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Review Article

Diagnosis and treatment of hereditary tyrosinemia in Japan

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Abstract Hereditary tyrosinemia is an autosomal recessive inherited disease that manifests as three types (types I–III). We conducted a nationwide survey of this disease in Japan, and here review the results in relation to prevalence, clinical characteristics, and treatment and diagnosis. A definitive diagnosis of tyrosinemia type I is difficult to obtain based only on blood tyrosine level. Detection of succinylacetone using dried blood spots or urinary organic acid analysis, however, is useful for diagnosis. In tyrosinemia type I, dietary therapy and nitisinone (Orfandin®) are effective. Prognosis is greatly affected by the complications of liver cancer and hypophosphatemic rickets; even patients that are treated early with nitisinone may develop liver cancer. Long-term survival can be expected in type I if nitisinone therapy is effective. Prognosis in types II and III is relatively good.

Key words liver cancer, liver transplantation, nitisinone, succinylacetone, tyrosine.

Tyrosine is an amino acid contained in food and is also obtained internally as a metabolite of phenylalanine. In the body, phenylalanine transforms into tyrosine via phenylalanine hydroxylase. Tyrosine breaks down into 4-OH-phenylpyruvate via tyrosine aminotransferase (TAT), then to homogentisate via 4-OH-phenylpyruvate dioxygenase (HPD), which breaks down to maleylacetoacetate via homogentisate dioxygenase and then to fumarylacetoacetate hydrolase (FAH) via maleylacetoacetase, and finally to fumarate and acetoacetate via FAH (Fig. 1).[1] Tyrosinemia results from elevated tyrosine in the blood, and although all hereditary forms show autosomal recessive inheritance, the causes are variable, and hereditary tyrosinemia is therefore genetically and clinically differentiated into three types: type I, type II, and type III (Table 1).[1,2] These diseases differ genetically and enzymatically, and clinical manifestations occur via different mechanisms.

Hereditary tyrosinemia type I (MIM276700) occurs from a defect of FAH (EC 3.7.1.2), type II (MIM276600) occurs from a defect of cytosol TAT (EC2.6.1.5), and type III (MIM276710) occurs from a defect of HPD (EC1.13.11.27). Hawkinsinuria is an autosomal dominant hereditary disease that also occurs due to HPD abnormality.[3] In the hawkinsinuria patient, the residual activity of HPD forms 1,2-epoxyphenyl acetic acid, which reacts with glutathione to produce hawkinsin (2-L-cystein-S-yl-1,4-dihydroxy-cyclohex-5-en-1-yl acetic acid).

We performed a nationwide survey of hereditary tyrosinemia in Japan. In the survey, we found that it is difficult to make a definitive diagnosis of tyrosinemia type I based only on blood tyrosine level. Detection of succinylacetone using dried blood spots or urinary organic acid analysis, however, is useful for diagnosis. Dietary therapy and nitisinone (Orfandin®) are effective therapies for tyrosinemia type I.

This study was approved by the ethics committee of the Faculty of Life Science, Kumamoto University. Briefly, we sent a questionnaire to 928 Japanese institutions, including the departments of pediatrics, endocrinology and metabolism, neonatology, genetics, and transplant surgery, asking doctors if they had diagnosed or provided medical care to tyrosinemia patients. Each institution was the medical center for a locality and had ≥300. Of the 928 institutions, 659 (71%) responded. Of these 659 institutions, 15 had treated patients with tyrosinemia.

Pathophysiology and epidemiology

In tyrosinemia type I, several pathologies occur due to the toxicity of fumarylacetoacetate, which builds up in cells due to the enzyme defect of FAH.[4–7] The defect results in abnormalities of gene expression, inhibited enzyme activity, apoptosis, chromosomal instability, and carcinogenesis in hepatocytes. In particular, decreased gene expression in patients with tyrosinemia type I can result in conditions such as hypoglycemia, metabolic disorders of amino acids, and reduction in coagulation factors. Chromosomal instability leads to a high incidence of juvenile liver cancer, and hepatocyte death due to apoptosis can cause progression to liver failure. Cellular damage also appears in the proximal renal tubules, causing Fanconi syndrome, including aminoaciduria, glycosuria, and metabolic acidosis, which ultimately result in hypophosphatemic rickets.

In tyrosinemia type II, clinical manifestations occur due to high concentrations of tyrosine in the body fluids.[8] Some cases are linked to the low solubility of tyrosine. The temperature of the skin and cornea can decrease more readily than in other areas, which could result in tyrosine crystal deposition leading to cellular damage. Delayed cognitive development is also often seen in cases of tyrosinemia type II. Although this is thought to be

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Phenylalanine

↓ phenylalanine hydroxylase **(phenylketuria)**

tyrosine

↓ tyrosine aminotransferase **(tyrosinemia type II)**

4-OH-phenylpyruvate

↓ 4-OH-phenylpyruvate dioxygenase **(tyrosinemia type III, Hawkinsinuria)**

Homogentisate

↓ Homogentisate dioxygenase

Maleylacetoacetate → Succinylacetoacetate → Succinylacetone

↓ Maleylacetoacetase

Fumarylacetoacetate → Succinylacetoacetate → Succinylacetone

↓ Fumalylacetoacetate hydrolase **(tyrosinemia type I)**

Fumarate + Acetoacetate

Fig. 1 Tyrosine metabolism: the metabolic pathway from phenylalanine through tyrosine to fumarate and acetoacetate. Tyrosinemia type I occurs due to a deficit of fumarylacetoacetate hydrolase, type II occurs from a deficit of tyrosine aminotransferase, and type III occurs from a deficit of 4-OH-phenylpyruvate dioxygenase.

linked to elevated tyrosine in the blood, the detailed mechanism has not been clarified. Moreover, large amounts of 4-OH-phenylpyruvate, the product of tyrosine transamination, and its oxides are excreted in urine.[9] This is due to the aspartate aminotransferase converting tyrosine to 4-OH-ohenylpyruvate when it is present at high concentration.

In tyrosinemia type III, the levels of tyrosine and 4-OH-phenylpyruvate, the α-keto acid of tyrosine, are increased. In addition, 4-OH-phenylpyruvate and its oxides excreted in the urine are markedly elevated.

We conducted a nationwide survey of hereditary tyrosinemia (71% response rate) in Japan, and received responses regarding five cases of type I, two cases of type II, and one case of type III. Responses were also received regarding 10 cases of tyrosinemia due to an unknown cause, which may be due to transient neonatal hepatitis.[10] Given that some hereditary tyrosinemia type III patients can be asymptomatic, we believe that there are more patients with tyrosinemia type III than were discovered through the survey. The prevalence of hereditary tyrosinemia type I in Europe is reported to be 1 in 125 000 people;[8] therefore, the incidence in Japan is clearly lower. Elevated tyrosine in the blood can be detected using tandem mass spectrometry, but because blood tyrosine can be high in newborns for a variety of reasons, it is difficult to identify true cases of hereditary tyrosinemia. For this reason, tyrosinemia is currently not a component of routine newborn screening. Screening of newborns for tyrosinemia, however, could be possible by measuring succinylacetone in the blood.[11] Nonetheless, there is a wide variety of genetic and biochemical disease groups that present with tyrosinemia. In the present survey, elevated tyrosine (>440 μmol/L; >8 mg/dL) was observed in 0.85% of newborns. In Europe, 0.5–1.8% of newborns have high tyrosine, indicating that it is a relatively frequent abnormality,[1] but, because hereditary tyrosinemia is only very rarely encountered, the diagnosis must be made on the basis of its characteristic clinical manifestations and specific tests.

Clinical manifestations

Tyrosinemia type I is clinically characterized by progressive liver dysfunction and renal tubule damage.[1,2] There are three subtypes of liver dysfunction: acute, subacute, and chronic. The acute type begins several weeks after birth with hepatomegaly, poor development, diarrhea, vomiting, and jaundice. Severe cases of dysfunction progress to liver failure; if left untreated, death occurs 2–3 months after birth. The subacute type manifests as liver dysfunction from several months to approximately 1 year after birth. In the chronic type, liver dysfunction progresses slowly, but can eventually result in cirrhosis or liver failure. Liver cancer occurs in many cases, and multiple tumors have been reported in such cases. With respect to the kidneys, renal tubular dysfunction can result in diseases such as hypophosphatemic rickets and

Table 1 Classification of tyrosinemia

Disease	Inheritance	Serum tyrosine elevation	Enzyme	Clinical manifestations
Type I	AR	Mild, >4 mg/dL (220 μmol/L)	Fumarylacetoacetate hydrolase	Liver failure, hypoglycemia, renal tubular disorder, galactosemia, neuropathy, hepatocellular carcinoma
Type II	AR	Very high, >20 mg/dL (1100 μmol/L)	Tyrosine aminotransferase	Mental retardation, abnormal keratinization, corneal ulcer
Type III	AR	Moderate, >10 mg/dL (550 μmol/L)	4-OH-phenylpyruvate dioxygenase	Ataxia, convulsion, mental retardation (mild)
Hawkinsinuria	AD	Transient	4-OH-phenylpyruvate dioxygenase	Growth retardation, appetite loss
Secondary tyrosinemia	–	Variable		Dependent on symptoms of the primary disease
Neonatal transient tyrosinemia	–	Variable		Asymptomatic

AD, autosomal dominant; AR, autosomal recessive.

vitamin D-resistant rickets. Moreover, succinylacetone inhibits aminolevulinate dehydratase, which causes bouts of abdominal pain, polyneuropathy, and other manifestations resembling acute intermittent porphyria.

Tyrosinemia type II involves higher blood tyrosine level than types I and III, but does not exhibit the liver and kidney dysfunction found in type I.[1,8] The skin lesions that appear in type II are due to deposition of needle-like tyrosine crystals, which cause excess keratinization and erosion that is limited to the palms and soles. Deposits of tyrosine crystals also appear on the cornea, leading to corneal erosion and ulceration. Corneal changes appear several months after birth, earlier than the skin manifestations, although in some cases clear manifestations do not appear until adolescence or later. Delayed mental development is observed in some cases of particularly high blood tyrosine concentration.

Manifestations in tyrosinemia type III are milder than those in types I and II, and some cases are asymptomatic.[1,3] Manifestations including ataxia, spasms, and mildly delayed cognitive development have been reported. These do not appear in types I or II, and may be linked to an increase in 4-OH-phenylpyruvate in body fluids. Many cases are diagnosed when these manifestations appear, although it is thought that some cases are asymptomatic and are likely undiagnosed.

Laboratory studies

Besides tyrosinemia types I, II, and III, high blood tyrosine can occur due to other causes, making differentiation and accurate diagnosis necessary.[1,10] The liver dysfunction associated with type I is particularly important for differential diagnosis. For differentiating between types II and III, transient tyrosinemia in newborns can be problematic. Blood amino acid analysis and tandem mass spectrometry are useful tools for monitoring blood tyrosine.

The presence or absence of liver dysfunction is important for diagnosing hereditary tyrosinemia type I. Liver dysfunction causes serum transaminase to rise, coagulation factor synthesis to decrease, and a reduction in albumin and cholinesterase.[1,8] Renal tubular dysfunction can cause hypophosphatemia, glycosuria, and proteinuria. An increase in α-fetoprotein is also a characteristic marker of type I. Blood amino acid analysis commonly indicates elevated levels of many amino acids, including tyrosine (>220 μmol/L, 4 mg/dL), methionine (>130 μmol/L, 2 mg/dL), and threonine (>170 μmol/L, 2 mg/dL). In urinary amino acid analysis, there is increased excretion of tyrosine and many other amino acids. Disorders of porphyrin metabolism result in an increase in δ-aminolevulinic acid in the urine. Hepatomegaly, cirrhosis, and fatty liver are common observations on imaging. Findings including abnormal liver tissue structure, abnormal hepatocyte morphology, and fatty liver are seen on liver biopsy, but these are non-specific, and are therefore not useful to make a definitive diagnosis. A definitive diagnosis requires the confirmation of increased succinylacetone in an analysis of urinary organic acids (Fig. 2). Elevated levels of the tyrosine metabolites 4-OH-phenylpyruvate, 4-OH-phenylacetate, and 4-OH-phenyllactate are also observed in urinary organic acid analysis. Enzyme diagnosis can be done by measuring hydrolase activity in samples of hepatocytes and cultured skin fibroblasts.

Skin and eye findings can be used to suggest hereditary tyrosinemia type II.[8] Tyrosine is extremely high in blood amino acid analysis, at ≥1100 μmol/L (20 mg/dL). Further, large amounts of 4-OH-phenylpyruvate, 4-OH-phenylacetate, and

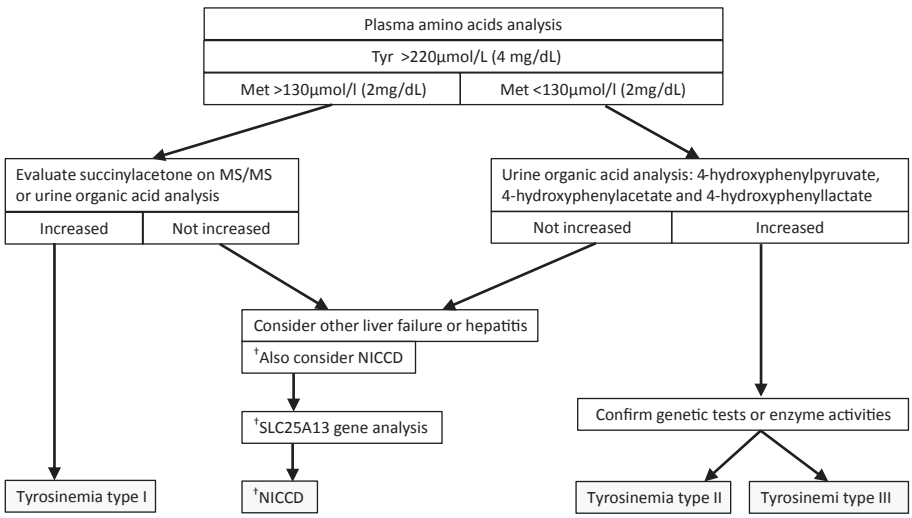

Fig. 2 Diagnosis of hereditary tyrosinemia. This algorithm can be used for differentiating among types of tyrosinemia. Increased succinylacetone in the blood or urine is used to diagnose tyrosinemia type I. Types II and III are diagnosed from elevated 4-OH-phenylpyruvate, 4-OH-phenylacetate, and 4-OH-phenyllactate, which are not accompanied by elevated succinylacetone on urinary organic acid analysis. It is generally easy to differentiate types II and III based on their clinical manifestations. When distinguishing between them is difficult, enzyme activity should be measured or genetic analysis should be performed. †If the disease is thought to be something other than hereditary tyrosinemia, it needs to be differentiated from neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). NICCD is diagnosed by analyzing *SLC25A*. MS/MS, tandem mass spectroscopy.

4-OH-phenyllactate are found on urinary organic acid analysis. Liver biopsy is required to measure specific enzyme activity. In addition, there are two forms of TAT: sTAT and mTAT, the former existing in the soluble fraction, and the latter localized to the mitochondria. Patients with type II tyrosinemia lack sTAT; therefore, these enzymes need to be measured separately during enzyme diagnosis.

Hereditary tyrosinemia type III does not have characteristic clinical manifestations.[1,3] For blood amino acids, tyrosine rises to 550–1100 μmol/L (10–20 mg/dL), and large amounts of 4-hydroxyphenylpyruvate and its oxides are detected in the urine. Liver enzymes can be measured for a definitive diagnosis. Hawkinsinuria, a mild form of type III, is diagnosed by detecting hawkinsin in the urine.

Treatment

When a patient has high tyrosine, it should be determined whether it is type I, type II, type III, or whether the tyrosinemia is due to some other cause, while simultaneously administering symptomatic treatment. In the absence of organ damage, newborns with high tyrosine are usually placed under observation. It is important to act quickly to prevent liver dysfunction from progressing in type I, for which the HPD inhibitor nitisinone (Orfandin®; 1 mg/kg/day) is used along with dietary therapy (formula without phenylalanine and tyrosine).[12] If nitisinone is effective, succinylacetone will decline to below the measurement sensitivity level, but tyrosine will rise. Thus, dietary therapy should aim to keep tyrosine at or below 500 μmol/L (9 mg/dL).[13] A formula without phenylalanine and tyrosine is available to help reduce the blood tyrosine level. Approximately 90% of patients are reported to respond to nitisinone if treatment is initiated early. The therapeutic effect is evaluated on liver function tests and by measuring serum α-fetoprotein. A positive prognosis can be expected if serum α-fetoprotein is maintained within the normal range (<10 ng/dL). Liver failure occurs in many patients who do not receive nitisinone, necessitating liver transplantation. Yet, in some cases, patients who are given nitisinone can nonetheless develop liver cancer or require a liver transplantation.

In type II, the aim of treatment is to reduce tyrosine in the blood, which will improve the skin and eye manifestations. To achieve this, patients are placed on a diet low in phenylalanine and tyrosine to maintain blood tyrosine <550 μmol/L (10 mg/dL). Formula without phenylalanine and tyrosine is recommended to reduce the blood tyrosine level. Similarly, type III patients are placed on a diet low in phenylalanine and tyrosine.

Complications and prognosis

In type I, prognosis is greatly affected by complicating liver cancer and hypophosphatemic rickets, which may result even in cases of early nitisinone treatment. Liver cancer was reported to occur within 10 years in 5% of children who began treatment at or before 2 years old.[13] Thus, regular imaging and other examinations should be performed during treatment so that liver cancer can be discovered early. Long-term survival can be expected for patients with type I if nitisinone treatment is effective. Types II and III have relatively good prognoses.

Conclusion

Although there are many causes of tyrosinemia, hereditary tyrosinemia can be diagnosed from clinical symptoms and biochemical analysis. Medical treatment requires nitisinone for type I, and formula without tyrosine and phenylalanine for types I, II and III. Early diagnosis is necessary for better prognosis.

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