

## WILSON'S DISEASE

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Wilson's disease is an autosomal recessive inherited disorder of copper metabolism resulting in accumulation of copper in various tissues. The hallmarks of the disease are the presence of liver disease, neurologic symptoms, and Kayser-Fleischer corneal rings. Until very recently, Wilson's disease was believed to be very rare. By a population-based approach, the incidence of Wilson's disease was estimated to be approximately 1:30,000 to 50,000 (Ireland: 17/106 live births<sup>34</sup>; former East-Germany: 29<sup>1</sup>) with a gene frequency of 1:90 to 1:150; however, these estimations were mostly based on adolescents or adults presenting with neurologic symptoms. More recent data, however, indicate that neurologic symptoms occur only in about half of patients with Wilson's disease. Thus, the incidence of Wilson's disease was underestimated by these studies.

### **PATHOGENESIS**

The basic defect in Wilson's disease is the impaired biliary excretion of copper resulting in the accumulation of copper in various organs, including the liver, the cornea, and the brain. Excess copper in tissues leads to the production of free radicals and to DNA cleavage. Probably the greatest source of damage is through the production of free radicals. Copper overload particularly affects mitochondrial respiration and causes a decrease in cytochrome C activity.<sup>43</sup> Damage to mitochondria is an early pathologic effect in the liver. Hepatocellular damage due to increased lipid peroxidation and abnormal mitochondrial respiration was shown both in copper-loaded dogs and in patients with Wilson's disease.<sup>44</sup> The mechanisms triggering copper-induced lipid peroxidation are unknown, but it is conceivable that hepatic copper accumulation renders patients with Wilson's disease susceptible to any oxidative stress. Copper may be directly toxic to neurons or may

exert its effects by selective inhibition of brain monoamine oxidase A (MAO-A).<sup>24</sup>

### **The Wilson's Disease Gene**

The Wilson's disease gene was identified on chromosome 13 by three independent groups in 1993.<sup>19, 32, 33, 50</sup> It encodes ATP7B, a copper-transporting P-type ATPase. Its functionally important regions are six copper-binding domains, a transduction domain (amino acid residues 837-864) involved in the transduction of the energy of ATP hydrolysis to cation transport, a cation channel and phosphorylation domain (amino acid residues 971-1035; with the highly conserved Asp-Lys-Thr-Gly-Thr motif), an ATP-binding domain (amino acid residues 1240-1291), and eight hydrophobic regions predicted to span the cell membrane.

More than 60 mutations<sup>50</sup> occurring throughout the whole gene have been documented so far, including missense and nonsense mutations, deletions, and insertions. Some are associated with severe liver disease very early in life, whereas other mutations appear to be associated with an attenuated presentation in mid-adulthood. Although most reported mutations occur in single families, a few are more common. For example, the His1069Gln missense mutation is found in 30% to 60% of patients of Eastern-, Northern-, and Central-European origin; it is less frequent in patients of Mediterranean<sup>16</sup> or non-European origin. About 10% of patients of French or British stock have a Gly1266Lys mutation in the ATP hinge domain. The 2299insC mutation can be detected in some patients of European and Japanese descent. The Arg778Leu mutation is present in 27% of Taiwanese patients but is not found in non-Asian patients.<sup>9</sup> In Sardinia, two frameshift mutations (1515insT, 2464delC) are found in about 20% of patients; these mutations have not been found in other populations. The study of genotype-phenotype correlations has

been hampered by the lack of clinical data, the rarity of some mutations, and the high frequency of the presence of two different mutations of the Wilson's disease gene in individual patients (compound heterozygotes). Sufficient information is available only for the His1069Gln mutation. This mutation is associated with late-onset neurologic disease with a female preponderance (female to male ratio, 3: 1).<sup>26</sup>

### **Hepatic Copper Metabolism and the Role of ATP7B**

Copper is an essential component of important enzymes, such as lysyl oxidase, superoxide dismutase, cytochrome C, tyrosinase, and DOPA-P-mono oxygenase. Dietary copper intake (about 1-4 mg/day) far exceeds the trace amounts required. Dietary copper is absorbed in the upper intestine and binds loosely to albumin, certain amino acids (histidine, cysteine, threonine), and peptides. Most ingested copper is taken up by the liver. The hepatic uptake of diet-derived copper appears to be an insaturable, carrier-mediated, energy-independent mechanism. Cellular copper transport processes are required by all organisms for correct use in cell biochemical processes and avoidance of the toxicity of copper excess." In the hepatocyte, glutathione (GSH) has an affinity to Cu<sup>+</sup>, and stable Cu<sup>+</sup>-GSH complexes are formed. Cu<sup>+</sup>-GSH complexes function in the intracellular copper transport and are effective copper donors to metallothionein and superoxide dismutase. Metallothionein is a cytosolic, low-molecular weight, cysteine-rich, metal-binding protein. The copper stored in metallothionein can be donated to other proteins, either following degradation in lysosomes or by exchange via GSH complexation. Copper is incorporated into apoceruloplasmin, possibly at the level of the Golgi compartment. Cu<sup>+</sup>-GSH is the only complex able to reconstitute ceruloplasmin at neutral pH. Thus, glutathione may function to shuttle the metal from the membrane copper pump (ATPase7B) and ceruloplasmin in the secretory compartments of the cell.<sup>30</sup> Ceruloplasmin contains six tightly bound copper atoms and its primary function is to carry copper to various tissues and to act as ferroxidase, converting Fe<sup>++</sup> to Fe<sup>+++</sup>.<sup>20,21</sup> Because hepatic uptake of dietary copper is not saturable, hepatic copper accumulation can easily be induced. Toxicity of copper, however, depends on its molecular association and subcellular

localization rather than on its concentration in the liver. Metallothionein-bound copper is nontoxic. Several metals, including zinc, can induce metallothionein synthesis.

Excess copper is secreted into the bile. There are at least three pathways for hepatobiliary excretion of copper<sup>14</sup>: lysosomal exocytosis, a GSH-dependent route at the canalicular membrane (probably by the mixed organic acid transporter c-MOAT), and secretion by ATP7B. The localization of ATP7B within the hepatocyte and its precise role in hepatic copper transport is unknown at present. Biochemically, an ATP-dependent copper carrier was identified at the canalicular membrane (canalicular copper transporter, c-COP). By immunohistochemistry using antibodies against ATP7B, this enzyme was localized in vesicular structures adjacent to the canalicular membrane.<sup>36</sup>

### **CLINICAL PRESENTATIONS**

Wilson's disease may present with a variety of clinical manifestations, the most common being liver disease and neuropsychiatric disturbances. None of the clinical signs are specific or diagnostic. One of the features of Wilson's disease is that no two patients, even within a family, are ever quite alike. With increased awareness for Wilson's disease, patients are generally diagnosed earlier; thus, late consequences of the disease, such as Kayser-Fleischer rings or severe neurologic symptoms, are less frequently seen. Early symptoms, if present at all, are uncharacteristic and nonspecific. Uncommon manifestations of Wilson's disease include hypercalciuria and nephrocalcinosis, chondrocalcinosis and osteoarthritis, and cardiac manifestations.

#### **Kayser-Fleischer Rings**

Characteristically, the ring starts as a small crescent of golden-brown granular pigment at the top of the limbus followed by the appearance of a lower crescent, and these two crescents meet laterally to form complete rings. The ring is not always detected by clinical inspection, and the cornea should be examined under a slit lamp by an experienced ophthalmologist. Kayser-Fleischer rings are present in 95% of patients with neurologic symptoms, in 50% to 60%, of patients without neurologic symptoms, and only in 10% of asymptomatic siblings.

## Liver Disease

Most patients with Wilson's disease have some degree of liver disease (Table 1). The most typical age hepatic manifestations occur is between 8 and 18 years old, but cirrhosis may already be present in children below the age of 5 or may only become symptomatic in patients over 60 years of age.<sup>3</sup> Chronic liver disease may precede manifestation of neurologic symptoms for many years.

### *Acute Wilsonian Hepatitis and Fulminant Wilson's Disease*

Acute Wilsonian hepatitis is indistinguishable from other forms of acute (viral or toxic) liver diseases. It should be suspected in young patients with acute non-A-E hepatitis. The initial episode may be self-limited and resolve without treatment, but may rapidly progress and resemble fulminant hepatic failure. Hemolysis induced by copper released from necrotic hepatocytes may complicate acute liver disease. Although fulminant and subfulminant liver failure due to Wilson's disease has several distinctive features, rapid diagnosis may be very difficult. The combination of anemia, marked jaundice, and relatively low aminotransferase activities in young patients should always raise the suspicion of acute Wilson's disease.<sup>4, 28</sup> Kayser-Fleischer corneal rings and a history of neurologic abnormalities are absent in most patients presenting with acute liver disease. Diagnostically the "gold standard" for diagnosis is the quantification of hepatic copper content in biopsy material or in the explanted liver. One puzzling feature of fulminant Wilson's disease is the preponderance of female sex (female:male ratio, 5:1).

Table 1. LIVER BIOPSY FINDINGS IN 64 PATIENTS WITH WILSON'S DISEASE

Liver Histology	Hepatic Presentation	Neurologic Presentation	Siblings	Total
Cirrhosis	19	7	1	27
Fibrosis	3	3	2	8
Chronic hepatitis, active	6	3	2	11
Chronic hepatitis, mild	1	6	3	10
Steatosis	--	2	1	3
Minimal changes	1	3	1	5
Total	30	24	10	64

All biopsies were obtained before initiation of therapy (Ferenci P, unpublished data).

## *Chronic Hepatitis Due to Wilson's Disease*

Wilson disease may present, particularly in young patients, with a syndrome indistinguishable from chronic active hepatitis of any cause.<sup>41</sup> Symptoms include malaise, fatigue, anorexia, and vague abdominal complaints. Arthralgias, amenorrhea, delayed puberty, and low-grade jaundice may be present. Frequently, Kayser-Fleischer rings are absent and plasma ceruloplasmin is in the normal range. Liver biopsy typically shows severe chronic active hepatitis, but diagnosis is missed if hepatic copper content is not measured. Without treatment, patients can deteriorate with ascites, edema, and jaundice within few months, and may eventually die of liver failure.

### Neuropsychiatric Presentation

Neurologic symptoms usually develop in the mid-teenage years or in the 20s,<sup>11</sup> but may occur in patients older than 45 years old. The initial symptoms, such as mild tremor and speech and writing problems, are subtle and frequently misdiagnosed as behavioral problems associated with puberty. The symptoms may remain constant or progress steadily. The hallmark of neurologic Wilson's disease is a progressive movement disorder with dysarthria, dysphagia, apraxia, and a tremor-rigidity syndrome. Because of increasing difficulty in controlling movement, patients may become bedridden and unable to care for themselves. Ultimately, the patient becomes helpless—usually alert, but unable to talk. In patients presenting with advanced liver disease, neurologic symptoms are often mistaken for signs of hepatic encephalopathy.

About one third of patients initially present with psychiatric abnormalities. Symptoms can include reduced performance in school or at work, depression, emotional lability, sexual exhibitionism, and frank psychosis. Frequently, adolescents with problems in school or work are referred for psychological counseling and psychotherapy. Among the author's patients, two were hospitalized in psychiatric institutions for psychosis (one having attempted suicide several times) and two for severe alcohol abuse before the diagnosis of Wilson's disease was made. The delay in diagnosis in one case was 12 years.

## DIAGNOSIS

The diagnosis of Wilson's disease is based on clinical findings and laboratory abnormalities (Table 2), and can be made if two of the following symptoms are present<sup>46</sup>: Kayser-Fleischer rings, typical neurologic symptoms, or low serum ceruloplasmin levels.

Table 2. ROUTINE TESTS FOR DIAGNOSIS OF WILSON'S DISEASE

Test	Typical Findings in Wilson's Disease	False-Negative	False-Positive
Serum ceruloplasmin	Decreased	Normal levels in patients with marked hepatic inflammation; overestimation by immunologic assay	Low levels in malabsorption, aceruloplasminemia, liver insufficiency, heterozygotes
24-hour urinary copper	> 100 µg/d	Normal Incorrect collection Children without liver disease	Increased hepatocellular necrosis, contamination
Serum-free copper	>10 µg/dL	Normal if ceruloplasmin overestimated by immunologic assay	
Hepatic copper	>250 µg/g dry weight	Due to sampling variation In patients with active liver disease In patients with regenerative nodules	Cholestatic syndromes (i.e., primary biliary cirrhosis)
Kayser-Fleischer rings by slit lamp	Present	In up to 40% of patients with hepatic Wilson's disease In most asymptomatic sibilings	Primary biliary cirrhosis

### Patients with Neurologic Disease

In a patient presenting with typical neurologic symptoms and having Kayser-Fleischer rings, the diagnosis is straightforward. No additional blood tests are required and routine laboratory parameters just confirm the diagnosis. Kayser-Fleischer rings are rarely absent in patients with neurologic involvement, although there are a few well-documented cases of neurologic Wilson's disease without demonstrable Kayser Fleischer rings.<sup>13</sup> In such patients, diagnosis is usually made by a low serum ceruloplasmin level.

Clinical neurologic examination is more sensitive than any other method to detect neurologic abnormalities. No further neurologic procedures are necessary to establish the diagnosis. Brain MR imaging may be useful to document the severity of changes in the central nervous system. The most common abnormalities are changes in signal intensity of gray and white matter and atrophy of the caudate nucleus, brain stem, cerebral, and cerebellar hemispheres.<sup>51</sup> The characteristic "face of the giant panda" sign is found only in a minority of patients. In Wilson's disease, an abnormal striatum or an abnormal pontocerebellar tract correlates with pseudoparkinsonianism and an abnormal dentatothalamic tract with cerebellar signs. The presence of portosystemic shunting is strongly associated with abnormalities of the globus pallidus. Auditory evoked brainstem potentials are helpful to document the degree of functional impairment and the improvement following treatment.<sup>17</sup>

### Patients with Liver Disease and Hemolytic Anemia

Diagnosis is far more complex in patients presenting with liver disease alone. None of the commonly used parameters alone allows a certain diagnosis of Wilson's disease. Usually a combination of various laboratory parameters is necessary to firmly establish the diagnosis. Kayser-Fleischer rings may be absent in up to 50% of patients with Wilsonian liver disease,<sup>45</sup> and even in a higher proportion in fulminant Wilson's disease. On the other hand, patients with cholestatic liver disease, such as primary biliary cirrhosis may occasionally have KayserFleischer rings.

### *Routine Laboratory Parameters of Liver Disease*

In general, aminotransferases are only mildly increased. Deep jaundice combined with mild elevation of liver enzymes in nonsurgical patients should raise the possibility of severe Wilson's disease; however, increased aminotransferases may be indistinguishable from findings seen in acute viral hepatitis. Sometimes alkaline phosphatase activities are relatively low in patients with Wilson's disease. An alkaline phosphatase-to-total bilirubin ratio below 2.0 has been claimed to provide 100% sensitivity and specificity to diagnose Wilsonian fulminant liver failure<sup>56</sup> but the usefulness of

this test has not been confirmed in larger series.<sup>35</sup>

### *Serum Ceruloplasmin*

Serum ceruloplasmin can be measured by the widely used immunologic assay or by the oxydase method, which is only performed in specialized centers. Serum ceruloplasmin is decreased in most patients with neurologic Wilson's disease, but is in the low normal range in up to 45% of patients with hepatic disease.<sup>45</sup> On the other hand, even a low ceruloplasmin level is not diagnostic for Wilson's disease in the absence of Kayser-Fleischer rings. It may be low in familial hypoceruloplasminemia,<sup>21</sup> in severely malnourished subjects, and in heterozygous carriers of the Wilson's disease gene.<sup>8</sup> Very low levels were found in a patient with autoimmune hepatitis, which increased following steroid treatment.<sup>22</sup> Thus, in patients with liver disease, a normal ceruloplasmin level cannot exclude, nor is a low level sufficient to make, the diagnosis of Wilson's disease.

### *Serum Copper*

Serum copper values parallel those of ceruloplasmin, and are frequently low in patients with Wilson's disease. Patients with fulminant Wilson's disease or hemolytic anemia may have markedly increased levels. Most of the copper in serum is bound to ceruloplasmin, and normally less than 5% circulates as free copper. The free copper concentration can be calculated by subtracting the ceruloplasmin-bound copper (ceruloplasmin times 3.3) from the total copper concentration. An increased free-copper level ( $>10$  jg/dL) is considered as a useful diagnostic test for Wilson's disease.

### *Urinary Copper Excretion*

Urine copper excretion is markedly increased in Wilson's disease (in 88% of patients in the author's experience<sup>45</sup>), but its usefulness in clinical practice is limited. The estimation of urinary copper excretion may be misleading because of incorrect collection of 24-hour urine volume or to copper contamination. In presymptomatic patients, urinary copper excretion may be normal. On the other hand, urinary copper excretion is also increased in any disease with extensive hepatocellular necrosis. The 24-hour urinary copper excretion after challenge with 500-Mg D-penicillamine was the most accurate single

diagnostic test in children with Wilson's disease<sup>12</sup> but even after D-penicillamine copper excretion was not elevated in 10% of the tested patients.

### *Hepatic Copper Content*

Hepatic copper content is increased in most patients with Wilson's disease and usually exceeds 250  $\mu$ g/g dry weight (normal, up to 50). In the absence of other tests suggestive for abnormal copper metabolism, the diagnosis of Wilson's disease cannot be made based on an increased hepatic copper content alone, as patients with chronic cholestatic diseases, neonates and young children, and possibly also subjects with exogenous copper overload have increased hepatic copper concentration  $>250$   $\mu$ g/g. On the other hand, hepatic copper content may be normal or borderline in about 10% of patients with unequivocal Wilson's disease due to sampling error given great regional differences in hepatic copper distribution, especially in the cirrhotic liver.<sup>29</sup>

### *Liver Biopsy*

**Light Microscopy.** Liver biopsy findings are nonspecific and not directly helpful to make the diagnosis of Wilson's disease. Early histopathologic changes include fatty intracellular accumulations, which often proceed to marked steatosis. At later stages, hepatic inflammation with portal and periportal lymphocytic infiltrates and presence of necrosis and fibrosis may be indistinguishable from other forms of hepatitis. Some patients have cirrhosis without any inflammation. The detection of focal copper stores by the Rhodanin stain is a pathognomic feature of Wilson's disease but is only present in the minority of patients.<sup>25</sup>

**Electron Microscopy.** The ultrastructural abnormalities include pathologic changes of mitochondria and peroxisomes. Hepatocellular mitochondria are pleomorphic, with various combinations of abnormalities, including enlargement, bizarre shapes, increased matrix density, separation of the normally apposed inner and outer membranes, widened intercrystal spaces, enlarged granules, and crystalline, vacuolated, or dense inclusions. Sometimes peroxisomes are abnormally enlarged, rounded, or misshapen, and contain a granular or flocculent matrix of various electron density

## **MRI of the Liver**

Hepatic T2-weighted MR imaging changes include multiple, tiny, low-intensity nodules surrounded by high-intensity septa in about 60% of patients.<sup>53</sup> Some also had low-intensity nodules in T1-weighted images. Patients with abnormal MR image findings had liver cirrhosis or fibrosis. Patients with low-intensity nodules and unknown cause of liver cirrhosis should be investigated for disorders of copper metabolism.

## **Radiocopper Test**

The basis of this test are the biphasic plasma kinetics of copper. Blood radioactivity is determined 1, 2, 24, and 48 hours after an oral dose of <sup>64</sup>Cu.<sup>47</sup> Initially, labeled copper is taken up by the liver and is completely removed from the serum. Later, the label reappears bound to ceruloplasmin. This second peak is absent in Wilson's disease patients. The test is only useful in patients and their siblings with ceruloplasmin levels in or close to the normal range. There is a considerable overlap between affected patients and heterozygotes. Several modifications (i.e., intravenous instead of oral administration, measurement of radiocopper incorporation into ceruloplasmin) did not improve the sensitivity of this test.

## **Mutation Analysis**

### *Direct Mutation Analysis*

Because of multiple mutations, direct molecular-genetic diagnosis of Wilson's disease can be problematic. Most patients are compound heterozygotes (i.e., carry two different mutations). Screening for mutations is typically done by single-strand conformation polymorphism analysis. Those samples showing a shift of one or both bands can then be sequenced to identify the exact mutation. This approach is quite useful as a research tool, but impractical for clinical diagnosis. In contrast, using allele-specific probes, direct mutation diagnosis is rapid and helpful if a mutation occurs with a reasonable frequency in the population. In Austria, the His1069Gln mutation is present in 61% of patients with Wilson's disease, and a two-step PCR-based test for this mutation has become very useful.<sup>26</sup> For example, the large family of an index patient homozygous for this mutation with more than 40 members was examined within 2 days. An asymptomatic, affected sibling and several

heterozygotes can be detected, even if first-degree relatives are not available for testing. Eventually, a multiplex polymerase chain reaction (PCR) for the most frequent mutations in Wilson's disease should make direct mutation analysis for diagnosis feasible.

### *Haplotype Analysis*

Because of the complexity in identifying the multiple mutations in Wilson's disease, haplotypes can be used to screen for mutations and to examine asymptomatic siblings of index patients. A number of highly polymorphic microsatellite markers have been described that closely flank the gene and are highly variable: D13S316, D13S314, DUS301, and D13S133.<sup>10</sup> If the allelic markers differ within members of a family, they allow a physician to trace the inheritance of a given mutation within the family. For such an analysis, testing of at least one parent or child of the patient is necessary to obtain the haplotype. The identification of unusual haplotypes can support, but is not sufficient to confirm, the diagnosis of Wilson's disease.

Microsatellite markers are also useful to study the segregation of the Wilson's disease gene in most families. By this approach, diagnostic dilemmas in differentiating heterozygote gene carriers and affected, asymptomatic siblings can be solved. For such analysis, at least one first-degree relative and the index patient is required. Haplotype analysis and restriction fragment length polymorphism of flanking genes (prior to the discovery of the Wilson's disease gene) proved to be extremely useful for prenatal testing and to confirm the diagnosis of affected siblings in Wilson's disease families.<sup>27,52</sup>

## **Family Screening**

Once diagnosis of Wilson's disease is made, an index patient evaluation of the family is mandatory. The likelihood of finding a homozygote among siblings is 25% (among children, 0.5%). Testing of second-degree relatives is only useful if the gene is found in one of the immediate members of his or her family. No single test has been able to identify affected siblings or heterozygote carriers of the Wilson's disease gene with sufficient certainty. Today, mutation analysis is the only reliable tool for family screening.

## An Algorithm for Diagnosis of Wilson's Disease

A diagnostic algorithm is suggested in Figure 1. In the absence of typical clinical findings, measurement of hepatic copper content is mandatory in every patient with unexplained elevations of liver enzymes and some abnormal parameters of copper metabolism. Furthermore, if the clinical suspicion is high, patients with ceruloplasmin levels in the normal range should be referred for a radiocopper test or PCRbased mutation analysis. In selected patients with unclear findings or in whom a liver biopsy cannot be performed, a test treatment with decoppering agents can be initiated for a period of 6 to 12 months.

### TREATMENT

#### Penicillamine

Penicillamine was first reported to be effective in treating Wilson's disease by Walshe and Yealland<sup>55</sup> in 1956, and is considered the gold standard for therapy. Penicillamine acts by reductive chelation: it reduces copper bound to protein and thereby decreases the affinity of the protein for copper, which facilitates binding of

copper to the drug. The copper mobilized by penicillamine is then excreted in the urine. Within a few weeks to months, penicillamine brings the level of copper to a subtoxic threshold and allows tissue repair to begin. The great majority of symptomatic patients, irrespective of predominantly hepatic, neurologic, or psychiatric manifestations, respond within months of starting treatment. Among patients with neurologic symptoms, a significant number may experience an initial worsening of symptoms before they get better.

The usual dose of penicillamine is 1 to 1.5 g/day. Initially, this dose will cause a large cupruresis, but copper excretion eventually decreases to 0.5 mg/d. To prevent deficiency induced by penicillamine, pyridoxine (vitamin B<sub>6</sub>) should be supplemented (50 mg/week). Once the clinical benefit is established, the dosage of penicillamine can be reduced to 0.5 to 1 g/d. A lower maintenance dose decreases the likelihood of late side-effects of the drug. A major problem of penicillamine is its high level of toxicity. In the author's series (Ferenci P, unpublished data), 20% of patients had major side-effects and were switched to other treatments. Other series report even higher frequencies of side effects. There are two broad classes of penicillamine toxicity: direct

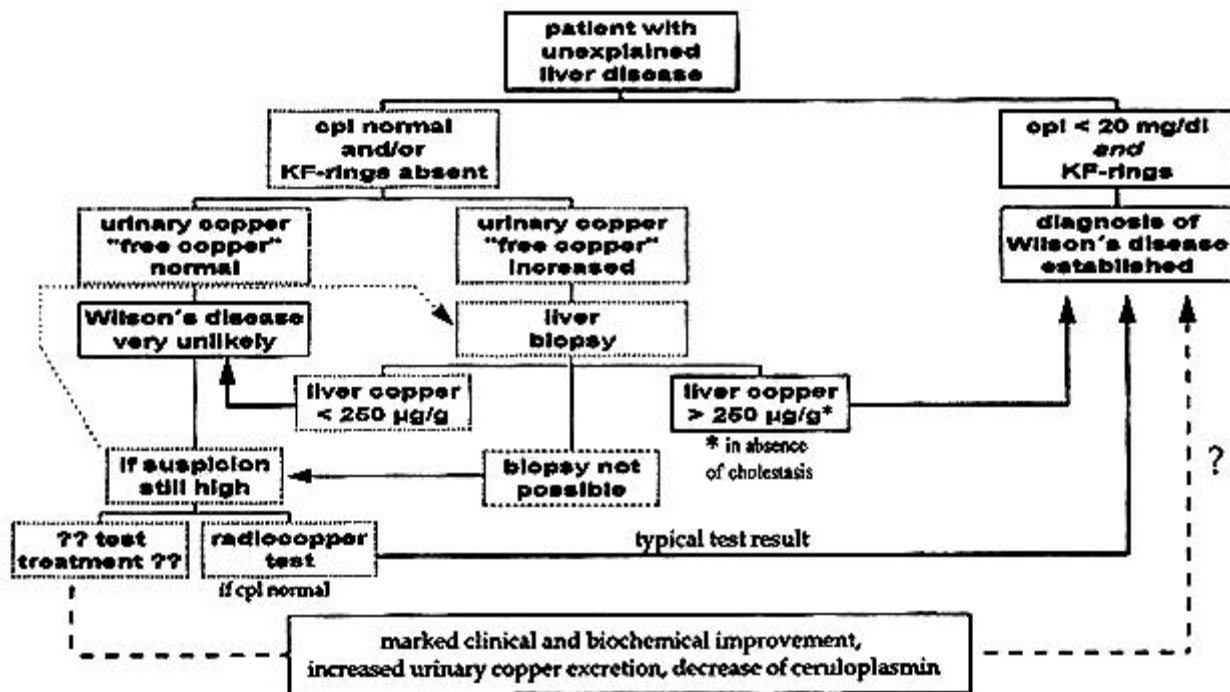


Figure 1. Flow diagram for diagnosis of Wilson's disease. cpl = ceruloplasmin; KF = Kayser-Fleischer.

dose-dependent side effects and immunologically induced lesions; direct side effects are pyridoxine deficiency and interference with collagen and elastin formation with the latter resulting in skin lesions, (i.e., cutis laxa and elastosis perforans serpiginosa). On routine skin biopsies 1 year after initiation of treatment signs of elastic and collagen fiber abnormalities in every patient were found, but none have developed symptomatic skin disease so far. These side-effects can be prevented or mitigated by decreasing the dosage of penicillamine. Immunologically mediated side-effects include leukopenia and thrombocytopenia, systemic lupus erythematosus, immune complex nephritis, pemphigus, buccal ulcerations, myasthenia gravis, optic neuritis, and Goodpasture's syndrome. Immunologically mediated side-effects occur within the first 3 months of treatment and require immediate cessation of penicillamine. To recognize these side-effects as soon as possible, patients should be monitored in weekly intervals during the first 6 weeks of therapy. If the drug is well tolerated, control intervals can be gradually prolonged.

### **Trientine**

Trientine is a copper chelator that primarily enhances urinary copper excretion. Trientine is licensed for treatment of Wilson's disease and is now generally available. Although experience with trientine is not as extensive as with penicillamine, it seems to be as effective as penicillamine with far fewer side-effects. Its efficacy has been evaluated in patients with intolerance to penicillamine<sup>31</sup>; however, the efficacy of trientine has not been compared with penicillamine as initial treatment of Wilson's disease. Uncontrolled anecdotal reports and the author's own experience indicate that trientine is a satisfactory first-line treatment for Wilson's disease. In the early phase of treatment trientine appears to be more potent at copper mobilization than penicillamine, but cupriuresis diminishes more rapidly than with penicillamine. The cupriuretic power of trientine may be disappointing, but is sufficient to keep the patient clinically well.

### **Ammonium Tetrathiomolybdate**

Ammonium tetrathiomolybdate has two mechanisms of action: it prevents the absorption of copper in the intestinal tract and the absorbed drug forms a complex with copper and albumin

in the blood, rendering the copper unavailable for cellular uptake. There is very limited experience with this drug. Tetrathiomolybdate appears to be useful as initial treatment of patients presenting with neurologic symptoms.<sup>6</sup> In contrast to penicillamine therapy, treatment with tetrathiomolybdate does not result in initial neurologic deterioration. This agent is particularly effective at removing copper from the liver. Because of its effectiveness, continuous use can cause copper deficiency. Bone marrow depression has been observed in a few patients.

### **Zinc**

Zinc interferes with the intestinal absorption of copper by two mechanisms: both metals share the same carrier in enterocytes and pretreatment with zinc blocks this carrier for copper transport. This effect of zinc has a half-life of about 11 days. In addition, zinc induces metallothionein in enterocytes, which acts as an intracellular ligand binding zinc, copper, and other metals, thereby rendering them unavailable for systemic absorption.<sup>57</sup> Instead, these metals are excreted in the feces with clesquarnated epithelial cells. Increased fecal excretion of copper was demonstrated in patients with Wilson's disease on treatment with zinc. Furthermore, zinc also induces metallothionein formation in hepatocytes, and binding of copper to metallothionein protects hepatocytes against copper toxicity.<sup>23</sup>

Data on zinc in the treatment of Wilson's disease<sup>15</sup> are derived mostly from uncontrolled studies using different zinc preparations (zinc sulfate, zinc acetate) at different doses (75-250 mg/d). The efficacy of zinc was assessed by four different approaches. First, patients successfully chelated by D-penicillamine were switched to zinc treatment and the maintenance of their asymptomatic condition was monitored. Most patients maintained a negative copper balance and no symptomatic recurrences occurred. A second group studied were symptomatic patients switched to zinc as alternate treatment due to intolerance to D-penicillamine. Sixteen case histories have been published so far. Liver function and neurologic symptoms improved in 3 and 5 patients, respectively. One patient further deteriorated neurologically and improved on retreatment with D-penicillamine. The remaining patients remained in stable condition. In a third group, zinc was used as first-line therapy.<sup>7</sup> About one third were asymptomatic

siblings of patients with Wilson's disease, and two thirds presented with neurologic or hepatic symptoms. Most patients remained free of symptoms or improved, but three patients died of progressive liver disease. Finally, in a prospective study" in 67 newly diagnosed cases of Wilson's disease, the effectiveness of long-term treatment with D-penicillamine and zinc was similar in those patients who were able to continue the initial therapy. Zinc was better tolerated than D-penicillamine; however, two zinc-treated patients died of progressive liver disease.

The usefulness of a combination of zinc with chelation therapy is unknown. Interactions in the maintenance phase of zinc therapy with penicillamine and trientine were investigated by Cu balance studies and absorption of orally administered <sup>64</sup>Cu as endpoints. The result on Cu balance was about the same with zinc alone as it is with zinc plus one of the other agents. Thus, there appears to be no advantages to concomitant administration.

### **Antioxidants**

As discussed previously, the main mechanism of hepatocellular injury by excess copper is the formation of free radicals resulting in lipid-peroxidation and impaired mitochondrial respiration. Thus, antioxidants, such as a-tocopherol, may be important adjuncts in the treatment of Wilson's disease.<sup>42</sup> There are no large experiences with a-tocopherol, but a few observations indicate that this therapeutic adjunct may be useful in severe liver disease.

### **Liver Transplantation**

Liver transplantation is the treatment of choice in patients with fulminant Wilson's disease and in patients with decompensated cirrhosis. The selection criteria for fulminant Wilson's disease and of decompensated Wilsonian cirrhosis are similar to those for fulminant hepatic failure or decompensated cirrhosis of other causes. Besides improving survival, liver transplantation also corrects defect of Wilson's disease.

Schilsky<sup>39</sup> analyzed retrospective data obtained on 55 patients with Wilson's disease transplanted at centers in the United States and Europe. Mean and median survivals after orthotopic liver transplantation were 2.7 and 2.5 years, respectively, with the longest survival being 20 years. Survival at 1 year was 79%. Fifty-one orthotopic liver transplants were

performed on 39 patients (16 pediatric, 23 adults) with Wilson's disease at the University of Pittsburgh.<sup>2</sup> The rate of primary graft survival was 730% and patient survival was 79.4%. Survival was better for those with a chronic advanced liver disease presentation (90%) than it was for those with a fulminant presentation (73%).

The outcome of neurologic disease following liver transplantation is uncertain. In the retrospective survey, four of the seven patients with neurologic or psychiatric symptoms due to Wilson's disease improved. There are few case reports documenting either dramatic improvement in neurologic function over a period of 3 or 4 months after transplantation or the development of postoperative central pontine and extrapontine myelinolysis or new extrapyramidal symptoms.<sup>19</sup>

### **PROGNOSIS**

Untreated, symptomatic Wilson's disease progresses to death in all patients. The overall mortality in medically treated patients (in most cases by D-penicillamine) has not been assessed prospectively. The mortality in 33 patients followed for 21 years by Scheinberg and Sternlieb<sup>37</sup> was approximately 20%. This figure is close to the cumulative mortality in 69 Austrian patients (Fig. 2). In 51 German patients<sup>48</sup> the cumulative survival was slightly reduced during the early period of follow-up, but was not different from an age- and sex-matched control population after 15 years of observation (about 96%). In Wilsonian chronic active hepatitis, medical treatment is highly effective.<sup>40</sup> Only two noncompliant patients required liver transplants. In contrast, in patients with fulminant Wilson's disease, medical treatment is rarely effective; without emergency liver transplantation, mortality is very high.

In general, patients presenting with neurologic symptoms have a better prognosis than those presenting with liver disease.<sup>5</sup> Neurologic symptoms are partly reversible. Improvement of neurologic symptoms tend to occur gradually over several months. Initially, neurologic symptoms may worsen, especially on treatment with D-penicillamine. In some patients, neurologic symptoms disappear completely, and abnormalities documented by evoked responses<sup>18</sup> or MR imaging may completely resolve within 18 to 24 months.

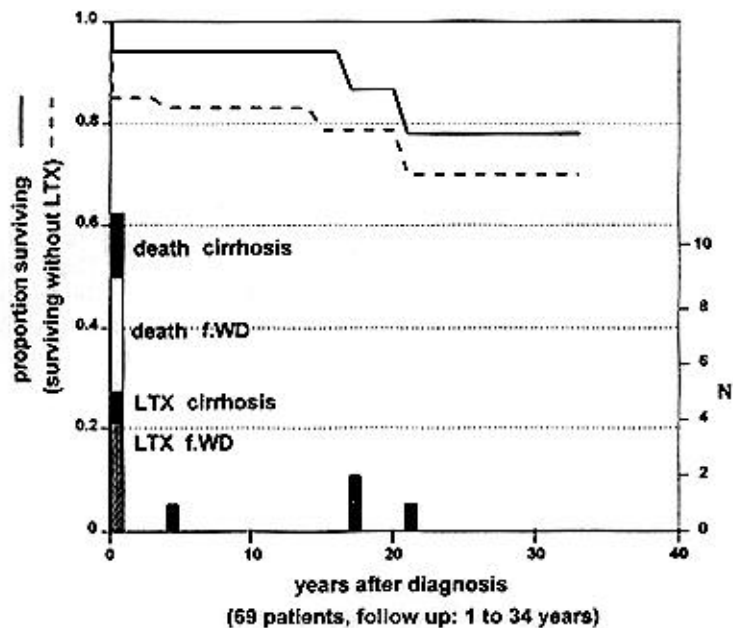


Figure 2. Cumulative survival in 69 Austrian patients with Wilson's disease. Dashed lines indicate survival rates if death or liver transplantation are chosen as endpoints. Bars refer to the number of patients dying from, or transplanted for, fulminant Wilson's disease or decompensated cirrhosis.

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