

4.1 Introduction

The tyrosine degradation pathway includes 5 enzymatic steps and inherited disorders have been identified in four of these steps. During normal conditions the level of tyrosine is regulated by the first enzyme (tyrosine aminotransferase) of the pathway, but acquired or inherited deficiency of the second enzyme (4-hydroxyphenylpyruvate dioxygenase) also results in hyper-tyrosinemia. Tyrosine is mainly degraded in liver, but also to a minor extent in the kidney. In tyrosinemia type I the primary defect is in the last enzyme of the pathway. This enzyme deficiency results in accumulation of toxic metabolites and the hypertyrosinemia in this disorder is caused by secondary deficiency of 4-hydroxyphenylpyruvate dioxygenase, which also is found in severe liver disease of other causes and in the immature liver. There is no common phenotype to the different disorders of tyrosine degradation. The occurrence of corneal and skin lesions, as seen in tyrosinemia type II, is a direct effect of high tissue tyrosine. High tyrosine concentration is associated with neurological symptoms and mental deficiency in tyrosinemia type II and III, but not in tyrosinemia type I. The liver and kidney disease of tyrosinemia type I is caused by accumulation of toxic metabolites (fumarylacetoacetate and its derivatives) and is prevented by an inhibitor (NTBC) of tyrosine degradation at the level of 4-hydroxyphenylpyruvate dioxygenase. In alkaptonuria there is no increase in tyrosine levels and the degradation of tyrosine proceeds at a normal rate to produce homogentisate, which upon oxidation forms reactive intermediates and pigment, which is deposited in various tissues preferably in joints and connective tissue.

■ Tyrosinemia Type I

The phenotype of tyrosinemia type I is highly variable and severity of the disease varies with age at onset of symptoms [1]. In the most common form the infant presents in liver failure often preceded by a period of failure to thrive at 2–3 months of age. There is in most cases only a moderate increase in transaminases, the jaundice is not pronounced but there is a

pronounced coagulopathy with highly increased prothrombin time and thrombocytopenia. There is a marked increase in α -fetoprotein concentration. Sepsis is not uncommon and there may be early signs of hypophosphatemic rickets secondary to renal tubulopathy. In this situation tyrosine is most often grossly elevated due to the catabolic state and the condition of the liver. In most cases there also is moderately to gross increase in methionine. In the more chronic forms of the disease symptoms may be subtle. However, there are most often clinical and laboratory signs of liver disease although there are children who present with rickets and few signs of liver disease. Another complication of tyrosinemia type I is occurrence of porphyria-like neurologic crises. This is caused by inhibition of heme synthesis by succinylacetone, which is a powerful inhibitor of porphobilinogen synthase. Reduced porphobilinogen synthase activity in erythrocytes and increased 5-aminolevulinate are useful complements in the diagnosis of tyrosinemia type I although the hallmark of the diagnosis of tyrosinemia type I is identification of increased concentration of succinylacetone or its precursors. The requirement for confirmation by enzymatic and/or DNA analysis is rarely needed although identification of mutations greatly facilitates prenatal diagnosis in future pregnancies [2].

The traditional treatment of tyrosinemia type I is restriction of dietary intake of tyrosine and phenylalanine to reduce production of the toxic metabolites. Although dietary and supportive therapy may alleviate acute symptoms and greatly improve the condition it does not prevent a high mortality rate during childhood due to liver failure or development of hepatocellular carcinoma [1]. During the last decade inhibition of 4-hydroxyphenylpyruvate dioxygenase by NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) has developed into a first line treatment of tyrosinemia type I [3]. Acute illness has resolved in 90% of the infants and the need for urgent liver transplantation, which is the ultimate treatment for tyrosinemia type I, has been reduced accordingly. The long-term follow-up also indicates that the incidence of early childhood development of liver malignancy is greatly reduced in patients with an early start of treatment. However, in patients who have started late there is still a substantial risk for development of hepatocellular carcinoma and decision on whether to transplant or not must be individualised with respect to the clinical findings. NTBC has now been used in >300 patients. Adverse effects are few and no patient has been withdrawn from therapy because of such effects. NTBC treatment results in increased tyrosine concentration and has to be combined with a diet restricted in tyrosine and phenylalanine. The rationale for restriction of tyrosine is not only to prevent the risk for eye lesions and to avoid possible neurologic effects of high tyrosine, but also to reduce the load on the tyrosine degradation pathway to further reduce the risk for production of toxic metabolites.

■ Tyrosinemia Type II

Tyrosinemia type II is characterised by eye lesions, skin disease and neurological complications or any combination of these symptoms. The disorder usually presents during infancy but may become manifest at any age. Tyrosine concentration is grossly elevated in an otherwise normal amino acid profile. When the eye and skin lesions are present it was generally not considered justified to confirm the diagnosis by enzyme analysis, which would require a liver biopsy specimen. However, mutations have now been identified and as methodologies evolve it might be feasible to get confirmation of the diagnosis by mutation analysis complemented by expression studies when required [4]. In patients with no symptoms e.g. picked up in neonatal screening programs or patients with only neurological symptoms a differentiation between tyrosinemia type II and III may offer a problem. The tyrosine level in tyrosinemia type II tends to be higher, but there is an overlap where differentiation based on the tyrosine level is impossible. Irrespective of the final diagnosis it seems reasonable to substantially reduce the tyrosine concentration (not only to $<800 \mu\text{mol/l}$ which is considered sufficient to avoid eye and skin lesions) by dietary means. Neurological complications and mental deficits are common in tyrosinemia type II and III and reduction of tyrosine level might reduce the risk for development of neurological complication. To evaluate the effects of tyrosine restriction and to delineate the phenotypes of these two disorders it seems important to get the diagnosis confirmed whenever possible.

■ Tyrosinemia Type III

Tyrosinemia type III has hitherto only been reported in 9 cases [5, 6] and 5 additional cases have been confirmed in our laboratory. The high tyrosine concentration in an otherwise normal amino acid profile has been found in patients presenting with neurological symptoms and mental retardation or in neonatal screening programs. Eye or skin lesions have not been reported. Some degree of mental retardation seems to be associated with the disorder, but there seems to be no correlation between either the severity of the enzyme deficiency or the recorded tyrosine level. Enzymatic diagnosis requires liver biopsy samples, but mutation analysis is possible and such analysis complemented with expression studies might be feasible in the near future. As for tyrosinemia type II it seems reasonable to reduce tyrosine levels by dietary means at least during early childhood.

■ Hawkinsinuria

Hawkinsinuria has only been reported in 4 families and 1 sporadic case. The disorder is characterised by failure to thrive and acidosis. Slight increase in plasma tyrosine has been reported but the diagnosis is based on identification of hawkinsin (2-cystenyl-1,4-dihydroxycyclohexenylacetate) which is supposed to be an abnormal reaction product of 4-hydroxyphenylpyruvate dioxygenase which is detoxified by reaction with glutathione. Depletion of glutathione and the resulting high excretion of 5-oxoproline is probably responsible for the acidosis in hawkinsinuria. The condition is benign and there are no symptoms after infancy. The condition responds to protein reduction and restriction of phenylalanine and tyrosine intake. In the published families there is dominant inheritance postulated to be caused by one defective 4-hydroxyphenylpyruvate dioxygenase allele. After infancy 4-hydroxycyclohexylacetate appear in urine in addition to hawkinsin. Oral loading tests with deuterated tyrosine has shown that hawkinsin is derived from tyrosine [7]. However, no enzyme studies have been performed. Further recent DNA analysis of 2 patients with hawkinsinuria has been reported [6]. No mutation was identified in the 4-hydroxyphenylpyruvate gene except for a polymorphism, which was present in 30% in our control alleles [5]. So at present the cause of this disorder is completely open.

■ Alkaptonuria

Early diagnosis of alkaptonuria is based on observation of darkening of urine on standing. Symptoms like scleral pigmentation and arthritis most often in the hip and knee joint do not appear until adulthood. Periods with acute inflammation may resemble rheumatoid arthritis. The arthritis may be severe and disabling. Ankylosis of the lumbosacral region is common and the roentgenological findings may be pathognomonic. Diagnosis is confirmed by identification of homogentisate, which is excreted in mmolar amounts in urine. There is no effective treatment of alkaptonuria at present, but treatment with NTBC would prevent production of homogentisate. The regime in tyrosinemia type I aims to completely block the flux through the pathway. This would probably not be necessary in alkaptonuria so the ideal treatment in this disorder would be to find a regime where a sufficient reduction of homogentisate production is obtained without an excessive increase in tyrosine concentration.

Enzymatic diagnosis is not required for a diagnosis and is not done. In recent years several mutations have been identified [8]. A peculiarity is that in an area of Slovakia where alkaptonuria is exceptionally common, several different mutations have been identified [9].

4.2 Nomenclature

No.	Disorder (affected component)	Tissue distribution	Chromosomal localization	OMIM
4.1	Tyrosinemia type I (fumarylacetoacetase, FAH)	General	15q23–q25	276700
4.2	Tyrosinemia type II (tyro- sine aminotransferase, TAT)	Liver	16.q22.1–q22.3	276600
4.3	Tyrosinemia type III (4-hydroxyphenylpyruvate dioxygenase, HPD)	Liver, kidney	12q24–qter	276710
4.4	Hawkinsinuria (unknown)	Unknown	Unknown	140350
4.5	Alkaptonuria (homogenti- sate dioxygenase, HGD)	Liver, kidney, in- testine, prostate	3q21–q23	203500

4.3 Metabolic Pathway

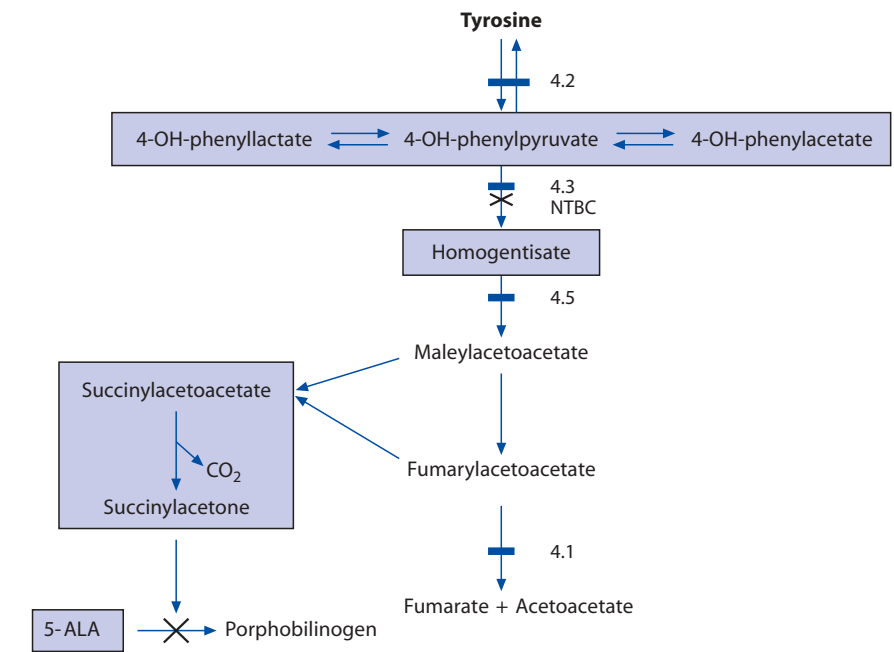


Fig. 4.1. Tyrosine degradation pathway. Metabolic markers are framed. Possible metabolic disorders are marked with boxes. 4.1, fumarylacetoacetase; 4.2, tyrosine aminotransferase; 4.3, 4-hydroxyphenylpyruvate dioxygenase; 4.5, homogentisate dioxygenase. Inhibition by succinylacetone and NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) are indicated by crosses. 5-ALA, 5-aminolevulinate

4.4 Signs and Symptoms

Table 4.1. Tyrosinemia type I [n > 400]

System	Symptoms/markers	Newborn	Infancy	Childhood	Adolescence	Adulthood
Liver	Macronodular cirrhosis	–	±	±	(+)	(+)
	Micronodular cirrhosis	–	±	±	±	±
	Hepatocellular carcinoma	–	±	±	±	±
	Acute liver failure with moderate jaundice and pronounced coagulopathy	±	±	–	–	–
Kidney	Tubulopathy	+	+	+	+	+
	Renal enlargement	–	+	+		
	Nephrocalcinosis				±	±
	Glomerulopathy				±	±
Skeleton	Rickets	–	±	±	±	+
Nervous system	Porphyria-like neurologic crisis (abdominal pain, hypertension, muscular weakness, paresis)	±	±	±	±	±
	Neuropathy	–	±	±	±	±
	Sepsis	+	+	–	–	–
General						
Special laboratory	Tyr (P)	↑	↑	↑	↑	↑
	Met (P)	(↑)	(↑)	(↑)	–	–
	Succinylacetone ^a (U, P)	↑	↑	↑	↑	↑
	4-Hydroxyphenylpyruvate (U)	↑	↑	↑	↑	↑
	4-Hydroxyphenyllactate (U)	↑	↑	↑	↑	↑
	4-Hydroxyphenylacetate (U)	↑	↑	↑	↑	↑
	5-Aminolevulinate (U)	↑	↑	↑	↑	(↑)
	Porphobilinogen synthase (RBC)	↓	↓	↓	↓	(↓)
	α-Fetoprotein (S)	↑↑	↑↑	↑	(↑)	(↑)

^a Succinylacetone and/or succinylacetoacetate and/or 4-oxo-6-hydroxyheptanoate (U).

Table 4.2. Tyrosinemia type II [n = 14]

System	Symptoms/markers	Newborn	Infancy	Childhood	Adolescence	Adulthood
Eye	Corneal erosion	–	±	+	+	+
	Photophobia	–	+	+	+	+
	Lacrimation	–	+	+	+	+
Skin	Blisters, erosion, hyperkeratosis on palms and soles	–	–	±	±	±
Central nervous system	Mental retardation/deficiency	–	±	±	±	±
Special laboratory	Tyrosine (P)	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
	4-Hydroxyphenylpyruvate (U)	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
	4-Hydroxyphenyllactate (U)	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
	4-Hydroxyphenylacetate (U)	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑

Table 4.3. Tyrosinemia type III

System	Symptoms/markers	Newborn	Infancy	Childhood	Adolescence	Adulthood
Central nervous system	Mental retardation/deficiency	-	±	±	±	±
Special laboratory	Tyrosine (P)	↑↑	↑↑	↑↑	↑↑	↑↑
	4-Hydroxyphenylpyruvate (U)	↑↑	↑↑	↑↑	↑↑	↑↑
	4-Hydroxyphenyllactate (U)	↑↑	↑↑	↑↑	↑↑	↑↑
	4-Hydroxyphenylacetate (U)	↑↑	↑↑	↑↑	↑↑	↑↑

Table 4.4. Hawkinsinuria

System	Symptoms/markers	Newborn	Infancy	Childhood	Adolescence	Adulthood
General	Failure to thrive, acidosis	-	+	-	-	-
Liver	Unspecified hepatopathy	-	+	-	-	-
Special laboratory	Tyrosine (P)		↑	-	-	-
	Hawkinsin (U)		↑	↑	↑	↑
	4-Hydroxycyclohexylacetate		-	↑	↑	↑
	4-Hydroxyphenylpyruvate (U)		↑	-	-	-
	4-Hydroxyphenyllactate (U)		↑	-	-	-
	4-Hydroxyphenylacetate (U)		↑	-	-	-
	5-Oxoproline		↑	-	-	-

Table 4.5. Alkaptonuria

System	Symptoms/markers	Newborn	Infancy	Childhood	Adolescence	Adulthood
Joints	Arthritis	-	-	-	-	+
	Ochronosis	-	-	-	-	+
	Lumbar-sacral disc degeneration					+
Eye	Scleral pigmentation	-	-	-	-	+
Skin	Pigmentation	-	-	-	-	±
Cardiovascular	Mitral and aortic valvulitis	-	-	-	-	±
Urine	Darkening on standing (alkalinisation and oxidation)	+	+	+	+	+
Special laboratory	Homogentisate (U)	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑

4.5 Reference Values

Age Years	Tyr (P) μmol/l	Met (P)	Succinyl- acetone activity ^a (P)	Porphobilinogen synthase activity ^b (RBC) nkat/g Hgb	mmol/mol Creatinine in random samples			
					Succinyl- acetone ^c (U)	5-Amino- levulinate (U)	Hawkinsin (U)	Homogen- tise (U)
Newborn	50–150	10–60	<0.1	0.58–1.25	<1	–	n.d.	<1
1–12	30–130	10–50	<0.1	0.58–1.25	<0.5	<12	n.d.	<1
>12	30–100	10–40	<0.1	0.58–1.25	<0.1	<3	n.d.	<1

^a Enzymatic method. Inhibition of porphobilinogen synthase by boiled plasma given in the equivalent succinylacetone concentration.

^b Enzymatic method. Reference values vary with the methodology.

^c Significant concentration of succinylacetone, succinylacetoacetate and 4-oxo-6-hydroxyheptanoate is not found in normal urine. With a sensitivity of 0.4 μmol/l for these substances <1% of tyrosinemia type I patients will be missed even when the urine sample is very dilute (urine creatinine <0.4 mmol/l).

n.d., not detectable

4.6 Pathological Values/Differential Diagnosis

No.	Disorder	Tyr (P) μmol/l	Met (P)	Succinyl- acetone activity (P)	Porphobilinogen synthase activity (RBC) % of normal mean	mmol/mol Creatinine in random samples			
						Succinyl- acetone (U)	5-Amino- levulinate (U)	Hawkinsin (U)	Homogen- tise (U)
4.1	Tyrosine- mia I	150–1300	20–1300	0.5–>100	1–50	0.5–>1000	20–>100	n.d.	<1–trace
4.2	Tyrosine- mia II	800–>2000	Normal	<0.1	Normal	<1	Normal	n.d.	<1
4.3	Tyrosine- mia III	500–1200	Normal	<0.1	Normal	<1	Normal	n.d.	<1
4.4	Hawkinsi- nuria	Normal – moderate increase	Normal	–	–	<1	–	200–2000	–
4.5	Alkapto- nuria	Normal	Normal	Normal	Normal	Normal	Normal	n.d.	>1000

n.d., not detectable

4.7 Diagnostic Flow Chart in Hypertyrosinemia

Clinical situation	Symptoms and signs	Diagnosis
Neonatal screening	Transaminases (\pm) α -Fetoprotein (+) Prothrombine time (\pm) Porphobilinogen synthase deficiency (+) Succinylacetone (+)	Tyrosinemia type I (tyr(P) 120-1300 $\mu\text{mol/l}$)
	Persisting	Tyrosinemia type II (tyr(P) 800->2000 $\mu\text{mol/l}$) Tyrosinemia type III (tyr(P) 500-1300 $\mu\text{mol/l}$) Liver enzyme analysis (DNA analysis)
Failure to thrive	Signs of liver disease (+) Succinylacetone (+)	Tyrosinemia type I
	Acidosis (+) Hawkinsin (+) 5-oxoproline (+)	Hawkinsinuria
Liver disease	Prothrombin time (+) α -Fetoprotein (+) Bilirubin (normal-100 $\mu\text{mol/l}$) Succinylacetone* (U) (1->1000 mmol/mol creatinine)	Tyrosinemia type I
In patients with rickets	Hypophosphatemia (+) General aminociduria (+) (Full Fanconi syndrome) (+) Signs of liver disease (+) Succinylacetone (U) (+)	Tyrosinemia type I
In mental retardation	Eye and/or skin symptoms (+) No other symptoms	Tyrosinemia type II Tyrosinemia type II or III

* The sum of urine succinylacetone, succinylacetoacetate and 4-oxo-6-hydroxyhepatanoate

Fig. 4.2. Increased tyrosine concentration is caused by inborn or acquired deficiency of the first two enzymes of the tyrosine degradation pathway; (the increased tyrosine concentration of tyrosinemia type I is caused by secondary deficiency of 4-hydroxyphenylpyruvate dioxygenase). Hypertyrosinemia in the newborn is in most instances not due to inborn errors of tyrosine metabolism, but rather to liver immaturity or other unspecific liver affections. However, whenever hypertyrosinemia is found, the pathognomonic sign of tyrosinemia type I should be excluded by a sufficiently sensitive analysis of succinylacetone and related metabolites. Decreased activity of porphobilinogen synthase activity in RBC is a sensitive and easily performed marker for increased concentrations of succinylacetone, which may be used as a first line diagnostic test before positive identification of succinylacetone and related metabolites by GC-MS can be achieved. It should also be noted that increased excretion of phenolic tyrosine metabolites is always found in hypertyrosinemia and is of no differential diagnostic value

4.8 Specimen Collection

Test	Precondition	Material	Handling ^a	Pitfalls
Tyr (P)	–	Plasma	Ambient temp.	Unspec. liver disease
Met (P)	–	Plasma	Ambient temp.	Unspec. liver disease
Succinylacetone (P)	–	Plasma (heparin)	Ambient temp.	Slight increase in severe liver disease
Porphobilinogen synthase (RBC)	–	Heparinized blood	Ambient temp.	May be close to normal Primary deficiency
Succinylacetone (U)	–	Urine	Ambient temp (Frozen –20 °C)	Method sensitivity, dilute urine
Hawkinsin	–	Urine	Frozen –20 °C	Not recognized
Homogentisate	–	Urine	Frozen –20 °C	–
FAH activity	–	Fibroblasts	Ambient temp.	Pseudodeficiency
		Lymphocytes	Frozen (–70 °C)	
		Liver	Frozen (–70 °C)	Patchy reversal of the genetic defect in liver
TAT activity	–	Liver	Frozen (–70 °C)	Highly regulated wide normal range
HPD activity	–	Liver	Frozen (–70 °C)	Deficient in cirrhotic liver

^a For diagnosis of tyrosinemia type I we require 2 ml of heparinized blood (the whole blood) and 20 ml of urine sent in ambient temperature by courier mail on the day of sampling.

4.9 Prenatal Diagnosis

Disorder	Material	Timing (WG)	Pitfalls
4.1	CV	12	Pseudodeficiency
	AF	12–	No increase in SA
	Amniocytes	12–	Pseudodeficiency

4.10 DNA Analysis

Disorder	Material	Tissue	Method
4.1	Genomic/cDNA	WBC/fibroblast culture	PCR RFLP/sequencing
4.2	Genomic	WBC	SSCP/sequencing
4.3	Genomic/cDNA	WBC/liver tissue	PCR RFLP/sequencing
4.4	See text	–	–
4.5	Genomic	WBC	SSCP/sequencing

4.11 Initial Treatment (Management while Awaiting Results)

It is obvious that the infants presenting with acute liver failure caused by tyrosinemia type I are in need of intensive care and general supportive therapy. Protein intake should be restricted/stopped and efforts should be made to reverse the catabolic state. Sepsis should be considered and the risk for bleeding should be diminished by substitution of coagulation proteins. Preparations should be made for institution of NTBC treatment as soon as the diagnosis is confirmed. It should be noted that in about 10% of these patients there is no clinical response to NTBC treatment. In these infants there has been no reversal of the coagulopathy in response to treatment, and in addition there has been increasing jaundice. In these infants urgent liver transplantation is required for survival. In rare instances, tyrosinemia type I may present as an acute porphyria-like neurologic crisis. In these patients it is essential to get the child into an anabolic state and institute NTBC treatment as soon as possible to prevent occurrence of respiratory paralysis. NTBC treatment has rapidly reversed the symptoms of acute neurologic crisis.

In tyrosinemia type I patients with more insidious symptoms there is no such urgency, although it is desirable to start the dietary treatment and NTBC treatment as soon as possible.

Whenever an exceedingly high tyrosine concentration is found, especially if it is accompanied by eye symptoms, it seems appropriate to reduce phenylalanine and tyrosine intake whether it is a transient tyrosinemia in the newborn or caused by an inherited disorder of tyrosine metabolism. Especially for tyrosinemia type II and III, positive confirmation of the diagnosis may be delayed considerably, and if a liver biopsy is considered, this should wait until persistency of the tyrosinemia has been established.

4.12 Summary/Comments

There are 4 defined inborn errors of tyrosine metabolism. The most common and serious of these disorders is tyrosinemia type I. For this disorder an efficient drug treatment based on inhibition of tyrosine degradation is available. Early institution of therapy, preferably before serious symptoms occur is desirable. Ideally an effective neonatal screen for this disorder, as has been effective for many years in Quebec where the incidence is very high, would be desirable. An approach based on second tear analysis of porphobilinogen synthase in the Guthrie cards of all samples with increased tyrosine concentration found in LC-MS-MS screening – seems promising and would reduce the number of false positives, which is essential, especially in areas with a low incidence of the disease [10]. For the other disorders, no specific therapy is available but improvement of diagnostic procedures and early detection might prevent some complications of these disorders.

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