

Wilson's disease

Wilsonova bolezen

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Izvleček

Wilsonova bolezen (WD) je genetsko pogojena, avtosomno recesivna motnja presnovek bakra. Gen ATP7B kodira nosilca bakra, ki skrbi za prenos bakra iz hepatocitov v žolč kot tudi za vgradnjo bakra v ceruloplazmin. WD se lahko pojavi v povezavi s katero koli vrsto bolezni jeter pri starosti v razponu od 4 do 12 let, ali pri nevrološki in psihiatrični simptomatiki v adolescenci. Manj pogosto je lahko začetni znak hemoliza in/ali fulminantna odpoved jeter. Diagnostično pomembni so nizka vrednost ceruloplazmina v plazmi, pozitivni penicilaminski test ter visoka vsebnost bakra v jetrih. Pri diagnosticiranju WD so v pomoč molekularne metode. Zgodaj odkrita bolezen se lahko zdravi s kelatorji in/ali cinkom in ima dobro prognozo. Pri fulminantni jetrni bolezni pa so brez presaditve rezultati slabi.

Abstract

Wilson's disease (WD) is a genetically determined, autosomal recessive disorder of copper metabolism. The gene ATP7B encodes a copper carrier that both transports copper from hepatocyte to bile and ceruloplasmin copper incorporation. WD may present with almost any variety of liver disease at an age ranging from 4–12 years, or with neurological and psychiatric symptoms in adolescence. Less commonly, haemolysis and/or fulminant hepatic failure may be an initial presentation. Low plasma ceruloplasmin, a positive penicillamine challenge test, and a high hepatic copper content suggest the diagnosis. Molecular methods help in diagnosing WD. If diagnosed early, it is treatable with chelators and/or zinc, and has a good prognosis. Fulminant hepatic disease has a poor outcome without transplantation.

Introduction

WD is increasingly recognized as an important cause of chronic liver disease in the adolescence. It is an important etiology of acute liver failure in school age-children as well. Normal serum ceruloplasmin does not preclude the diagnosis of WD at all. High liver copper content is essential in diagnosing WD. In a selected number of children with uneven liver parenchyma copper deposition and "false" borderline values of liver copper content, and in their relatives, genetic studies are highly necessary. It is of great importance to diagnose WD in pre-cirrhotic stage, thus providing the children with WD full recovery.

Epidemiology

The incidence rate is 1:100 000/year. The prevalence is 1:30 000 births. The heterozygote carrier state is approximately 1:90 persons. Initial manifestations before the age of 4 or after 35 years of life are an exception.^{1,2} Most patients develop the first clinical manifestations around the age of 15. Higher incidence is found in regions with strong consanguinity.^{1,2}

Patients present with a variety of clinical manifestations, including hepatic presentation common in childhood, and later-onset, predominantly neurological form.^{1,3} Forty to 60% of patients demonstrate a primary hepatic involvement during the first and second decades of life, the remainder later

on with a primarily neurological (34 %) or psychiatric (10 %) symptomatology and signs.^{1,3,4} Another important presenting feature may be haemolysis.

Genetics

WD gene designated as ATP7B is located on chromosome 13. Approximately 500 different mutations of ATP7B have been identified.⁵ Most of these are small deletions or missense mutations. Missense mutations are associated with a predominance of neurological symptoms and later presentation. Deletions are associated with earlier presentation predominated by liver disease.⁶ The most common mutation in Europe is H1069Q in approximately 40 % cases.^{6,7} H1069Q homozygotes are more likely to present at later age and with neurological rather than liver disease. Over a half of all mutations occur rarely in any population. Because of the wide variety of mutations, genetic techniques to establish the diagnosis of WD may have some limitations.⁷ Numerous mutations make mutation analysis as routine diagnostic test impractical, except in areas where one or two mutations predominate, such as Eastern Europe, Sardinia etc.⁷ The absence of any of these mutations does not exclude the diagnosis.

Pathogenesis

Mutations in ATP7B are responsible for impaired biliary copper excretion.^{8,9} Progressive hepatic copper accumulation is followed by subsequent deposition in other organs. At the end of the first decade of life, hepatic copper load is exceeded, causing release of free copper into the circulation that penetrates other tissues.⁶

The WD gene, ATP7B encodes a copper transporting ATPase, the Wilson's disease protein (WDP).⁸ WDP is found only in the liver, brain, and kidneys. WDP resides in the membrane of the trans-Golgi apparatus and is bifunctional. It transports copper for incorporation into ceruloplasmin, and traffics it to pericanalicular vesicles that sequester excess copper for export.⁹ Therefore, it is clear that mutations in ATP7B gene cause

decreased biliary excretion of copper and defective hepatocyte copper incorporation into ceruloplasmin.⁵

Ceruloplasmin is copper-containing enzyme, ferroxidase in nature. The low plasma ceruloplasmin seen in WD is not associated with a disturbance in iron metabolism. Ceruloplasmin is synthesized at first as a precursor, which is glycosylated to the apoceruloplasmin, into which copper is introduced.¹⁰

Apoceruloplasmin has a short half-life. Serum ceruloplasmin is low in WD because of decreased synthesis of holoceruloplasmin and rapid clearance of apoceruloplasmin. The rate of transcription of the ceruloplasmin gene is reduced in WD (translation or posttranslation defect). However, the ceruloplasmin gene in WD is normal.¹⁰

Ceruloplasmin has apparently little role in copper homeostasis. Approximately 5 %–17 % of WD patients have plasma ceruloplasmin above 0.2 g/L.¹¹ Although a useful biomarker for WD, the decreased ceruloplasmin synthesis is a result of, rather than the cause of the disturbance of copper metabolism in WD. It is not related to any of the manifestations of WD.

The lack of WDP as copper exporter in the liver cell explains the principal biochemical features of WD. Copper is toxic to hepatocytes. There is no clear relationship between liver copper concentrations and liver damage.¹² The presymptomatic toddler with WD but minimal histologic abnormality may have a higher liver copper than the child with acute liver failure.¹³ There are phenotypic differences between WD patients with similar liver copper concentrations.¹⁴ The possible explanation is that the most cellular copper is either safely bound to glutathione, metallothionein, copper-chaperones, WDP or incorporated into enzymes.^{12,14} This means that very little is available to cause free radical-mediated macromolecular damage.

Copper causes free radical-mediated oxidative DNA damage. Nuclear DNA lesions and large deletion of mitochondrial DNA are demonstrated.¹⁵

It is considered that in the brain copper is deposited mainly in the basal ganglia due to copper "overflow" from the liver. It seems

more likely that basal ganglia copper deposition results from defective action of WDP in neurons.

There is no correlation between the severity of hepatic and CNS damage. Kayser-Fleischer (KF) ring, which is caused by deposition of copper in the iris, represents simple overflow of copper from the liver. This is usually present in patients with neurological involvement, but not in younger children with Wilsonian liver disease only. There is no correlation between the presence/absence of KF ring and the degree of urine copper or serum ceruloplasmin.¹¹

Clinical features

The majority of patients presents at the age of 4–12 years with liver disease or, during adolescence or early adult life, with neurological or psychiatric manifestations. The two forms may overlap.

Hepatic forms – Hepatic presentations include acute hepatitis, acute hepatic failure, chronic active hepatitis, and cirrhosis.¹⁶

An acute hepatic illness may be the first presentation.^{14,17,18} Clinical signs mimic infectious hepatitis. If viral etiology is excluded, the ceruloplasmin should be measured in these cases. Associated haemolysis is highly suggestive of WD.

Wilsonian acute liver failure may appear identical to that encountered in acute fulminant viral hepatitis or following ingestion of hepatotoxins.^{17,18} It is characterized with progressive jaundice, ascites, hepatic and renal failure. Indicators of WD are KF ring, family history of WD, neurological features of WD, jaundice, haemolysis, high bilirubin, relatively low transaminases, and low alkaline phosphatase.¹⁰ Without transplantation survival is unlikely.

In children with WD, a more insidious onset of liver disease mimicking autoimmune hepatitis may occur.¹⁹ In terms of clinical and laboratory examinations, it can be only distinguished by the way of etiology-specific findings. The presence of a low titer of autoantibodies may cause confusion. In these cases liver histology may also show features seen in autoimmune hepatitis, including interface hepatitis.¹⁹ The presence of steatohe-

patitis in particular should trigger the search for WD.

The patient may present with insidiously developing cirrhosis, typically well compensated. Clinical features include vascular spiders, splenomegaly, ascites and portal hypertension.⁵ Obvious neurological dysfunction, psychiatric symptoms, or a family history of WD should raise suspicion of WD.

Cholelithiasis is relatively uncommon in WD and results from haemolysis in the presence of cirrhosis.

In patients with WD abdominal pain is an indication for ultrasound examination.

Neurological disease – It is important to suspect WD in adolescents presenting with deteriorating school performance, psychiatric abnormalities, or neurological features.¹⁰ The most common neurological manifestations are dystonia and parkinsonism. Cognitive ability initially remains intact. The neurological manifestations are easily misdiagnosed as deteriorating performance at school, worsening handwriting, and behavioral problems attributed to adolescence.¹⁰ Among children with a neurological presentation common findings are hepatosplenomegaly, abnormal liver function tests, and histological liver changes. KF ring is usually present. Neurological abnormalities may emerge after starting treatment with penicillamine.¹⁰

EYES – Copper is deposited in the form of copper-sulphur complex in Descemet's membrane on the back of cornea. This causes the development of brown-green ring around the periphery of the cornea (KF ring). KF ring may not be detectable in patients with hepatic presentation.

Sunflower cataracts are another ocular manifestation of excess copper deposition.

Other clinical features – Renal tubular abnormalities found in WD include glycosuria, aminoaciduria, renal tubular acidosis, impaired phosphate reabsorption, or full-blown renal Fanconi syndrome. These are the consequences of renal tubular copper deposition. Renal tubular acidosis may manifest with the development of nephrocalcinosis and renal stones. Glomerular function is not compromised. Proteinuria may be exacerbated by penicillamine.

Skeletal manifestations include copper-mediated oxidative damage to collagen, causing arthritis that occurs rarely in WD. Rickets and osteoporosis are secondary effect of renal tubular phosphate leak associated with hypercalciuria and hepatic osteodystrophy.

Diagnosis

Detection of an acute or chronic liver disease of unclear aetiology, in particular fatty liver, and/or haemolysis, and/or neurological or psychological peculiarities in children, suggests the suspected presence of WD. Therefore it is essential that the possibility of WD is considered in the first place!

The diagnosis is based on three laboratory tests: low serum ceruloplasmin ($< 0.2 \text{ g/L}$), increased urinary copper above $25 \text{ } \mu\text{mol}/24\text{h}$ following penicillamine challenge test, and high liver copper levels ($> 250 \text{ } \mu\text{g/g}$ dry weight).²

If the serum ceruloplasmin is higher than 0.2 g/L and lower than 0.3 g/L the diagnosis of WD is not excluded if clinical circumstances are consistent with it.¹¹ Up to 17 % of patients have serum ceruloplasmin level in this range.¹¹ Ferenci group from Vienna reported that 40 % of patients with Wilsonian liver disease had normal ceruloplasmin, some exceeding 0.3 g/L .¹¹ A significant number of WD heterozygotes have a serum ceruloplasmin below 0.2 g/L .

An elevated 24h-urine copper excretion above $1.5 \text{ } \mu\text{mol}$ and an at least five-fold increase during a penicillamine challenge (in particular exceeding $25 \text{ } \mu\text{mol}/24\text{h}$) indicates WD.²⁰ To improve the diagnostic accuracy of urinary copper excretion, the measurement during the second and third day of penicillamine treatment, may demonstrate further increase in copper excretion, which further supports the diagnosis of WD.²¹ It should be noted that this test may show similar results in cholestatic liver diseases.

The diagnosis of WD is confirmed by the determination of liver copper content (normal $< 50 \text{ } \mu\text{g/g}$ dry weight).²² The inhomogeneous distribution of copper in the liver, particularly in cases of cirrhosis, has to be

taken into account. Concentrations greater than $250 \text{ } \mu\text{g/g}$ are diagnostic for WD.²² Recent observations indicate that lower values, $70\text{--}100 \text{ } \mu\text{g/g}$ may occur in WD as well. Elevated values are also found with long-standing cholestasis.

The earliest liver histologic changes comprise microvesicular and macrovesicular fatty deposition.¹²

Peroxisomes are dense and enlarged. Mallory's hyaline may be present in hepatocyte cytoplasm. As disease progresses, portal fibrosis and inflammation develop.^{1,12} Copper storage is demonstrated with rhodanine, rubeanic acid and orcein staining. However, despite elevated hepatic copper, these stains are frequently negative.¹ This is because copper is not present in the hepatocytes of regenerating nodules that have no time to accumulate copper; copper has been released from hepatocyte injury or cytosolic copper is difficult to identify by histochemistry. Cirrhosis develops in untreated patients and is of either macronodular or micro/macronodular pattern.¹

The presence of KF ring in a patient with hepatic or neurological disease establishes the diagnosis of WD.

Genetic testing strategy has been developed for the population served. The H1069Q is the most common mutation in patients from central, eastern, and northern Europe.²³ Therefore the direct testing for most locally common mutations is rapid and supports or allows primary diagnosis. By direct sequencing of exons coding region of ATP7B gene, we can detect disease causing mutation.

Treatment

Early diagnosis is best, and treatment is life-long. At present three drugs are commonly available to treat WD: D-penicillamine, trientine and zinc. Ammonium tetrathiomolibdate may have a role in neurological cases refractory to other therapies.

Penicillamine – This drug chelates copper and increases urinary copper excretion. Beside decoppering it is considered that the drug acts by inducing methallothionein

complexing with copper.^{5,24} Thus unbound copper is not available for injury.

Penicillamine treatment is started with 20mg/kg/day by mouth in divided doses taken 1 hour before meal. All treated patients should also receive 50 mg vitamin B6 daily. Of patients with neurological disease, about 25 % experience neurological disease worsening which, however, ultimately results in an improvement.^{5,25}

Success during the initial period of penicillamine therapy is judged by improvement of hepatic synthetic functions. Improvement is marked by disappearance of KF ring as well. Evidence of significant decoppering is estimated by measuring 24-h urinary copper excretion. This will rise in the first 3 months, and decline after 1 year of continued treatment. After this time, urine copper should be measured 6-monthly. If the expected improvement occurs, the dose of D-penicillamine has to be reduced to 10 mg/kg/day (up to 1 g/day).

Side-effects are observed in 20 %–25 % of cases. These are: hypersensitivity reactions with fever and rash, leucopenia, thrombocytopenia, aplastic anemia, lymphadenopathy, dermatopathy etc.¹² Therefore, white-cell and platelet counts, and urinalysis should be checked regularly. D-penicillamine may also cause proteinuria and SLE-like syndrome. In an event of any serious adverse effect, it has to be discontinued and alternative trientine therapy should be started.

Trientine – This drug was initially introduced as a second-line drug for those intolerant of penicillamine.²⁶ Trientine has few side effects.²⁶ A common side effect is iron deficient anemia.

Nephrotoxicity, skin and mucosal lesions are reported occasionally. The dose used is 20 mg/kg/day. Patients are monitored as they are on penicillamine therapy.

Zinc therapy – Treatment with zinc is considered to be an alternative therapy for mobilizing copperdeposits. Zinc causes inhibition of intestinal copper resorption and stimulates the synthesis of methallothionein in the liver and intestinal mucosa.²⁷ It is of low toxicity. The dosage of Zn-acetate in children is: < 5 years 25 mg twice daily, 6–15 years 25 mg three times daily, > 16 years 50 mg three times daily. Side-effects in the form of gastric irritation and unpalatability have been reported.

There are encouraging reports of combination therapy with chelator plus zinc in decompensated hepatic disease or a severe neurological form of WD.⁵ The chelator dose is alternated with the zinc dose, usually with 5–6-hour interval between the administration of either drug. If the treatment gives results, monotherapy (chelator or zinc) can be instituted after 3 months.¹⁶

A low-copper diet has to be advised. Copper-containing foods (liver, shellfish, nuts, chocolate, mushrooms) should be avoided.

A child with acute liver failure and encephalopathy due to WD should be referred for transplantation.²⁸ Albumin dialysis and MARS may be a bridge to liver transplantation. Children with decompensated cirrhosis unresponsive to medical therapy also have to undergo transplantation.

King's group from London developed an index based on serum bilirubin, INR, AST, and white cell count at presentation.¹³ A score greater than 11 of possible 20 was predictive of death. No patients with score less than 11 died. This index is sensitive and specific in predicting mortality without transplantation in children with WD.

In unaffected siblings, it is possible to diagnose WD with genetic study. Zinc treatment may begin after the age of 3 years. This will prevent hepatic and neurological disease.

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