

Diagnosis and treatment of Wilson's disease

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Abstract: Wilson's disease (WD) has moved on from being a recognized syndrome that was uniformly fatal to a curative disease for which the genetic basis has been discovered. Most pediatric patients present with hepatic manifestations, but some may have neurologic or psychiatric features. Clinical and biochemical screening, including liver biopsy for hepatic copper analysis, remain the standard for diagnosis, but haplotype analysis for siblings is now available and should be considered for family screening when possible. Lifelong medical therapy remains the mainstay of treatment, but treatment preferences are changing from penicillamine to alternative agents such as trientine and zinc. OLT remains lifesaving for those with fulminant WD and those in whom initial medical therapy fails. The future will probably see the application of rapid and accurate molecular diagnostic testing for this disorder and new therapeutic modalities such as hepatocyte transplantation, gene replacement therapy, and gene modification.

Michael L. Schilsky

Division of Liver Diseases and Recanati/Miller
Transplantation Institute, The Mount Sinai Medical
Center, New York, New York, USA

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Michael L. Schilsky, MD, Associate Professor of
Medicine Division of Liver Diseases and Recanati/
Miller Transplantation Institute, The Mount Sinai
Medical Center, One Gustave L. Levy Place, Box
1633, New York, NY 10029, USA
Tel.: 212-241-1424
Fax: 212-996-5149
E-mail: michael.schilsky@mssm.edu

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Copper is an essential trace element that acts as a cofactor for a variety of enzymatic reactions by virtue of its ability to accept and donate electrons under physiologic conditions. A deficiency of copper results in the reduction of activity of many of the copper-dependent cellular enzymes, while its excess may result in cellular toxicity. Approximately 50% of ingested copper (≈ 1.5 – 3 mg/day) is absorbed in the upper small intestine. The absorbed copper is mainly transported in the circulation as copper–albumin. This copper is extracted avidly by hepatocytes where it is utilized for cellular metabolism, exported back into the circulation in the form of copper-containing ceruloplasmin, or excreted into bile. Biliary excretion represents the main pathway for elimination of excess copper as biliary copper does not undergo significant enterohepatic recirculation. Approximately 90% of the serum copper is bound to ceruloplasmin in adults. Circulating levels of ceruloplasmin, measured as the oxidase activity of the protein toward specific substrates, are low in newborns and rise to normal levels (20–40 mg/dL)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAL, British Anti-Lewisite; KF, Kayser-Fleischer; OLT, orthotopic liver transplantation; WD, Wilson's disease.

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within months of birth. Newborn livers contain an elevated content of copper, which returns to normal adult levels (<40 $\mu\text{g/g}$ dry wt) over time. The normal adult liver contains $\approx 10\%$ of the 100–150 mg total body content of copper. A review of hepatic copper metabolism, as it relates to WD, is found in refs (1) and (2).

WD is an autosomal recessive disorder in which copper pathologically accumulates primarily within the liver and subsequently in the neurologic system and other tissues. Unless specific treatment is instituted, copper accumulation is progressive and ultimately fatal. The reduced biliary excretion of copper, essential for eliminating excess copper in normal individuals, is the basis for the accumulation of copper within hepatocytes of patients with WD. The timely and appropriate utilization of the current modes of treatment offer patients excellent long-term survival for this once fatal illness. Transplantation offers a cure for the underlying metabolic defect.

The prevalence of WD in almost all populations is $\approx 1 : 30,000$ individuals, with exceptions found in specific subgroups with consanguinity. The gene for WD, *ATP7B*, is located on chromosome 13. The gene product is a copper transporter that is similar to other ATP-dependent transporters for heavy metals and highly homologous to the gene for Menkes' disease (see Fig. 1) (3). There are multiple mutations of the gene which give rise to WD, and most patients have two different muta-

tions of the gene on each allele encoding the WD gene (compound heterozygotes).

Diagnosis

WD may be detected in the asymptomatic stage in siblings or offspring of patients with WD, or when the disease is considered for evaluation of abnormal biochemical parameters prior to the onset of signs or symptoms. Symptomatic presentations of WD include liver disease, which may manifest itself as hepatic insufficiency, chronic active hepatitis, or as a fulminant hepatitis associated with hemolysis. WD may also present with extra hepatic manifestations in the form of neurologic or psychiatric disease.

The diagnosis of WD may be established by a combination of clinical, biochemical, and histochemical evaluations [see refs (1) and (4), and Table 1]. Patients with WD may have the characteristic corneal copper deposition within Descemet's membrane, known as KF rings, which are visible by slit lamp examination. KF rings may be absent early on in the disorder. About 50% of patients with liver disease lack KF rings (5); however, these are almost invariably present when neurologic or psychiatric manifestations of WD are observed.

Circulating levels of the blue copper-protein ceruloplasmin are reduced in nearly 95% of patients with WD, to <20 mg/dL. This characteristic reduction in ceruloplasmin may be reliably detected only in patients older than 1 yr of age as the levels of this protein are low at birth and do not reach adult levels until late in the first year of life. About 20% of individuals heterozygous for WD have reduced circulating levels of this protein. Levels of this protein in the circulation may also be decreased in patients with severe copper deficiency, in patients with severe fulminant hepatitis with hepatic insufficiency, in patients with significant protein-losing nephropathy or enteropathy, and in patients with hereditary hypoceruloplasminemia or aceruloplasminemia. Ceruloplasmin is an acute-phase reactant and may be elevated in inflammatory states. Levels of ceruloplasmin increase in response to exogenous administration of estrogens and during pregnancy, and in some individuals with WD these stimuli can increase levels above the lower limits of normal.

Urinary copper excretion is elevated in symptomatic patients to levels >100 $\mu\text{g}/24$ h, and may provide supporting evidence for the diagnosis of WD. Other causes of increased urinary copper include concurrent administration of metal chelators (penicillamine, BAL, Trientine, or tetrathiol-

molybdate), the presence of acute liver failure, and cirrhosis with cholestasis or nephrotic syndromes. The dramatic increase of urinary copper content to >1500 μg of Cu/24 h following administration of the chelating agent penicillamine to untreated patients with WD may be utilized as an adjunctive test to help establish the diagnosis of WD (6).

The presence of KF rings and a low level of ceruloplasmin is sufficient to diagnose WD. However, as KF rings are often absent in younger patients, the liver biopsy and copper quantitation play an important role in the diagnosis of WD.

Liver biopsy with histologic evaluation and copper quantitation play an important role in the diagnosis of WD. Fatty changes are the most common finding in the hepatocytes of asymptomatic patients. Nuclei may appear vacuolated and can contain invaginations of cytoplasm. Steatosis may progress and be followed by fibrosis and ultimately progress to cirrhosis by passage through a clinically fulminant hepatitis or chronic active hepatitis.

The presence of copper staining in histologic sections by rhodanine, rubeanic acid or by other means provides supportive evidence for the diagnosis of WD. In the early stages of WD, when hepatic copper contents are maximal, it is difficult to detect copper histochemically, and only very sensitive stains may reveal its diffuse distribution in the cytoplasm. Later on, copper is present within hepatic lysosomes and may be detected by the less sensitive rhodanine or rubeanic acid stains, as well as by orcein staining of lysosomal copper-protein. Characteristic of WD at this later stage is the presence of copper staining of cells throughout a liver nodule while being absent from others. This distinguishes WD from other cholestatic disorders, such as advanced PBC in which copper may accumulate in the periphery of most lobules.

Ultrastructural examination of liver tissue is not routine but may help adjunctively in distinguishing heterozygotes from homozygotes. Large pleomorphic mitochondria with increased matrix density and widened inter-cristal spaces are unique to WD (7). These mitochondrial abnormalities are only present in the early stages of fatty infiltration of the liver and disappear spontaneously with the progression of the pathologic process toward cirrhosis or with treatment specific for WD.

Determination of hepatic copper content remains the standard for diagnosing WD, and that of patients with WD typically exceeds 250 $\mu\text{g}/\text{g}$ dry weight. Normal hepatic copper content is less than 40 $\mu\text{g}/\text{g}$ dry weight, and some intermediate

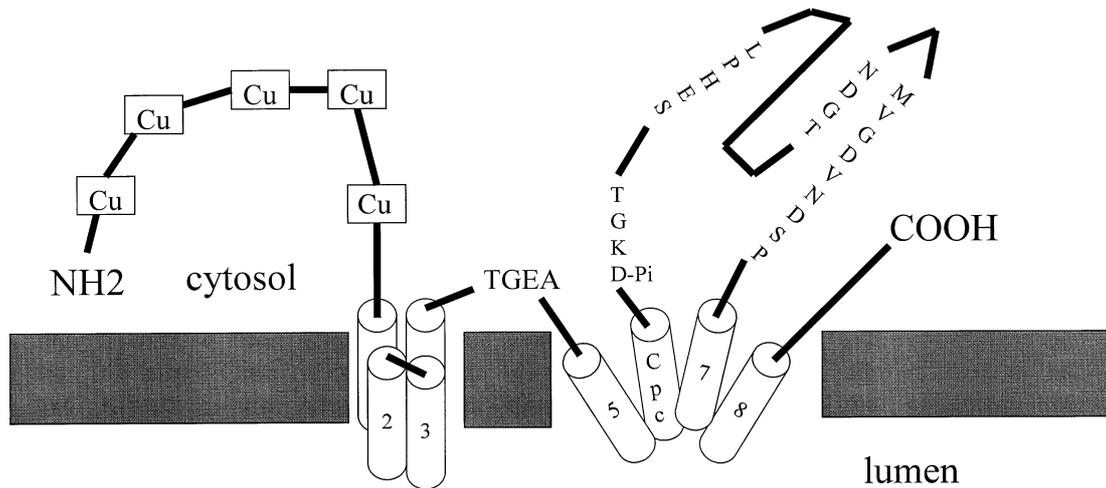


Fig. 1. The ATP7B protein includes general features of P-type ATPases: phosphatase domain (TGEA), phosphorylation domain (DKTGT), ATP binding domain (TGDN) and hinge region (MVG DGVNDSP) that connects ATP binding domain to the transmembrane segment. Specific to metal-transporting ATPases includes copper-binding domains (Cu), transmembrane CPC and cation-translocation region (SEHPL). This diagram is based on the figure of Terada *et al.* (3).

values are seen in heterozygotes. Patients with long-standing cholestasis and those with idiopathic copper toxicosis may also have elevated hepatic copper above 250 µg/g dry weight, but clinical and histologic parameters help differentiate between these disorders.

Radiographic imaging studies of the brain (magnetic resonance imaging or computerized tomography) are often performed because the presence of abnormal neurologic signs may suggest WD. Most commonly, alterations in

basal ganglia are noted; however, atrophy, subcortical white matter, midbrain, and pons, may be seen in most (but not all) symptomatic patients (8).

The genetic localization of the gene for WD to a specific region of chromosome 13 has permitted molecular identification of the disease by haplotype analysis or genotyping around the disease locus. Such analysis is limited to the screening of families of affected individuals, as DNA polymorphisms are too varied amongst

Table 1. Diagnostic testing for Wilson's disease

| Test | Wilson's disease | Comment |
|--|--|---|
| Slit lamp examination | Kayser-Fleischer rings | Often absent early in the disease |
| Serum ceruloplasmin | <20 mg/dL | Decreased in newborns, 20% of heterozygous carriers, and 95% of patients |
| Serum copper | <100 µg/dL | Normally decreased in proportion to serum ceruloplasmin, markedly elevated in Wilsonian fulminant hepatitis |
| 24-h urinary copper | >100 µg | Normal < 50 µg/dL, increased in most symptomatic patients and following treatment with chelators |
| Hepatic copper concentration | >250 µg/g dry weight | May be increased in other cholestatic disorders and idiopathic copper toxicosis |
| Hepatic histology | Abnormal | Steatosis, glycogen nuclei, fibrosis, chronic active hepatitis, cirrhosis |
| Hepatic ultrastructure | Abnormal | Stage-specific mitochondrial and lysosomal abnormalities |
| Rhodanine histochemistry | Positive | Present in some, but not all, nodules; may be absent |
| MRI or CT brain scan | Abnormal | Atrophy, alterations in basal ganglia, subcortical white matter, midbrain, pons, in most but not all patients with neuro/psychiatric symptoms; abnormalities can be present in some asymptomatic patients |
| Genetic testing: Haplotype analysis for siblings | Haplotype: same as proband | Haplotype: requires identification of proband |
| Mutation analysis for select populations | Mutation: known disease-specific mutation on each allele | Mutation: useful in select populations with dominant mutations |

CT, computerized tomography; MRI, magnetic resonance imaging.

the general population to allow comparison of non-related individuals. This testing is now commercially available.

Direct mutational analysis for index cases remains difficult as there are numerous mutations (now over 200) that are scattered across the coding region for the gene (see the listing of *ATP7B* mutations at <http://www.medgen.med.ualberta.ca/database.html>). There is a single dominant mutation (H1069Q) that is present in many patients of Slavic background, but in only up to one-third of individuals in North America (reviewed in ref. 9). The majority of known mutations do occur in a limited number of exons, and screening can be undertaken for these as an initial attempt to identify patients; however, this analysis is not generally available. Failure to identify the mutation of both alleles remains a significant problem, and even screening of the remaining exons may still fail to reveal intronic mutations and mutations in the precoding region. Advances in methodology in DNA sequencing that permit high-throughput screening of amplified regions for mutations should soon permit the *de novo* identification of suspected patients.

Acute fulminant presentation of WD

The presentation of acute fulminant hepatitis caused by WD deserves special mention. The recognition of this entity is important so that the severity of the disease can be properly assessed. These individuals, most often female (by a ratio of almost 2 : 1) are typically in their second decade of life, and present acutely with signs of jaundice, ascites that progresses to encephalopathy, and liver failure. Key signs that aid in the recognition of WD as the etiology include the presence of a Coombs-negative hemolytic anemia (which also contributes to the hyper-bilirubinemia), markedly elevated serum and urine copper contents, low serum ceruloplasmin, elevated INR, and KF rings. KF rings may only be present in $\approx 50\%$ of these individuals (10). Elevations of ALT and AST are typically much lower than those seen for other causes of acute liver failure, and specifically the alkaline phosphatase is disproportionately low (leading to the use of the alkaline phosphatase : bilirubin ratio of <2.0 as an indicator for this entity). Renal insufficiency is frequently present but is reversible.

Treatment

Pharmacological treatment of WD aims to prevent the accumulation of copper or reverse the

toxic effects of this metal by reducing copper absorption, inducing synthesis of endogenous cellular proteins such as metallothionein (which is capable of sequestering copper in a non-toxic manner within cells), by promoting the excretion of copper in the urine or bile, or by a combination of these. Pharmacological agents that function to remove copper (chelating agents) include D-penicillamine, trientine, and BAL and tetrathiomolybdate. Zinc stimulates enterocyte metallothionein and blocks absorption of copper from the diet. Zinc therapy is the safest and easiest regimen for long-term maintenance and the experience with its use in pediatric patients was recently reported (11). Trientine is a good choice for initial treatment. The relative roles of these agents in the treatment of WD was recently reviewed (12). Medical therapy is effective for treating this disorder, and most patients with abnormalities in liver function demonstrate improvement within 6–12 months of treatment. Therapy must be maintained for the life of the patient.

Combinations of therapy (chelator and zinc) may be useful in treating symptomatic patients with liver or neurologic WD. We have managed 11 patients with the combination of trientine and zinc spaced temporally, including several with severe decompensated liver disease that were initially listed for OLT, having Child-Pugh Turcotte scores of >10 (13). While none of these had acute fulminant hepatic failure and had Nazer prognostic scores of <7 (14), they demonstrated significant improvement biochemically and clinically. With the exception of two patients who had recurrent variceal bleeding, the first undergoing OLT and the other a splenorenal shunt, the nine others thus far have avoided transplant or surgery and remain well on maintenance therapy with zinc.

Indications and outcome for liver transplantation for WD

OLT has been shown to correct the WD phenotype and provide excellent long-term survival. Results of a series of 21 patients, transplanted at our center for WD from 1989 to 2000, were reviewed (12). Of these, 11 were transplanted for fulminant hepatitis and six for severe hepatic insufficiency, despite therapy, many of whom had been non-compliant with prior treatment. Survival at 1 yr was 87.5%, and those who survived had excellent long-term survival. Renal insufficiency is a common occurrence in many of these patients as a result of tubular injury from copper as well as part of the multi-organ failure that can

occur during acute liver failure; however, renal function uniformly recovered with time in these individuals. This study reconfirmed that indications for transplant for WD include individuals with acute fulminant hepatic failure, those with progressive hepatic insufficiency, despite therapy, and patients who suffer complications of portal hypertension (such as recurrent bleeding) as a result of cirrhosis. Neurologic symptoms, if present prior to OLT, may improve following transplant. OLT is not recommended for those with neurologic symptoms alone, as improvement is possible with medical treatment.

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