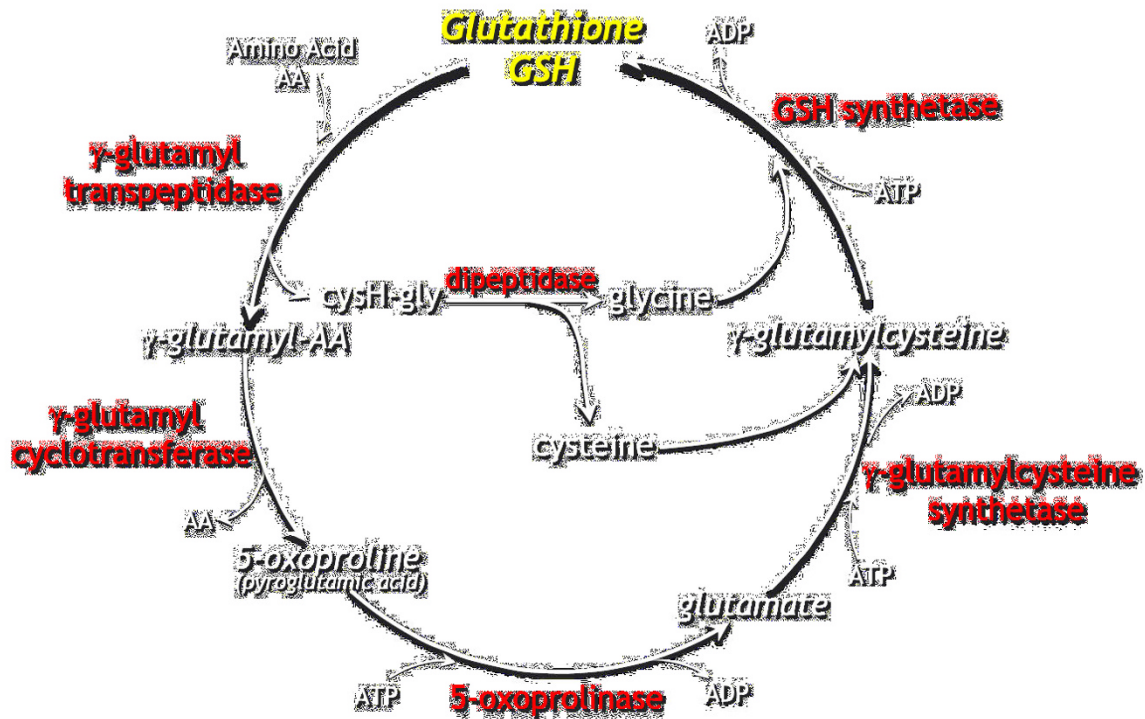


Figure 1
The gamma-glutamyl cycle.

B. Glutathione synthetase deficiency

Disease name and synonyms

Glutathione synthetase deficiency (OMIM #266130).

5-oxoprolinuria (pyroglutamic aciduria) is sometimes used when referring to glutathione synthetase deficiency; it should be borne in mind however that 5-oxoprolinuria may have other causes (see 'Differential diagnosis').

Definition and diagnostic criteria

Hereditary glutathione synthetase deficiency is a rare autosomal recessive disease characterized by hemolytic anemia, metabolic acidosis, 5-oxoprolinuria, progressive neurological symptoms and recurrent bacterial infections. The diagnosis usually involves the following: clinical findings, the finding of 5-oxoprolinuria, low levels of glutathione, low activity of glutathione synthetase, and mutation

analysis of the glutathione synthetase gene. The diagnosis of glutathione synthetase deficiency is established by:

- Low activity of glutathione synthetase in cultured skin fibroblasts and/or red blood cells.
- Low levels of glutathione in red blood cells and/or cultured skin fibroblasts.
- Urinary 5-oxoprolinone (up to 1 g/kg/day)
- Mutation(s) in the glutathione synthetase (GSS) gene.

Heterozygous carriers have an enzyme activity of about 55% of the normal mean and normal levels of glutathione [18].

Epidemiology

More than seventy patients have been reported in more than 50 families worldwide.

Clinical description

According to the clinical symptoms, glutathione synthetase deficiency can be classified as mild, moderate or severe [19]:

Mild glutathione synthetase deficiency

These patients show mild hemolytic anemia as their only clinical symptom. In very rare cases, they may excrete excessive amounts of 5-oxoproline in their urine (reference range < 0.1 mol/mol creatinine) but they usually maintain sufficient cellular levels of glutathione to prevent accumulation of 5-oxoproline in body fluids.

Moderate glutathione synthetase deficiency

Patients with the moderate variant usually present in the neonatal period with severe and chronic metabolic acidosis, 5-oxoprolinuria, and mild/moderate hemolytic anemia.

Severe glutathione synthetase deficiency

Patients have symptoms as in moderate glutathione synthetase deficiency and, in addition, they develop progressive neurological symptoms, *e.g.* psychomotor retardation, mental retardation, seizures, spasticity, ataxia, and intention tremor. Some patients with severe glutathione synthetase deficiency also develop recurrent bacterial infections, probably due to defective granulocyte function. Retinal dystrophy has also been observed in adult patients [20].

Several patients died in early life.

Etiology

Glutathione synthetase catalyses the last step in the synthesis of glutathione and a deficiency results in low levels of glutathione (Figure 1). Acidosis is due to reduced feed-back inhibition of γ -glutamyl cysteine synthetase in the gamma-glutamyl cycle, which ultimately leads to overproduction and accumulation of 5-oxoproline. The human glutathione synthetase enzyme is a homodimer with a subunit size of 52 kDa. The three-dimensional structure of the glutathione synthetase enzyme has been established [21]. The gene has been localized to chromosome 20q11.2 and its structure determined [22,23]. Since the human genome contains only one glutathione synthetase gene, the various clinical forms of glutathione synthetase deficiency reflect different mutations or epigenetic modifications in the glutathione synthetase gene. Several mutations have been identified.

The mechanism of metabolic acidosis and 5-oxoprolinuria is the following: decreased levels of cellular glutathione lead to decreased feed-back inhibition of γ -glutamylcysteine synthetase. This results in excessive formation of the dipeptide γ -glutamylcysteine, which is converted by γ -glutamyl cyclotransferase into 5-oxoproline. The overproduction of 5-oxoproline exceeds the capacity of 5-oxoprolinase, and 5-oxoproline therefore accumulates in body fluids and is excreted in the urine.

Patients with glutathione synthetase deficiency accumulate the dipeptide γ -glutamylcysteine and cysteine in fibroblasts [24]. As γ -glutamylcysteine contains both reactive groups of glutathione (*i.e.* the sulphhydryl and γ -glutamyl groups) it may to some extent compensate for glutathione in the cellular defence against oxidative stress.

Glutathione is participating in leukotriene C4 (LTC₄) synthesis, the primary cysteinyl leukotriene. It has been shown that the synthesis of cysteinyl leukotriens is impaired in patients with glutathione synthetase deficiency [25].

Diagnostic methods

Urinary 5-oxoproline can be determined by gas chromatography-mass spectrometry [26]. Glutathione and sulphhydryls in erythrocytes can be measured using 5,5'-dithiobis (2-nitrobenzoic acid), while glutathione in fibroblasts can be measured with the 5,5'-dithiobis (2-nitrobenzoic acid) glutathione recycling assay [16], or using HPLC [17].

Glutathione synthetase in erythrocytes and/or cultured fibroblasts can be measured as described elsewhere [1].

Mutation analysis of the glutathione synthetase gene (GSS) can be made as described by Shi *et al.* 1996 and Njålsson *et al.* 2003 [27,28].

Differential diagnosis

For other causes of 5-oxoprolinuria, beside glutathione synthetase deficiency and 5-oxoprolinase deficiency [9,29], please see Table 1.

Genetic counseling

Families with glutathione synthetase deficiency can be referred for testing in order to analyze the enzyme activity as well as their mutations. It is essential to find out whether it is possible to correlate the genotype with the phenotype.

Table 1: Causes of 5-oxoprolinuria beside glutathione synthetase deficiency and 5-oxoprolinase deficiency.

Etiology	Possible mechanism	Ref.
Diet	Certain infant formulas and tomato juice may contain proteins modified by preparation that have increased 5-oxoprolin content	[52]
Severe burns Stevens-Johnson syndrome	Increased metabolism of collagen, fibrinogen or other proteins that contain substantial amounts of 5-oxoprolin	[53]
Other inborn errors of metabolism	Inborn errors of metabolism not involving the gamma-glutamyl cycle, e.g. X-linked ornithine transcarbamylase deficiency, urea cycle defects, tyrosinemia. In critical organs (e.g. liver, kidney), lack of ATP, which is needed for conversion of 5-oxoprolin into glutamate, may lead to 5-oxoprolinuria	[29] [9]
Homocystinuria	Patients with homocystinuria may have excessive formation of 5-oxoprolin	[54]
Drug metabolism	Paracetamol, vigabatrin and antibiotics (flucloxacillin, netimicin) probably interact with the gamma-glutamyl cycle	[55-57]
Prematurity	Transient 5-oxoprolinuria has been observed in very preterm infants. The cause is unknown	[58] [29, 59]
Malnutrition, pregnancy	Limited availability of glycine	[60]
Nephropatic cystinosis	Nephropatic cystinosis patients: may have 5-oxoprolinuria probably because of decreased availability of free cysteine, resulting in a secondary impairment of the γ -glutamyl cycle. Cysteamine therapy normalizes the 5-oxoprolinuria	[61]

Antenatal diagnosis

Antenatal diagnosis is possible and can be made by:

- Mutation analysis of chorionic villi (the method of choice if the mutation in the family is known).
- Analysis of 5-oxoprolin in amniotic fluid [30,31].
- Analysis of glutathione synthetase activity in cultured amniocytes or chorionic villi [30].

Management including treatment

The clinical goals of management are correction of acidosis and early supplementation with vitamin C (ascorbic acid) and vitamin E (alpha-tocopherol) [19]. The acidosis is corrected with bicarbonate. The recommended dose of vitamin C is 100 mg/kg/day and of vitamin E 10 mg/kg/day. N-Acetylcysteine used to be recommended because it may protect cells from oxidative stress. However, cysteine has been shown to accumulate in tissues of patients with glutathione synthetase deficiency – at least in cultured fibroblasts [24]. Since cysteine is known to be neurotoxic in excessive amounts [32], treatment with N-acetylcysteine should not be recommended for patients with glutathione synthetase deficiency as this may increase the intracellular cysteine levels even more.

Patients with glutathione synthetase deficiency should avoid drugs known to precipitate hemolytic crises in patients with glucose-6-phosphate dehydrogenase deficiency.

Prognosis

A long-term follow up study of 28 patients with glutathione synthetase deficiency has showed that the factors

most predictive of survival and long-term outcome are early diagnosis, correction of acidosis and early supplementation with vitamin C and vitamin E [19].

C. Gamma-glutamyl transpeptidase deficiency**Disease name**

Gamma-glutamyl transpeptidase deficiency (OMIM 231950).

Definition and diagnostic criteria

Gamma-glutamyl transpeptidase deficiency is a very rare autosomal recessive disease characterized by increased glutathione concentration in plasma and urine. Central nervous system involvement may also be present. The diagnosis is established by:

- Low activity of γ -glutamyl transpeptidase in nucleated cells such as leukocytes or cultured skin fibroblasts. Erythrocytes also lack γ -glutamyl transpeptidase under normal conditions.
- High levels of glutathione in plasma and urine (up to 1 g/day in urine; controls < 10 mg). Cellular levels of glutathione are normal.

Epidemiology

Gamma-glutamyl transpeptidase deficiency is a very rare disease, which has been reported in seven patients in five families worldwide [33-37].

Clinical description

Five out of seven reported patients had central nervous system involvement [33-37]. Whether these symptoms are part of the clinical picture remains to be established. All patients have had glutathionuria (up to 1 g/day; con-

trols < 10 mg). In addition, patients have increased urinary levels of gamma-glutamylcysteine and cysteine. Three patients with γ -glutamyl transpeptidase deficiency have been studied and found to have a complete deficiency of leukotriene D4 biosynthesis [38].

Etiology

The human gamma-glutamyl transpeptidase gene family is composed of at least seven different gene loci and several of them are located on the long arm of chromosome 22 [9]. Gamma-glutamyl transpeptidase is a heterodimer with subunits of 21 kDa and 38 kDa. The enzyme is membrane-bound with its active site facing the external side of the cell. Erythrocytes lack gamma-glutamyl transpeptidase and this enzyme activity also varies in other tissues. Gamma-glutamyl transpeptidase catalyses the first step in the degradation of glutathione (Figure 1). No mutations have been identified in patients with gamma-glutamyl transpeptidase deficiency. A knock-out mouse for γ -glutamyl transpeptidase has been developed. Gamma-glutamyl transpeptidase deficiency leads to glutathionuria, glutathionemia, growth failure, cataracts, lethargy, shortened life span, and infertility. A closer study of the reproductive phenotype of γ -glutamyl transpeptidase deficient mice show that they are hypogonadal and infertile [39].

Diagnostic methods

Glutathione in plasma and urine can be determined by various chromatographic or calorimetric techniques.

Gamma-glutamyl transpeptidase in nucleated cells can be determined using the method described by Wright *et al.* [36].

Genetic counseling

The disease is transmitted as an autosomal recessive trait. Patients should be offered genetic counseling.

Antenatal diagnosis

Antenatal diagnosis has not been reported.

Management including treatment

No specific treatment has been proposed or tried. However, administration of N-acetylcysteine to γ -glutamyl transpeptidase deficient mutant mice for two weeks restored their fertility [39].

D. 5-Oxoprolinase deficiency

Disease name

5-Oxoprolinase deficiency (OMIM 260005)

Definition and diagnostic criteria

5-Oxoprolinase deficiency is a very rare autosomal recessive disease characterized by 5-oxoprolinuria and very heterogeneous clinical presentation (renal stone formation, enterocolitis, mental retardation, neonatal hypoglycemia, microcytic anemia and microcephaly).

The diagnosis of 5-oxoprolinase deficiency is established by:

- Low activity of 5-oxoprolinase in nucleated cells such as leukocytes or cultured skin fibroblasts (5-oxoprolinase is not present in erythrocytes).
- Elevated levels of 5-oxoprolin in body fluids.
- Urinary 5-oxoprolin.

Epidemiology

Eight patients have been reported worldwide.

Clinical description

All patients with 5-oxoprolinase deficiency have been identified because of 5-oxoprolinuria (4 to 10 g/day. Reference range < 0.1 mol/mol creatinine), but they lack a consistent clinical picture [9]. They have normal acid-base balance. Different clinical symptoms reported in individual patients with 5-oxoprolinase deficiency are renal stone formation, enterocolitis, mental retardation, neonatal hypoglycemia, microcytic anemia and microcephaly [40-45].

Etiology

5-Oxoprolinase catalyses a step in the gamma-glutamyl cycle (glutathione metabolism), the ring-opening of 5-oxoprolin to yield glutamate. 5-Oxoprolinase is the enzyme in the gamma-glutamyl cycle with the lowest capacity (Figure 1).

The mammalian enzyme is not well studied, but it is apparently composed of two identical subunits. The mechanism leading to 5-oxoprolinuria is the following: decreased activity of 5-oxoprolinase leads to decreased conversion of 5-oxoprolin to glutamate. Therefore, 5-oxoprolin accumulates in body fluids and is excreted in the urine. The quantities are less than those found in patients with glutathione synthetase deficiency and therefore acid-base balance is usually normal.

Diagnostic methods

The level of 5-oxoprolin in urine can be determined by gas chromatography-mass spectrometry [26]. Activity of 5-oxoprolinase in leukocytes and/or cultured fibroblasts can be measured according to the method described by Larsson *et al.* [46].

Differential diagnosis

For other causes of 5-oxoprolinuria, besides 5-oxoprolinase deficiency and glutathione synthetase deficiency [9,29], please see Table 1.

Genetic counseling

Families with 5-oxoprolinase deficiency can be tested in order to analyze the enzyme activity. They should be referred for genetic counseling.

Antenatal diagnosis

Antenatal diagnosis of 5-oxoprolinase deficiency has not been reported to date.

Management including treatment

No specific treatment has been proposed or tried.

E. Dipeptidase deficiency**Disease name and synonyms**

Dipeptidase deficiency

Cysteinylglycinuria

Cysteineglycinuria deficiency

Definition and diagnostic criteria

Dipeptidase deficiency is an extremely rare disease autosomal recessive manner, characterized by increased urinary excretion of cysteinylglycine and a pathological excretion pattern of leukotrienes. The diagnosis is based on the finding of cysteinylglycinuria and decreased activity of dipeptidase, and is established by:

- Increased urinary excretion of cysteinyl glycine
- Normal concentration of cysteinyl glycine in plasma
- Low activity of dipeptidase in cultured skin fibroblasts and/or red blood cells.

Epidemiology

Dipeptidase deficiency has been suggested in only one patient worldwide. The diagnosis has not been confirmed by enzyme analysis in this patient.

Clinical description

One patient with a suspected membrane-bound dipeptidase deficiency has been described [47]. This was a 15-year-old boy who presented with mental retardation, mild motor impairment, and partial deafness. Biochemical investigations showed a normal level of cysteinyl glycine in plasma, an abnormal urinary profile with increased excretion of cysteinyl glycine (4972 mmol/mol of creatinine) and leukotrienes with increased leukotriene D4 and complete absence of LTE4. The concentration of leukot-

riene D(4) (LTD(4)), which is usually not detectable, was highly increased, whereas LTE(4), the major urinary metabolite in humans, was completely absent. These data suggest membrane-bound dipeptidase deficiency [47].

Etiology

Membrane-bound dipeptidase (E.C. 3.4.13.19) is the enzyme that hydrolyzes dipeptides, including cysteinylglycine compounds, such as the oxidized γ -glutamyl-transpeptidase product cystinyl-bis-glycine and the conversion of leukotriene D4 to E4 [48]. Dipeptidase also hydrolyzes certain β -lactam antibiotics. The protein is 42 kDa unglycosylated and 63 kDa when glycosylated. The crystal structure of human membrane-bound dipeptidase has been reported [49]. Renal dipeptidase has been mapped to human chromosome 16 at q24 [50]. A deficiency of dipeptidase has been suspected in only one patient.

Diagnostic methods

The diagnosis is made by quantitative amino acid analysis of urine.

The activity of dipeptidase can be assayed with a HPLC method using glycyl-D-phenylalanine as described by Littlewood *et al.* 1989 [51]. To date, the corresponding enzyme deficiency has not been assayed in the patient with suspected dipeptidase deficiency.

Genetic counseling

Patients should be offered genetic counseling.

Antenatal diagnosis

Not relevant.

Management including treatment

No specific treatment has been proposed or tried.

Unresolved questions

- It remains to be established whether CNS symptoms reported in some patients with gamma-glutamylcysteine synthetase are related to the enzyme defect or not. Also, strategies for treatment need to be investigated.
- It remains to be established if free radicals are involved in the pathogenesis of glutathione synthetase deficiency and if patients with low levels of glutathione are more sensitive to oxidative stress.
- The relationship between genotype and phenotype in gamma-glutamyl transpeptidase deficiency remains to be established. Are the symptoms in the identified patients merely a coincidence?

- The relationship between genotype and phenotype in 5-oxoprolinase deficiency remains to be established. It is unknown whether symptoms in identified patients are merely a coincidence.
- In dipeptidase deficiency, it remains to be established if there is a relationship between the biochemical defect and the clinical symptoms.
- Prognosis of gamma-glutamylcysteine synthetase deficiency, gamma-glutamyl transpeptidase deficiency, 5-oxoprolinase deficiency and dipeptidase deficiency is unclear and difficult to predict as very few patients have been reported worldwide.

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References

- Ristoff E, Augustson C, Geissler J, de Rijk T, Carlsson K, Luo JL, Andersson K, Weening RS, van Zwieten R, Larsson A, Roos D: **A missense mutation in the heavy subunit of gamma-glutamylcysteine synthetase gene causes hemolytic anemia.** *Blood* 2000, **95(7)**:2193-2196.
- Mañú Pereira M, Gelbart T, Ristoff E, Bergua JM, Kalko S, García Mateos E, Beutler E, J.L. VC: **Chronic nonspherocytic haemolytic anaemia associated with severe neurological disease due to gamma-glutamylcysteine synthetase deficiency in a boy of Muslim origin.** *Manuscript* 2006.
- Richards F, Cooper MR, Pearce LA, Cowan RJ, Spurr CL: **Familial spinocerebellar degeneration, hemolytic anemia, and glutathione deficiency.** *Arch Intern Med* 1974, **134(3)**:534-537.
- Hamilton D, Wu JH, Alaoui-Jamali M, Batist G: **A novel missense mutation in the gamma-glutamylcysteine synthetase catalytic subunit gene causes both decreased enzymatic activity and glutathione production.** *Blood* 2003, **102(2)**:725-730.
- Beutler E, Gelbart T, Kondo T, Matsunaga AT: **The molecular basis of a case of gamma-glutamylcysteine synthetase deficiency.** *Blood* 1999, **94(8)**:2890-2894.
- Hirono A, Iyori H, Sekine I, Ueyama J, Chiba H, Kanno H, Fujii H, Miwa S: **Three cases of hereditary nonspherocytic hemolytic anemia associated with red blood cell glutathione deficiency.** *Blood* 1996, **87(5)**:2071-2074.
- Beutler E, Morooso R, Kramer L, Gelbart T, Forman L: **Gamma-glutamylcysteine synthetase deficiency and hemolytic anemia.** *Blood* 1990, **75(1)**:271-273.
- Konrad PN, Richards F, Valentine WN, Paglia DE: **Gamma-glutamyl-cysteine synthetase deficiency. A cause of hereditary hemolytic anemia.** *N Engl J Med* 1972, **286(11)**:557-561.
- Larsson A, Ristoff E, Anderson ME: **Glutathione synthetase deficiency and other disorders of the gamma-glutamyl cycle.** In *The Metabolic and Molecular Bases of Inherited Disease* Edited by: Scriver CR, Beaudet AL, Sly WS, Valle D, Vogelstein B, Childs B, Kinzler KW. New York, McGraw-Hill; 2005:Online. <http://genetics.accessmedicine.com>.
- Sierra-Rivera E, Summar ML, Dasouki M, Krishnamani MR, Phillips JA, Freeman ML: **Assignment of the gene (GLCLC) that encodes the heavy subunit of gamma-glutamylcysteine synthetase to human chromosome 6.** *Cytogenet Cell Genet* 1995, **70(3-4)**:278-279.
- Sierra-Rivera E, Dasouki M, Summar ML, Krishnamani MR, Meredith M, Rao PN, Phillips JA 3rd, Freeman ML: **Assignment of the human gene (GLCLR) that encodes the regulatory subunit of gamma-glutamylcysteine synthetase to chromosome 1p21.** *Cytogenet Cell Genet* 1996, **72(2-3)**:252-254.
- Huang CS, Chang LS, Anderson ME, Meister A: **Catalytic and regulatory properties of the heavy subunit of rat kidney gamma-glutamylcysteine synthetase.** *J Biol Chem* 1993, **268(26)**:19675-19680.
- Shi ZZ, Osei-Frimpong J, Kala G, Kala SV, Barrios RJ, Habib GM, Lukin DJ, Danney CM, Matzuk MM, Lieberman MW: **Glutathione synthesis is essential for mouse development but not for cell growth in culture.** *Proc Natl Acad Sci U S A* 2000, **97(10)**:5101-5106.
- Dalton TP, Dieter MZ, Yang Y, Shertzer HG, Nebert DW: **Knock-out of the mouse glutamate cysteine ligase catalytic subunit (Gclc) gene: embryonic lethal when homozygous, and proposed model for moderate glutathione deficiency when heterozygous.** *Biochem Biophys Res Commun* 2000, **279(2)**:324-329.
- Yang Y, Dieter MZ, Chen Y, Shertzer HG, Nebert DW, Dalton TP: **Initial characterization of the glutamate-cysteine ligase modifier subunit Gclm(-/-) knockout mouse. Novel model system for a severely compromised oxidative stress response.** *J Biol Chem* 2002, **277(51)**:49446-49452.
- Anderson ME: **Determination of glutathione and glutathione disulfide in biological samples.** *Methods Enzymol* 1985, **113**:548-555.
- Luo JL, Hammarqvist F, Andersson K, Wernerman J: **Surgical trauma decreases glutathione synthetic capacity in human skeletal muscle tissue.** *Am J Physiol* 1998, **275(2 Pt 1)**:E359-65.
- Njalsson R, Ristoff E, Carlsson K, Winkler A, Larsson A, Norgren S: **Genotype, enzyme activity, glutathione level, and clinical phenotype in patients with glutathione synthetase deficiency.** *Hum Genet* 2005, **116(5)**:384-389.
- Ristoff E, Mayatepek E, Larsson A: **Long-term clinical outcome in patients with glutathione synthetase deficiency.** *J Pediatr* 2001, **139(1)**:79-84.
- Ristoff E, Burstedt M, Larsson A, Wachtmeister L: **Progressive retinal dystrophy in two sisters with glutathione synthetase (GS) deficiency.** *J Inher Metab Dis* 2007, **30(1)**:102.
- Polekhina G, Board PG, Gali RR, Rossjohn J, Parker MW: **Molecular basis of glutathione synthetase deficiency and a rare gene permutation event.** *Embo J* 1999, **18(12)**:3204-3213.
- Gali RR, Board PG: **Sequencing and expression of a cDNA for human glutathione synthetase.** *Biochem J* 1995, **310(Pt 1)**:353-358.
- Whitbread L, Gali RR, Board PG: **The structure of the human glutathione synthetase gene.** *Chem Biol Interact* 1998, **111-112**:35-40.
- Ristoff E, Hebert C, Njalsson R, Norgren S, Rooyackers O, Larsson A: **Glutathione synthetase deficiency: is gamma-glutamylcysteine accumulation a way to cope with oxidative stress in cells with insufficient levels of glutathione?** *J Inher Metab Dis* 2002, **25(7)**:577-584.
- Mayatepek E, Hoffmann GF, Carlsson B, Larsson A, Becker K: **Impaired synthesis of lipoxygenase products in glutathione synthetase deficiency.** *Pediatr Res* 1994, **35(3)**:307-310.
- Hoffmann G, Aramaki S, Blum-Hoffmann E, Nyhan WL, Sweetman L: **Quantitative analysis for organic acids in biological samples: batch isolation followed by gas chromatographic-mass spectrometric analysis.** *Clin Chem* 1989, **35(4)**:587-595.
- Njalsson R, Carlsson K, Winkler A, Larsson A, Norgren S: **Diagnostics in patients with glutathione synthetase deficiency but without mutations in the exons of the GSS gene.** *Hum Mutat* 2003, **22(6)**:497.
- Shi ZZ, Habib GM, Rhead WJ, Gahl WA, He X, Sazer S, Lieberman MW: **Mutations in the glutathione synthetase gene cause 5-oxoprolinuria.** *Nat Genet* 1996, **14(3)**:361-365.
- Mayatepek E: **5-Oxoprolinuria in patients with and without defects in the gamma-glutamyl cycle.** *Eur J Pediatr* 1999, **158(3)**:221-225.
- Erasmus E, Mienie LJ, de Vries WN, de Wet WJ, Carlsson B, Larsson A: **Prenatal analysis in two suspected cases of glutathione synthetase deficiency.** *J Inher Metab Dis* 1993, **16(5)**:837-843.
- Manning NJ, Davies NP, Olpin SE, Carpenter KH, Smith MF, Pollitt RJ, Duncan SL, Larsson A, Carlsson B: **Prenatal diagnosis of glutathione synthetase deficiency.** *Prenat Diagn* 1994, **14(6)**:475-478.

32. Janaky R, Varga V, Hermann A, Saransaari P, Oja SS: **Mechanisms of L-cysteine neurotoxicity.** *Neurochem Res* 2000, **25(9-10)**:1397-1405.
33. Goodman SI, Mace JW, Pollack S: **Serum gamma-glutamyl transpeptidase deficiency.** *Lancet* 1971, **1(7692)**:234-235.
34. Hammond JW, Potter M, Wilcken B, Truscott R: **Siblings with gamma-glutamyltransferase deficiency.** *J Inherit Metab Dis* 1995, **18(1)**:82-83.
35. O'Daley S: **An abnormal sulphhydryl compound in urine.** *Irish J Med Sci* 1968, **7**:578-579.
36. Wright EC, Stern J, Ersser R, Patrick AD: **Glutathionuria: gamma-glutamyl transpeptidase deficiency.** *J Inherit Metab Dis* 1980, **2(1)**:3-7.
37. Iida M, Yasuhara T, Mochizuki H, Takakura H, Yanagisawa T, Kubo H: **Two Japanese brothers with hereditary gamma-glutamyl transpeptidase deficiency.** *J Inherit Metab Dis* 2005, **28(1)**:49-55.
38. Mayatepek E, Okun JG, Meissner T, Assmann B, Hammond J, Zschocke J, Lehmann WD: **Synthesis and metabolism of leukotrienes in gamma-glutamyl transpeptidase deficiency.** *J Lipid Res* 2004, **45(5)**:900-904.
39. Kumar TR, Wiseman AL, Kala G, Kala SV, Matzuk MM, Lieberman MW: **Reproductive defects in gamma-glutamyl transpeptidase-deficient mice.** *Endocrinology* 2000, **141(11)**:4270-4277.
40. Schulman JD, Goodman SI, Mace JW, Patrick AD, Tietze F, Butler EJ: **Glutathionuria: inborn error of metabolism due to tissue deficiency of gamma-glutamyl transpeptidase.** *Biochem Biophys Res Commun* 1975, **65(1)**:68-74.
41. Henderson MJ, Larsson A, Carlsson B, Dear PR: **5-Oxoprolinuria associated with 5-oxoprolinase deficiency; further evidence that this is a benign disorder.** *J Inherit Metab Dis* 1993, **16(6)**:1051-1052.
42. Roesel RA, Hommes FA, Samper L: **Pyroglutamic aciduria (5-oxoprolinuria) without glutathione synthetase deficiency and with decreased pyroglutamate hydrolase activity.** *J Inherit Metab Dis* 1981, **4(2)**:89-90.
43. Bernier FP, Snyder FF, McLeod DR: **Deficiency of 5-oxoprolinase in an 8-year-old with developmental delay.** *J Inherit Metab Dis* 1996, **19(3)**:367-368.
44. Cohen LH, Vamos E, Heinrichs C, Toppet M, Courtens W, Kumps A, Mardens Y, Carlsson B, Grillner L, Larsson A: **Growth failure, encephalopathy, and endocrine dysfunctions in two siblings, one with 5-oxoprolinase deficiency.** *Eur J Pediatr* 1997, **156(12)**:935-938.
45. Mayatepek E, Hoffmann GF, Larsson A, Becker K, Bremer HJ: **5-Oxoprolinase deficiency associated with severe psychomotor developmental delay, failure to thrive, microcephaly and microcytic anaemia.** *J Inherit Metab Dis* 1995, **18(1)**:83-84.
46. Larsson A, Mattsson B, Wauters EA, van Gool JD, Duran M, Wadman SK: **5-oxoprolinuria due to hereditary 5-oxoprolinase deficiency in two brothers--a new inborn error of the gamma-glutamyl cycle.** *Acta Paediatr Scand* 1981, **70(3)**:301-308.
47. Mayatepek E, Badiou S, Bellet H, Lehmann WD: **A patient with neurological symptoms and abnormal leukotriene metabolism: a new defect in leukotriene biosynthesis.** *Ann Neurol* 2005, **58(6)**:968-970.
48. Anderson ME, Allison RD, Meister A: **Interconversion of leukotrienes catalyzed by purified gamma-glutamyl transpeptidase: concomitant formation of leukotriene D4 and gamma-glutamyl amino acids.** *Proc Natl Acad Sci U S A* 1982, **79(4)**:1088-1091.
49. Nitanai Y, Satow Y, Adachi H, Tsujimoto M: **Crystal structure of human renal dipeptidase involved in beta-lactam hydrolysis.** *J Mol Biol* 2002, **321(2)**:177-184.
50. Nakagawa H, Inazawa J, Inoue K, Misawa S, Kashima K, Adachi H, Nakazato H, Abe T: **Assignment of the human renal dipeptidase gene (DPEP1) to band q24 of chromosome 16.** *Cytogenet Cell Genet* 1992, **59(4)**:258-260.
51. Littlewood GM, Hooper NM, Turner AJ: **Ectoenzymes of the kidney microvillar membrane. Affinity purification, characterization and localization of the phospholipase C-solubilized form of renal dipeptidase.** *Biochem J* 1989, **257(2)**:361-367.
52. Oberholzer VG, Wood CB, Palmer T, Harrison BM: **Increased pyroglutamic acid levels in patients on artificial diets.** *Clin Chim Acta* 1975, **62(2)**:299-304.
53. Tham R, Nystrom L, Holmstedt B: **Identification by mass spectrometry of pyroglutamic acid as a peak in the gas chromatography of human urine.** *Biochem Pharmacol* 1968, **17(8)**:1735-1738.
54. Stokke O, Marstein S, Jellum E, Lie SO: **Accumulation of pyroglutamic acid (5-oxoprolin) in homocystinuria.** *Scand J Clin Lab Invest* 1982, **42(4)**:361-369.
55. Ghauri FY, McLean AE, Beales D, Wilson ID, Nicholson JK: **Induction of 5-oxoprolinuria in the rat following chronic feeding with N-acetyl 4-aminophenol (paracetamol).** *Biochem Pharmacol* 1993, **46(5)**:953-957.
56. Bonham JR, Rattenbury JM, Meeks A, Pollitt RJ: **Pyroglutamic aciduria from vigabatrin.** *Lancet* 1989, **1(8652)**:1452-1453.
57. Croal BL, Glen AC, Kelly CJ, Logan RW: **Transient 5-oxoprolinuria (pyroglutamic aciduria) with systemic acidosis in an adult receiving antibiotic therapy.** *Clin Chem* 1998, **44(2)**:336-340.
58. Jackson AA, Persaud C, Hall M, Smith S, Evans N, Rutter N: **Urinary excretion of 5-L-oxoprolin (pyroglutamic acid) during early life in term and preterm infants.** *Arch Dis Child Fetal Neonatal Ed* 1997, **76(3)**:F152-7.
59. Goto A, Ishida A, Goto R, Hayasaka K, Nanao K, Yamashita A, Yamaguchi S, Takada G: **Transient 5-oxoprolinuria in a very low-birthweight infant.** *J Inherit Metab Dis* 1992, **15(2)**:284-285.
60. Jackson AA, Badaloo AV, Forrester T, Hibbert JM, Persaud C: **Urinary excretion of 5-oxoprolin (pyroglutamic aciduria) as an index of glycine insufficiency in normal man.** *Br J Nutr* 1987, **58(2)**:207-214.
61. Rizzo C, Ribes A, Pastore A, Dionisi-Vici C, Greco M, Rizzoni G, Federici G: **Pyroglutamic aciduria and nephropathic cystinosis.** *J Inherit Metab Dis* 1999, **22(3)**:224-226.

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