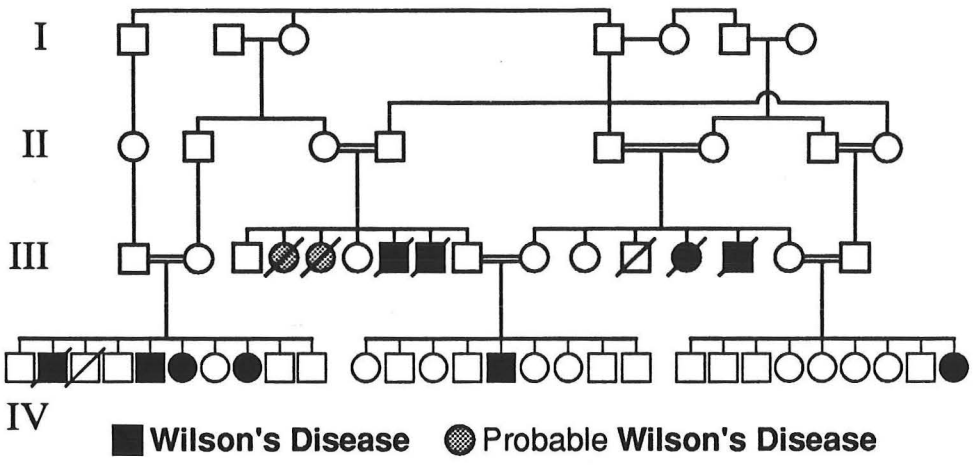
Wilson('s) Disease in 1994: A New Gene and an Animal Model for an Old Disease



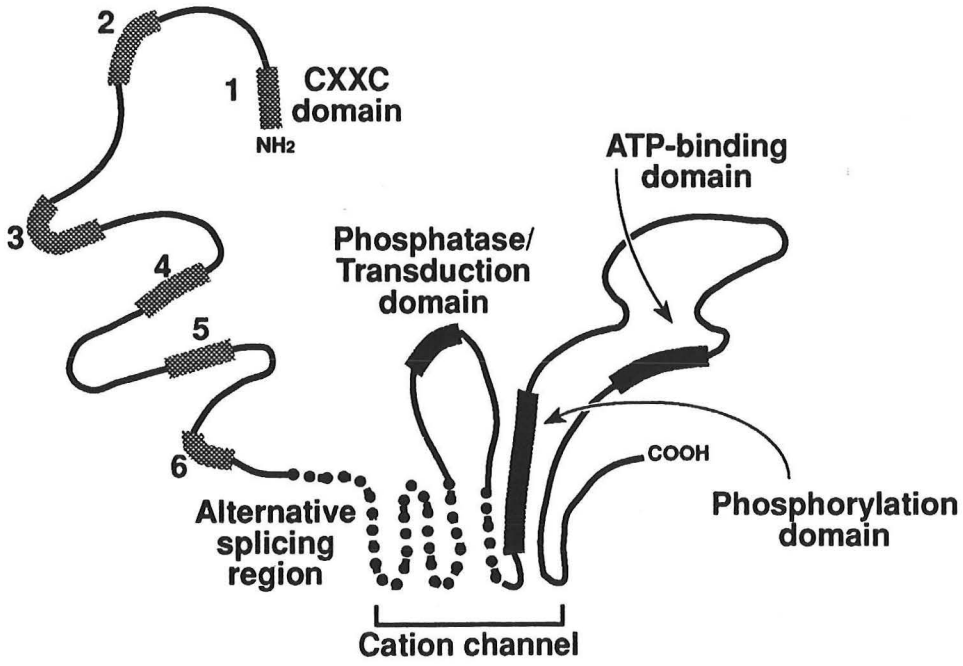


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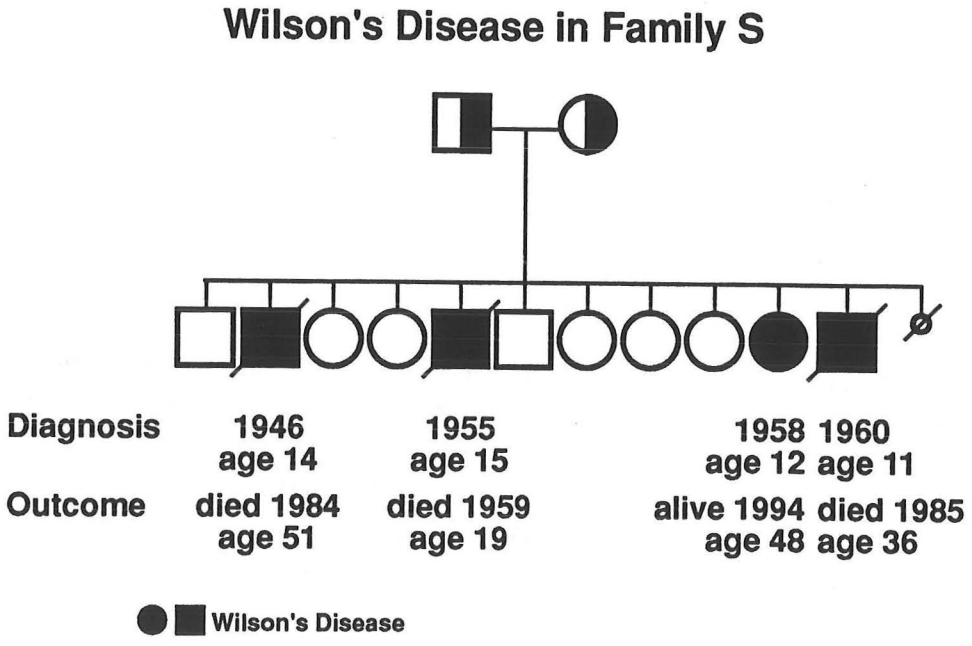
Table of Contents

This contribution concentrates on progress in the understanding of the molecular basis of Wilson's disease since it was last reviewed at this venue (Dwain L. Thiele, M.D., "Wilson's Disease", July 25, 1985). Interested readers are directed to his presentation for more detailed discussions of areas only briefly considered herein. Original references for statements not specifically referenced are contained in the detailed monograph of Scheinberg and Sternlieb[1] and recent reviews in major textbooks of Hepatology[2] and Metabolism.[3]

Case Report

In 1958, a 12 year old girl was evaluated at Parkland Memorial Hospital. An episode of swelling attributed to liver disease was noted at age 9. Since then, there was intermittent dysarthria and gait disturbance and deterioration of school performance. Family history was relevant for two older brothers with Wilson's disease. Examination was remarkable for Kayser-Fleischer rings and laboratory investigations revealed ceruloplasmin 6mg/dl (normal range 18-35). An open liver biopsy demonstrated cirrhosis. A diagnosis of Wilson's disease was made and she was treated with penicillamine. Two pregnancies were completed uneventfully on penicillamine therapy. Intermittent periods of non-compliance were followed by the transient development of tremor, mild ataxia and dysarthria that improved with re-institution of therapy. Unfortunately, her liver disease progressed during non-compliance and she has had variceal hemorrhage and ascites complicating her course. However, she is alive (age 48 years) with little disability at present (related to ascites and abdominal wall hernias). She illustrates the classic family presentation of a sibling with Wilson's disease, demonstrating the excellent prognosis with early recognition and long-term maintenance on D-penicillamine.

In 1958, her younger brother was not symptomatic and had a normal physical examination, no Kayser-Fleischer rings were visible, ceruloplasmin 22.4mg/dl. A radio-copper study was abnormal and he was considered to be a heterozygote for the Wilson's disease genetic defect. He presented 18 months later with frequent epistaxes. Investigation revealed repeat ceruloplasmin 25.2 mg/dl, 24 hour urine copper 300 μg (normal 0-10). A liver biopsy demonstrated cirrhosis, fatty metamorphosis and increased hepatic copper. He was commenced on penicillamine therapy but was intermittently non-compliant and died of hepatic failure at age 36. His brothers also succumbed to the disease, one early after recurrent variceal hemorrhage, the other later from sepsis and hepatic failure. These family members emphasize the need for continued therapy to remove the excess, toxic copper.

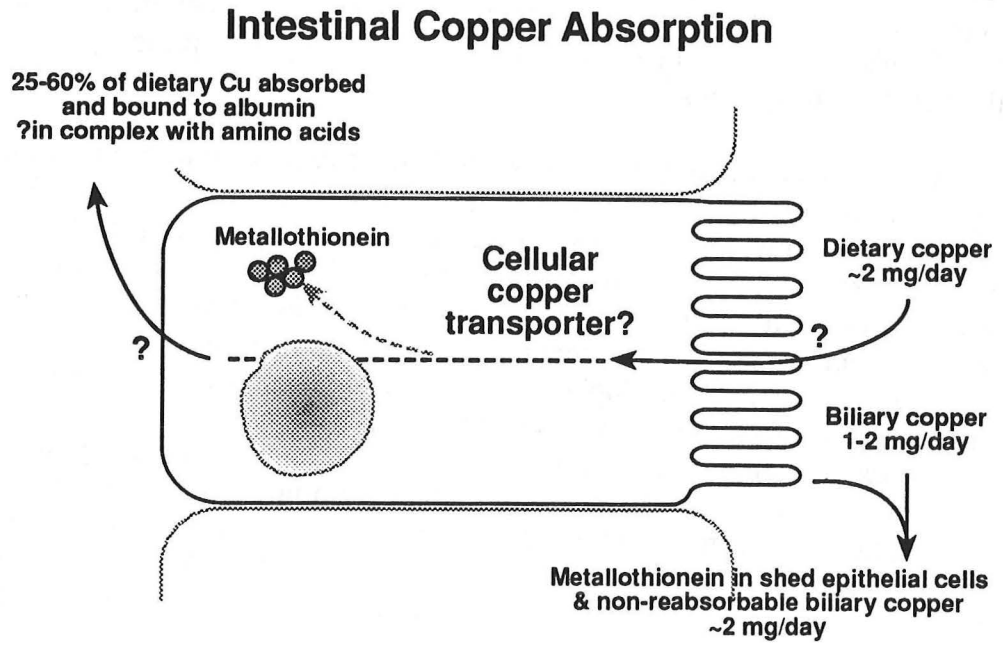


Normal copper homeostasis

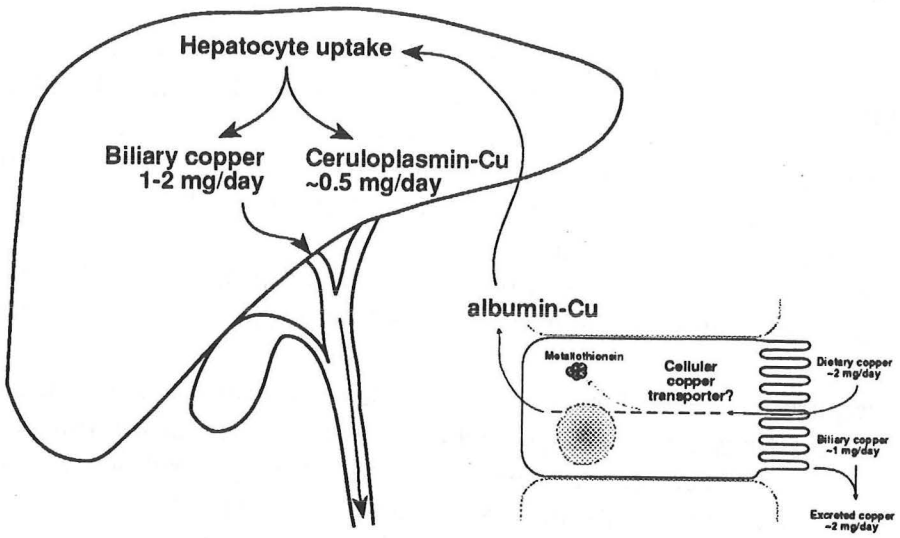
Copper is one of at least 12 heavy metals essential for life (reviewed in reference 4). Metabolism of copper and of two other metals, iron and zinc, is known to be abnormal in inherited metabolic diseases; Wilson's and Menkes' diseases (disorders of copper metabolism), hereditary hemochromatosis (iron metabolism) and hereditary acrodermatitis (zinc metabolism). Inherited disease processes related to the other essential heavy metals, arsenic, chromium, cobalt, manganese, molybdenum, nickel, selenium, tin and vanadium, have not been reported. Additional heavy metals that are potentially important in metabolic processes include antimony, bismuth, cadmium, lead, mercury, silver, tellurium and thallium.[4]

Copper is required as a co-factor in ~30 redox enzymes.[2-4] These include lysyl oxidase (collagen and elastin cross-linking), cytochrome *c* oxidase (electron transport chain), superoxide dismutase (free radical detoxification), dopamine β-hydroxylase (catecholamine production) and tyrosinase (pigmentation). When copper accumulates above stringently maintained levels, toxicity ensues. Copper is implicated in deleterious oxidation of lipids and proteins and in free radical formation in cells containing high levels of copper.[2-4] Regulation of intra-cellular copper levels is apparently critical, as evidenced by the invariably fatal nature of copper deficiency (Menkes' disease) and copper overload (Wilson's disease) without appropriate therapy.[1, 3]

Normal dietary copper varies from 1-5mg/day.[2] Foods rich in copper include shellfish, liver, nuts, chocolate and mushrooms. Dietary copper is rapidly transported into epithelial cells of the upper small intestine with characteristics of facilitated uptake,[2] however the precise molecular mechanisms of uptake are unknown. Within the intestinal epithelial cell, the copper can either be bound to cellular metal-binding polypeptides (metallothioneins) or transported across the baso-lateral membrane.[5] Transcription of metal-free metallothionein (apo-thionein, Mr 6-7kDa), a small polypeptide of 60 amino acids 20 of which are cysteine, is up-regulated by various metals.[6, 7] Copper can induce metallothionein synthesis,

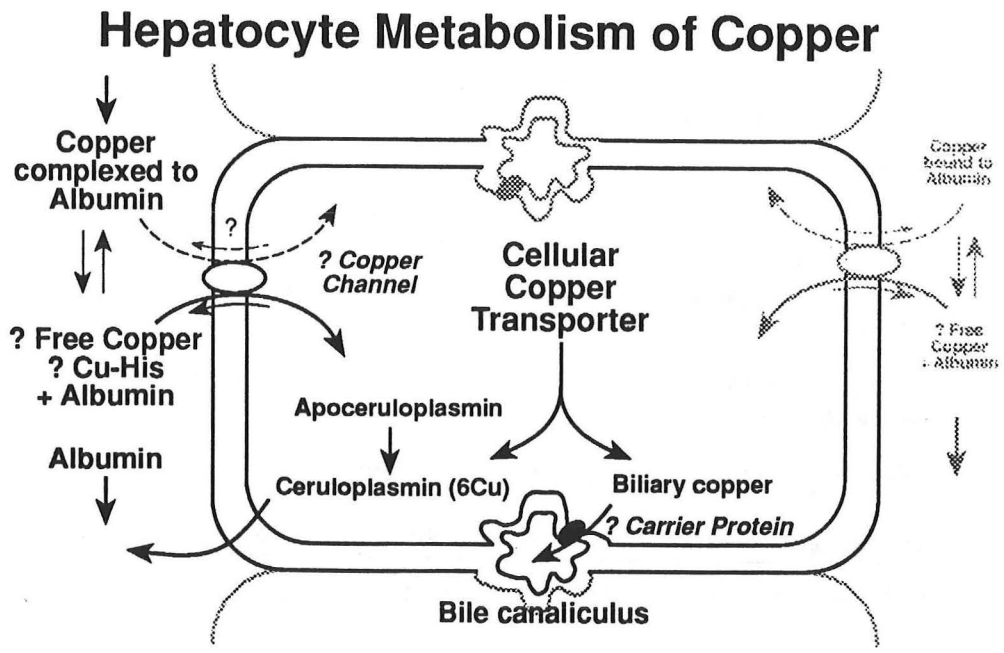


Hepatic Copper Metabolism



zinc is a more potent inducer (reviewed in reference 6). Metallothioneins generally bind 7-8 divalent metal ions/molecule, using the -SH groups to with copper to generate Cu(I)-thiolate clusters.[8] Copper-metallothionein has a longer half-life than other metallothioneins forms[6] and is likely excreted when the epithelial cells are shed with normal turnover. The exit of copper from the baso-lateral membrane of the intestinal epithelial cell is abnormal in Menkes' disease and copper accumulates in the intestine.[3] Thus, the intestine, by controlling uptake and transport into the portal circulation, functions as one of two major sites of normal regulation of copper metabolism (the other being liver).[5] From the baso-lateral membrane, the 25-60% of dietary copper that reaches the circulation is bound to albumin, one atom/molecule, with high affinity (K_d = 7 x 10^{-17} M).[2] A complex of copper-bound albumin with plasma amino acids, eg histidine, may form.[5] Histidine has the highest affinity for copper of the plasma amino acids (K_d = 10^{-18} M for $Cu(His)_2$).[2] The histidine residue (third from the amino-terminal end) appears to be essential for albumin to bind copper.[2, 5] Copper is rapidly transported from albumin into hepatocytes, by mechanisms that are not completely defined.[2, 5] A specific uptake process is likely to exist, particularly since hepatic clearance is rapid and complete. Intestinal copper absorption and transport to hepatocytes is normal in Wilson's disease.[2, 3] Typically, little copper escapes hepatic uptake and is excreted in the urine (<40 μg/day).[2, 3]

Once copper reaches hepatocytes, it is available for complexing with apo-thionein to produce Cu-metallothionein, incorporation into ceruloplasmin or excretion into bile. The latter two processes are interrupted in Wilson's disease.[1-3] Ceruloplasmin (Mr 132kDa), an α2-migrating glycoprotein is encoded on chromosome 3q.[5, 9] Apo-ceruloplasmin is synthesized in the liver and normally 6-7 copper atoms/molecule are incorporated, probably post-translationally.[2] Whether ceruloplasmin functions as a copper transporter to the remainder of the body is under debate.[2, 5] Ceruloplasmin also has ferroxidase, amine oxidase and superoxide dismutase activities, the importance of which are unknown but do not appear to be essential. Some experimental evidence suggests that ceruloplasmin may transfer Cu(I) to other cells since exchange is blocked by Cu(I) chelators but not Cu(II) chelators *in vitro*.[5] Other investigators, however, consider that ceruloplasmin does not release its bound copper except during degradation.[2] Ceruloplasmin normally circulates at levels of ~30mg/dl, accounting for 70-95% of serum copper (1 μg/dl).[5] Increased ceruloplasmin is synthesized during acute phase responses and when induced by hormones (estrogens in oral contracep-



tives and during pregnancy).[1-3] Ceruloplasmin levels may decrease non-specifically with protein-losing states such as nephrotic syndrome and enteropathy, in protein malnutrition and in severe liver disease.[1-3] Ceruloplasmin levels are specifically reduced in neonates, hereditary hypoceruloplasminemia, Menkes' disease (because hepatic copper is decreased) and in Wilson's disease where there is failure of copper incorporation.[1-3]

Biliary excretion of copper, in a state that prevents reabsorption, is the other major regulatory control mechanism in copper metabolism.[2] In normal individuals, 1-2mg/day is excreted via the biliary tract.[2] In Wilson's disease, this is reduced to ≤20-40% of normal.[2] Copper in bile is reported in association with a number of different moieties and the exact nature of its excretion remains obscure. Biliary copper excretion is impaired in cholestatic processes such as primary biliary cirrhosis and primary sclerosing cholangitis. It is also absent in neonates, leading to increased hepatic copper.[3] Neonates thus resemble patients with Wilson's disease, having impairment of both incorporation of copper into ceruloplasmin and excretion of copper into bile.

Hepato-biliary Copper Metabolism
Development & Disease

Characteristic	Normal Neonate	Normal Adult	Wilson's Disease
Hepatic copper	High <295µg/g dry wt	Low <50µg/g dry wt	High >250µg/g dry wt
Incorporation of Cu into ceruloplasmin	Abnormal?	Normal	Abnormal
Ceruloplasmin	Low 2-13mg/dl	Normal 20-40mg/dl	Low in 95%
Biliary copper transport	Abnormal?	Normal	Abnormal

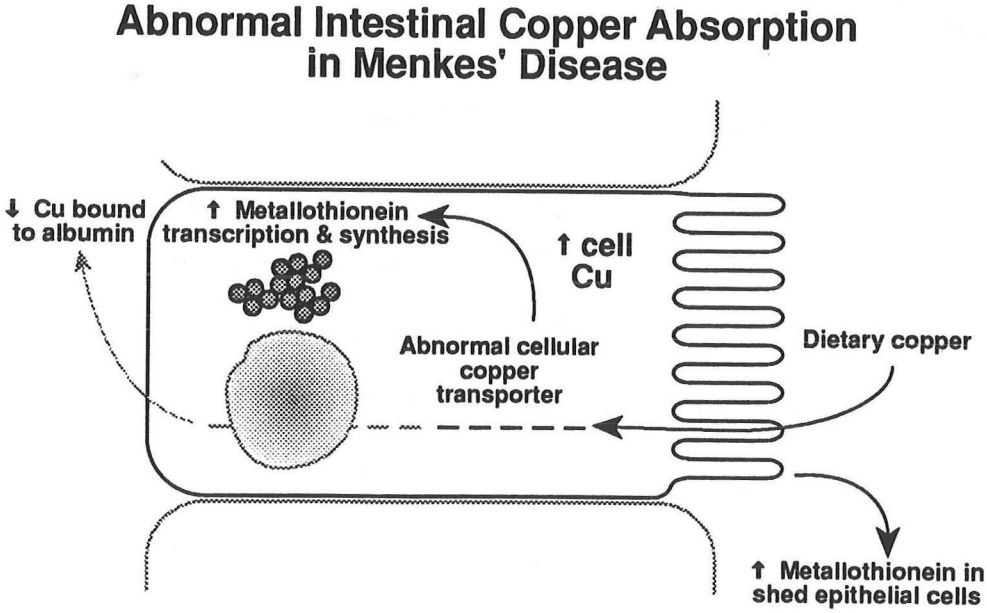
Wilson's and Menkes' Diseases
Inherited Disorders of Copper Metabolism

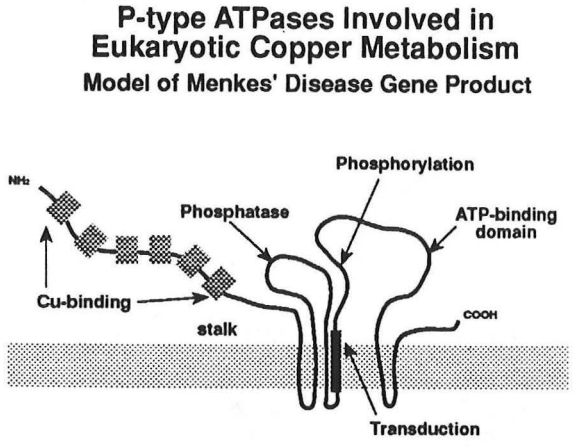
Characteristic	Wilson's Disease	Menkes' Disease
Movement of dietary copper from intestine	Normal	Abnormal
Incorporation of Cu into critical cellular enzymes	Normal	Abnormal
Incorporation of Cu into ceruloplasmin	Abnormal	Normal*
Biliary copper transport	Abnormal	Normal*

*low hepatic copper → low ceruloplasmin-Cu, low biliary copper

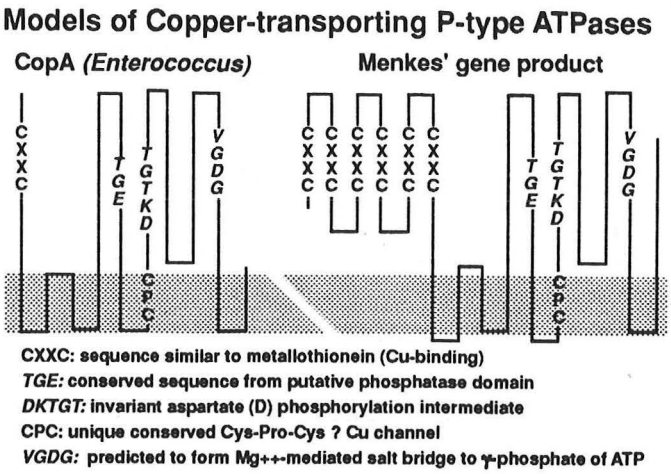
Inherited Disorders of Copper Metabolism: Menkes' and Wilson's diseases

In order to understand the nature of the defect in Wilson's disease, it is helpful to understand the defect in the other inherited disorder of copper metabolism, Menkes' disease. Menkes' disease, named for an author of the first description, is a rare (~1:300,000 live births), X-linked recessive disorder.[3] It is characterized by progressive neurologic degeneration, hypothermia, connective tissue defects, steely hair and pallor. Abnormal copper metabolism was demonstrated by Danks *et al* in 1972, after recognizing similarities to copper-deficiency in sheep and pigs (reviewed in reference 2). The copper metabolic defect results in trapping of copper in the intestine and kidney, with deficits in cellular copper elsewhere leading to a deficiency in critical copper-containing enzymes and the characteristic findings. A similar mutation in mice (the mottled mutation) is also X-linked.[3] The characteristic pigment pattern in heterozygous females led to the Lyon hypothesis of X-inactivation.





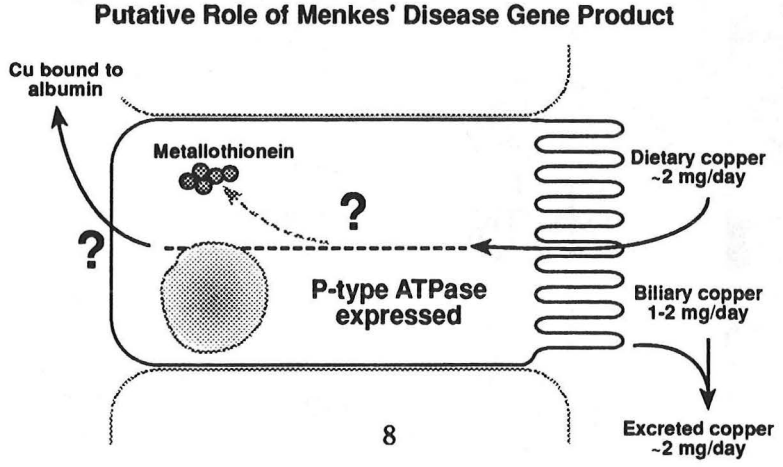
From: Vulpe *et al, Nat Genet* **3:** 7-13, 1994

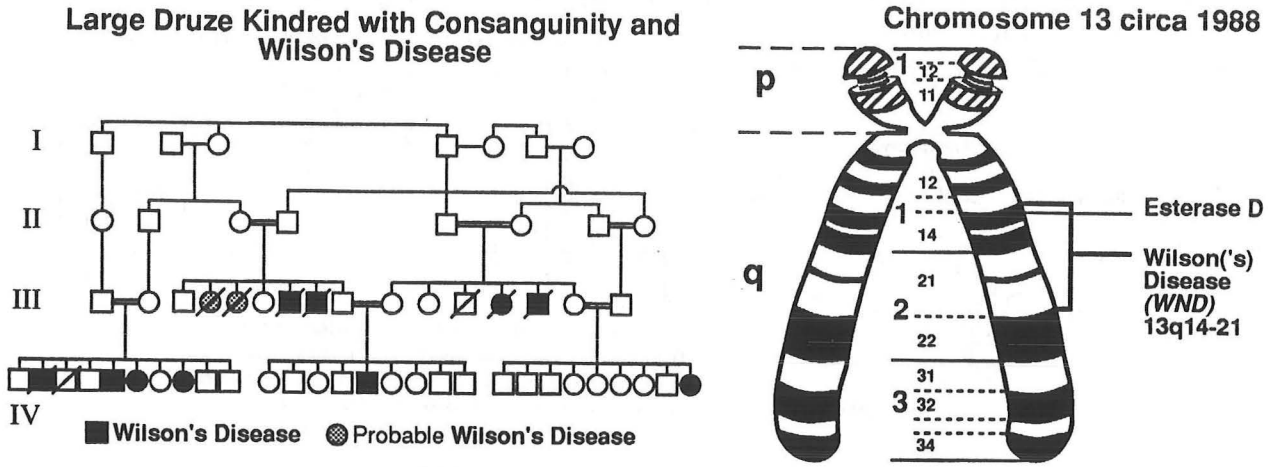


From: Solioz*et al, FEBS Lett* **346:** 44-47, 1994

Cloning the Menkes' disease gene, *MNK* or ATP7A, was greatly aided by the identification of an unusual female patient with Menkes' disease and a balanced X:2 translocation (detailed in reference 10). The gene was identified by searching at the translocation breakpoint for coding sequences.[10-12] The gene is predicted to encode a 1,500 amino acid protein with motifs suggesting function as a P-type ATPase and action as a copper-transporter.[4, 13, 14] P-type ATPases transport H^+, Na^+, K^+ and Ca^{++}, using an aspartyl Phosphate intermediate to move cations across membranes.[4] The conserved domains include those involved in ATP binding and phosphatase action as well as the aspartyl residue forming the intermediate. In addition to sequences homologous to P-type ATPases, the Menkes' disease gene product also contains 6 tandem copies of a bacterial heavy metal binding sequence, GMTCxxC.[14] These are found in the amino-terminus of the protein. The sequence CxxC is characteristic of Cu-, Fe-, and Zn-binding protein, eg metallothioneins, ferredoxin and DNA binding proteins. The mouse homolog of *MNK* is altered in the dappled and blotchy forms of the mottled mouse.[15, 16]

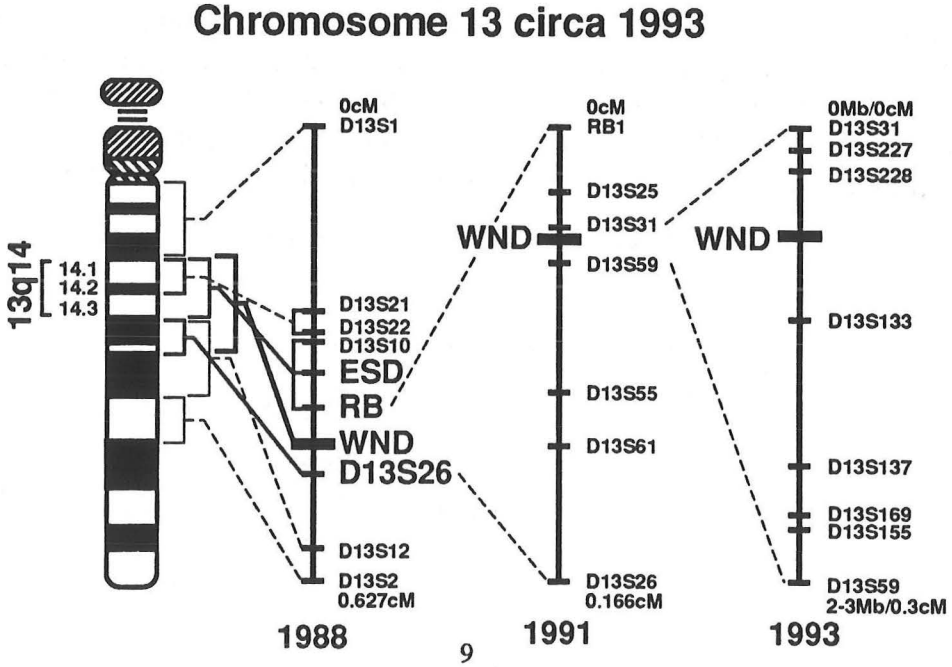
The Menkes' gene product is widely expressed, including intestine, brain, kidney, heart, fibroblasts and lymphoblasts.[10] The liver has low or no expression. The expression profile explains the observation that cultured fibroblasts and lymphoblasts from Menkes' patients accumulate copper *in vitro*, although they are copper-deficient *in vivo* secondary to the failure of normal intestinal transport. The expression of the mRNA for the *MNK* gene is abnormal in some Menkes' patients whereas others (~16%) harbor large deletions including the gene.[10, 11] The *MNK* gene product is proposed to function in the intra-cellular movement of copper or in the transport of copper across the plasma membrane.

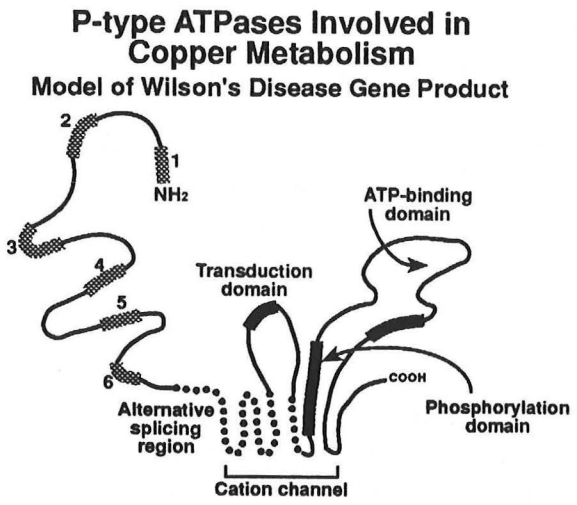




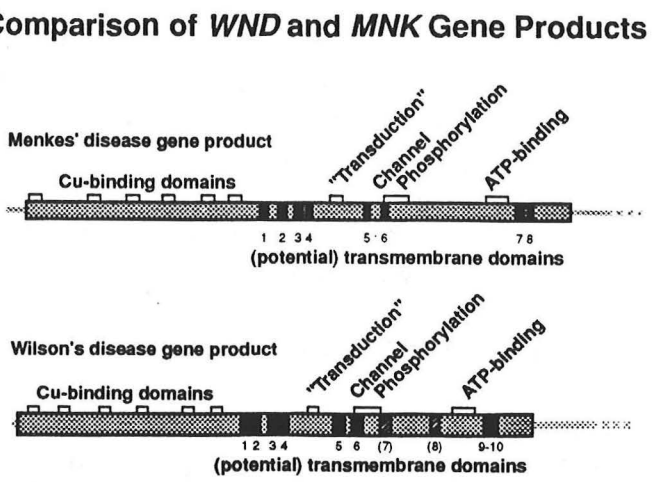
Cloning the Wilson's Disease Gene

The familial nature of Wilson's disease is the result of an autosomal recessive inheritance pattern. The estimated gene frequency is 5.2 per 1,000 in Japan, however, in areas of the world where consanguinity was previously common, the rate tends to be much higher. The disease frequency ranges from 1:30,000 (Japan) to 1:100,000 (Australia).[17] In 1985, the disease gene was linked to the esterase D locus,[18] a polymorphic enzyme of red cells which was previously mapped to chromosome 13. Using DNA from additional large, inbred kindreds,[19, 20] and anonymous markers on chromosome 13, the disease locus was defined as 13q14-21, near the retinoblastoma gene, in 1988.[21] With polymorphic microsatellite markers the gene was mapped to chromosome 13q14.3[22, 23] and in 1993 the gene was cloned by three groups.[24-26] Two groups used the Menkes' gene sequence to identify a similar product in the area mapped to the Wilson's disease gene *WND*.[24, 26] The other group was using heavy metal-binding motifs to identify genes potentially involved in neurologic disease and then discovered that the gene retrieved by this technique mapped to the Wilson's disease area of chromosome 13.[25]





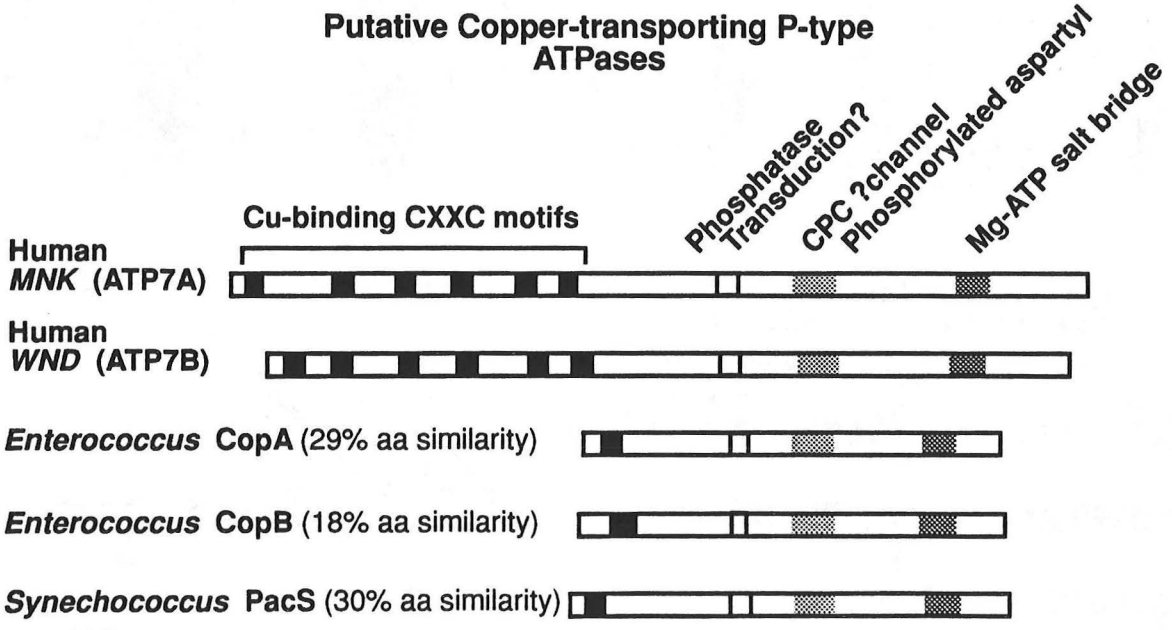
From: Bull and Cox, *Trends Genet* **10:** 246-252, 1994



From: Bull *et al*, *Nat Genet* **5:** 327-337, 1993

The Wilson's disease gene product is expressed in liver and kidney with low or absent expression elsewhere. It encodes a 1,411 amino acid protein with 56% overall similarity to the Menkes' gene product. Thus, both appear to encode P-type ATPases with similarity to bacterial copper transporters. In *Enterococcus hirae* (previously termed *Strep. fecalis*), CopA has a proposed role in uptake of copper and CopB in extrusion of copper.[14] Both CopA and CopB resemble the Wilson's and Menkes' gene products, displaying 29% and 18% amino acid similarity in the ATPase domain by one method of calculation.[4] The degree of similarity was even higher using another method (43%).[14] This remarkable conservation between bacteria and man likely relates to the overwhelming need for tight control of copper levels to prevent toxicity.

Unlike Menkes' disease, preliminary studies indicate that defects are missense mutations and small deletions and insertions rather than large deletions that could be detected by DNA hybridization.[24, 25] In some patients, the mRNA is decreased below

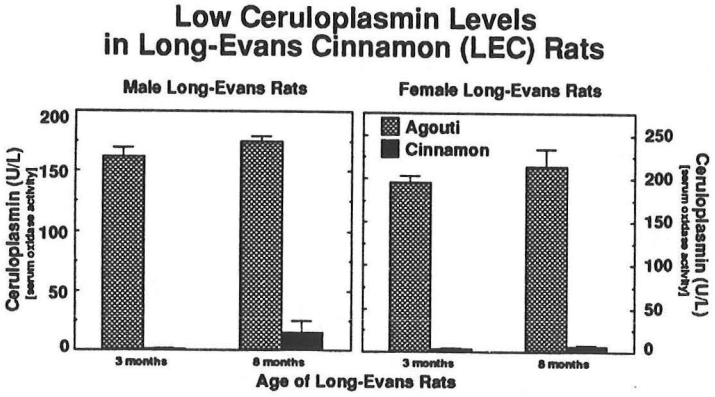


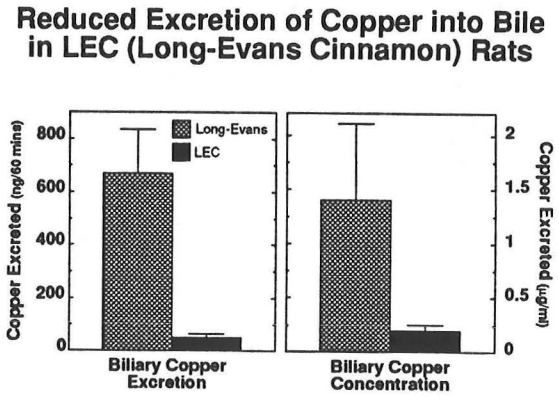
Modified from Bull and Cox, *Trends Genet* **10:** 246-252, 1994

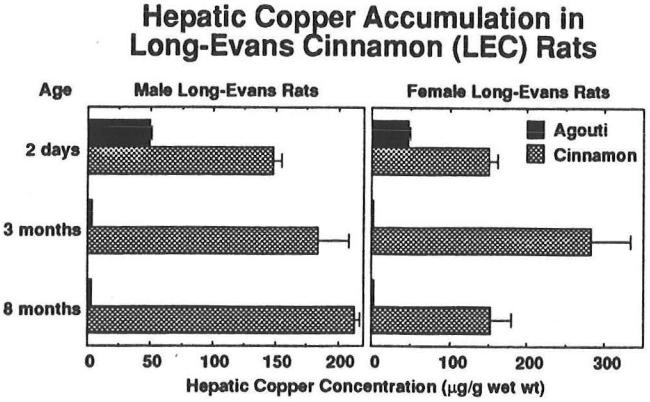
expected[26] whereas other patients demonstrate mutations predicted to interfere with copper transport.[24, 25] Whether allelic heterogeneity (ie multiple different mutations at the same locus) accounts for the clinical heterogeneity long apparent in Wilson's disease is not yet determined. However, the observations that manifestations are different within sibships sharing identical disease genes[1, 27] and between identical twins[28] strongly suggests that other genes and environmental factors are also likely important, perhaps altering the age of onset and characteristic presentation.

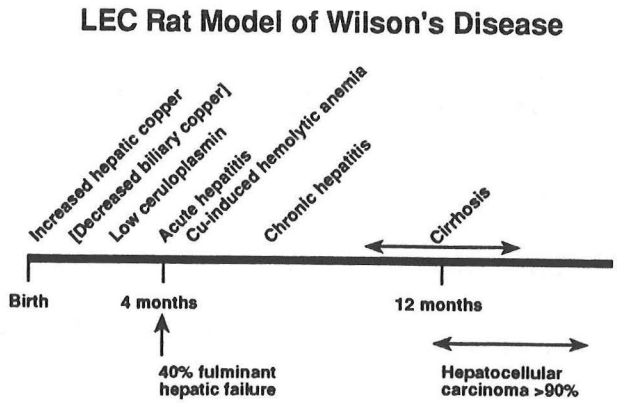
Animal Models of Wilson's Disease

Many experimental studies cannot be carried out in patients and an animal model that reflects the human disease can be extremely useful in determining molecular mechanisms and testing therapeutic regimens. In the Bedlington terrier, an autosomal recessive disorder leads to copper accumulation and fatal liver disease. However, there are no neurologic manifestations and ceruloplasmin levels are normal. Similarly, in toxic milk mice copper accumulates in the liver and cirrhosis ensues but there are no neurologic abnormalities. Recently, a new animal model for Wilson's disease was discovered. The Long-Evans Cinnamon rat derives from a closed colony of Long-Evans Agouti rats in Japan and is distinguished by coat color.[29] Initially, the rats were observed to develop acute hepatitis at ~4 months of age, from which ~40% died. The remainder progressed to chronic hepatitis, cirrhosis and hepatocellular carcinoma. On realizing that the histologic appearance resembled that of Wilson's disease, researchers examined copper metabolism in the rats.[30-32] They found low ceruloplasmin levels,[30] defective incorporation of copper into ceruloplasmin,[31] reduced excretion of biliary copper[32, 33] and markedly elevated hepatic copper concentrations.[30-33]









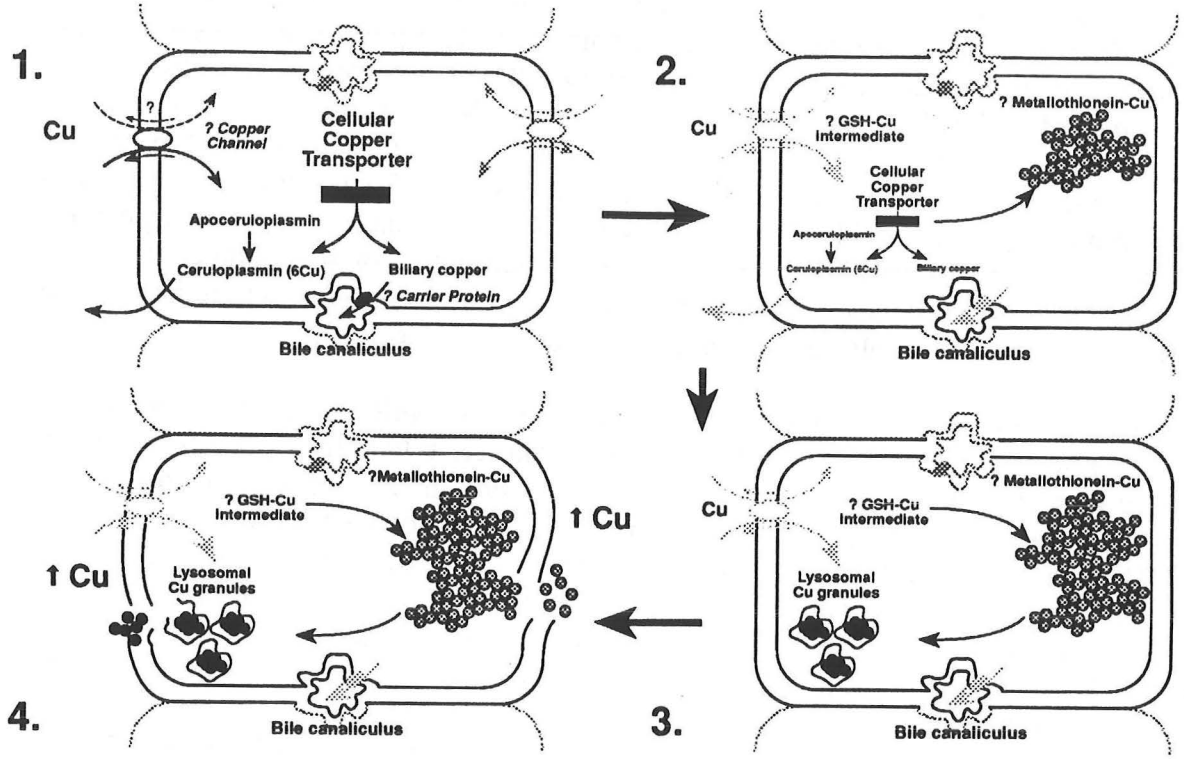
Once the Wilson's gene was identified, the sequence was used to examine the equivalent gene in the LEC rat. The gene responsible for hereditary hepatitis in the LEC rat, *hts*, (linked to rat chromosome 16) was cloned using the polymerase chain reaction and oligonucleotides from the conserved copper-binding domains.[34] The partial rat cDNA is shares 91% identity with the human counterpart. No transcript was detected in LEC liver RNA, either before or after development of cirrhosis whereas mRNA was present in other rat liver tissue. DNA hybridization analysis did not reveal any gross rearrangements. Further studies are needed to determine the nature of the defect in LEC rats. Regardless, this naturally-occurring animal model resembles Wilson's disease in the defects in copper incorporation into ceruloplasmin and biliary copper excretion with development of acute and chronic liver disease from toxic copper damage. Furthermore, the rat responds to D-penicillamine therapy and therefore will be a suitable model for therapeutic intervention.[35]

The rat disease is unlike the human disease in the low levels of copper in non-hepatic tissues, even after cirrhosis, and less neurologic disturbance. Whether this is a reflection of the specific mutation in the rat, thereby mimicking "pure" hepatic disease in man, or is explained by other differences, should be forthcoming now that the tools are available for analysis. The other major difference is the development of hepatocellular carcinoma in the survivors of the acute hepatitic episode. Hepatocellular carcinoma is so rare in Wilson's disease that each case, now numbering ~11, is reported together with a review of the literature.[1, 28, 36-38] Potential explanations for the low rate in Wilson's disease include quiescent liver disease when treated, however, before treatment was introduced there was not a high rate of hepatocellular carcinoma. Alternatively, increased iron (~3-fold) in LEC rat livers[39] may serve as an amplifying factor or an increased susceptibility to environmental carcinogen promoters may explain the findings. Rodent tissues are more readily transformed than human tissues, which may also contribute to the difference. The LEC rat does manifest other alterations (failure of $CD4^+CD8^+$ cells to mature to $CD4^-CD8^+$) and other, as yet unidentified, changes may account for the malignant transformation. Regardless, a new and exciting animal model for Wilson's disease studies is now available.

History of Wilson's Disease
Clinical Landmarks

1902	Pigmented corneal rings in "multiple sclerosis" - Kayser
1903	Pigmented corneal rings in "pseudosclerosis" - Fleischer
1912	Progressive lenticular degeneration and cirrhosis - Kinnier Wilson
1948	Excess liver & brain copper in Wilson's patients (JN Cumings)
1951	Copper chelation with BAL (JN Cumings)
1956	Penicillamine oral therapy (JM Walshe)

Evolution of Hepatocyte Copper Metabolism in Wilson's Disease



Clinical Presentations in Wilson's Disease

When the *WND* gene product is abnormal, copper may not be incorporated into ceruloplasmin correctly and biliary copper excretion is markedly diminished. The liver remains in a "neonatal" state, which predicts that the Wilson's gene product is not expressed until later in infancy. Copper initially is diffuse in the cytoplasm, likely complexed in a non-toxic form with metallothionein, the transcription of which is induced by copper.[6] Cu-metallothionein is also long-lived compared with other metallothioneins.[6] In this form, copper cannot be stained histochemically, despite being present in quite high amounts.[1]

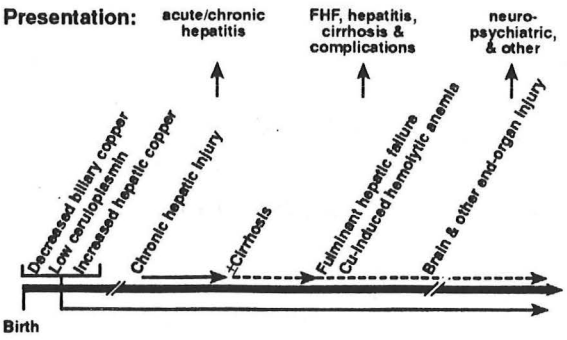
Later, copper appears in dense granules in lysosomes, perhaps in polymerized complexes of Cu-metallothionein.[1] The copper in these granules stains with rhodanine and copper-associated protein is detected with orcein and victoria stains. Hepatocytes sustain injury from the accumulated copper and chronic hepatitis ensues. Copper released into the circulation from damaged hepatocytes can also then affect other tissues. Finally, sufficient disease in the liver, brain or other organs leads to the development of symptoms and signs of copper-induced toxicity.

Clinical Presentations of Wilson's Disease
Recent Case Series*

Series	Hepatic	Neuropsychiatric
U.S. (Brewer 1992)	17/70 (24%)	53/70 (76%)
U.K. (Walshe 1989)	87/220 (40%)	133/220 (60%)
Sardinia (Giagheddu 1985)	30/53 (57%)	23/53 (43%)
Israel (Bonné-Tamir 1990)	35/48 (73%)	13/48 (27%)

* excluding pre-symptomatic individuals detected by family screening

Wilson's Disease - Evolution of Clinical Manifestations

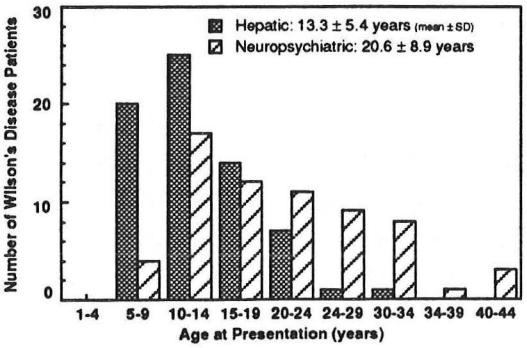


The vast majority of patients present with hepatic or neuropsychiatric illness. The distribution of cases is linked to the age of the patient, with hepatic disease presenting an average of 5-10 years earlier. Thus, in the patients reported by Scheinberg and Sternlieb,[1] the average age for hepatic presentation was 13.3 years whereas for neuropsychiatric symptoms it was 20.6 years. Similarly, in Israel hepatic disease was seen from ages 18 months to 19 years (average 10.4 years) and onset of neuropsychiatric disorders was from 12 years to 28 years (average 19.1 years).[27] Findings were analogous in Sardinia, where a review of patients with Wilson's disease from 1902 to 1983 found an average age of 12 years 11 months for hepatic onset and 19 years 4 months for neurologic onset.[40]

Hepatic Presentations of Wilson's Disease

Clinically, hepatic presentations of Wilson's disease can be abrupt and devastating or insidious. Of 87 cases reviewed by Walshe, with either initial hepatic illness or presentation with symptomatic liver disease, 32 (36%) had an acute onset.[41] Liver disease had resolved in 22/87 (25%) when they presented with neuropsychiatric disease and the remainder had symptomatic chronic liver disease without an acute decompensation. Pathologic changes in the liver are subtle early, with only glycogen nuclei and fatty infiltration (triglyceride) apparent on light microscopy.[1, 42]

Age Distribution of Patients at Presentation with Wilson's Disease



Age at Presentation with Wilson's Disease
Effect on Clinical Type

Presentation	<10 years	10-19 years	>19 years
Liver Disease	20/25 (80%)	39/84 (46%)	9/44 (20%)
Neuropsychiatric Disease	4/25 (16%)	29/84 (35%)	32/44 (73%)
Other: (hematologic, endocrine, renal)	1/25 (4%)	16/84 (19%)	3/44 (7%)

Data from Scheinberg and Sternlieb, Wilson's Disease *Major Problems in Internal Medicine* **vol. XXIII**, 1984

Characteristic mitochondrial changes are seen on electron microscopy,[1] but like all other pathologic findings they may not be pathognomonic.[43, 44] Progression to chronic hepatitis, fibrosis and cirrhosis occurs at a variable rate. In some patients (20/320 or 6%), the findings on liver biopsy are indistinguishable from chronic hepatitis resulting from other etiologic agents.[45] More commonly, inflammatory changes are less marked and fibrosis progresses more insidiously. When cirrhosis is established, the pattern can be micronodular, macronodular or mixed and rhodanine staining may be positive (usually patchy) as well as staining for copper-associated protein.[42, 46]

The trigger for an abrupt onset of liver disease is not known. Sometimes, a viral illness or viral hepatitis may precede the severe acute hepatitis.[47, 48] Hemolytic anemia (Coombs negative) is often part of the clinical picture, from copper-mediated red cell injury. A disproportionately high bilirubin may then ensue. The combination of high bilirubin (>10mg/dl), increased hepatic aminotransferases (>200IU/L) and prolonged prothrombin time (>12 seconds prolonged) portends a poor prognosis.[49] Some of the patients have an abnormally low alkaline phosphatase,[50, 51] the mechanism for which is unknown. Investigators have suggested that the combination of low alkaline phosphatase and disproportionately high bilirubin (ratio of alkaline phosphatase to bilirubin >2) may distinguish patients with Wilsonian fulminant hepatic failure from those with other etiologies.[52] Unfortunately, this was not confirmed.[53] The outcome for this presentation is dismal unless liver transplantation is available.[41]

Autopsy or examination of the explanted liver almost always reveals cirrhosis,[54] occasionally nodular regeneration[55] or chronic hepatitis changes[56] are observed. At King's College Hospital, 8/11 were micronodular, 2/11 mixed and 1/11 was macronodular, all were cirrhotic. Other features of Wilsonian liver injury, including fat, cell necrosis, inflammation, Mallory bodies, cholestasis and ductular proliferation, are seen. Staining for copper-associated protein is usually positive whereas rhodanine staining for copper is variable.[54]

Neuropsychiatric Wilson's Disease

Excess copper released from the liver accesses the central nervous system and can be found throughout the brain.[1] Specific clinical symptoms then appear in patients. However, Walshe has observed that "no two patients are ever the same, even in a sibship"[57] with respect to neurologic disease. Notwithstanding, he divided 136 patients into 4 categories.[57] The "Parkinsonian" patients (61/136; 45%) were distinguished by paucity of expression and movement. The "pseudosclerotic" patients (33/136; 24%) had a tremor resembling multiple sclerosis. The dystonic group (21/136; 15%) were characterized by hypertonicity, this was often associated with abnormal limb movements whereas in the choreic group (15/136; 11%) choreo-athetoid or choreic abnormal movements predominated but these were often associated with dystonia. Only 6 patients with intermediate symptomatology could not be classified into one of these 4 groups.

Anatomic changes, degeneration and cavitation, principally involve the putamen, globus pallidus, caudate nuclei and thalamus.[1, 2] Less commonly, the brain stem and frontal cortex are involved. Specific structural changes can be detected neuroradiologically by computed tomography or magnetic resonance imaging,[58] in addition these modalities often demonstrate generalized atrophy and ventricular dilatation.[59, 60] Correlation between imaging defects and symptom complexes is primitive.[58, 59] The sparing of sensory and motor

(strength) pathways in most patients with diffuse increases in brain copper is unexplained as is the devastating dysfunction of the basal ganglia and cerebellum.[1] Most patients with neurologic disease have established cirrhosis,[1] however, some (7/34 reviewed by Stromeyer and Ishakin reference 42) may only have fatty change on liver biopsy.

Psychiatric features are common being present in 51% (99/195) of the "Cambridge series" of whom 39/195 (20%) had seen a psychiatrist.[61] Abnormal behavior, personality change, depression and cognitive impairment were the frequent manifestations. Not unexpectedly, psychiatric features were related to neurologic symptoms rather than hepatic symptoms. Similarly, 65% (24/37) of U.S. patients reported psychiatric symptoms on initial presentation.[62] A continuing awareness of Wilson's disease as a cause of psychiatric and neurologic symptoms is obviously necessary.

Kayser-Fleischer rings are generally considered a *sine qua non* of neuropsychiatric Wilson's disease.[1] However, occasional patients do not have K-F rings despite neuropsychiatric symptoms.[42, 63] Copper in the cornea probably forms complexes with protein (?metallothionein) in Descemet's membrane. The rings appear initially in the superior quadrant, then inferior quadrant followed by medial and lateral quadrants and disappear in reverse order with chelation therapy. Copper contents are uniformly elevated throughout the cornea and the ring appears to depend on the presence of the protein that forms the visible complex. Any cause of increased copper, either local or from systemic release, can lead to their formation.[64, 65]

Renal and Other Manifestations of Wilson's Disease

Since the Wilson's disease gene is expressed in kidney tissue, renal manifestations may be primary, or secondary to release of copper from the liver. Clinically, patients may resemble Fanconi's syndrome with aminoaciduria, glucosuria, (also fructose, galactose and pentose), uricosuria and accompanying low uric acid, hyperphosphaturia, hypercalciuria and distal renal tubular acidosis.[1, 66] Urolithiasis, hematuria and nephrocalcinosis[67, 68] are reported and proteinuria and peptiduria can occur both before treatment as part of the disease process and after therapy as a side-effect of D-penicillamine. The distinction between primary and secondary effects of the Wilson's disease gene abnormality may be answered in the LEC rat which also demonstrates an increase in copper in the kidneys.[30, 69]

Almost any organ system can be damaged by excess copper and lead to symptoms in Wilson's disease. Musculoskeletal complaints include osteopenia (both osteoporosis and osteomalacia),[70] arthritis or arthralgias with chondrocalcinosis or osteoarthritic changes[71] and spinal degeneration.[72] Cardiac arrhythmias and cardiomyopathy were previously underestimated.[73] Coagulopathy from liver disease, leukopenia or thrombocytopenia from hypersplenism as well as the hemolytic anemia of acute disease can lead to a hematologic presentation.[74] Primary or secondary amenorrhea[75] and recurrent abortion can result from Wilson's disease and be reversed by therapy.[1] Other uncommon presentations and complications include hypoparathyroidism[76] and exocrine pancreatic insufficiency.[1] Any internist or medical specialist may be presented with a new patient with Wilson's disease!

"Atypical" Cases of Wilson's Disease

As noted above, most patients with Wilson's disease present in the first two decades of life and few patients are more than 40 years of age at presentation. Thus, 3/153 (2%) patients reported by Scheinberg and Sternlieb and none of 136 patients reviewed by Walshe were older than 40. However, there are isolated case reports, particularly of neuropsychiatric illness presenting in the late forties to mid-fifties,[28, 77] sometimes with symptoms dating back years.[28] Of particular note, Danks *et al* reported four patients, aged between 43 and 58 years, who had hepatic disease alone.[78] Together with a similar patient, manifesting only hepatic disease at age 55 years,[79] these cases illustrate some of the clinical heterogeneity associated with Wilson's disease. Hopefully, the molecular explanation for such observations will soon be known.

The other "atypical" cases are those with neuropsychiatric illness yet lacking Kayser-Fleischer rings. A unilateral K-F ring was observed in one patient who had sustained an injury to the other eye resulting in low pressure and presumed decreased deposition of copper.[28] In another atypical case, there was marked arcus senilis,[80] possibly obscuring or preventing K-F ring formation. In two series, however, K-F rings were absent in 4/27 and 3/18 patients with neuropsychiatric disease.[42, 63] Clearly, no broad generalizations hold true for every patient currently diagnosed with Wilson's disease. In the future, identification of the exact genetic defect(s) may permit a more complete understanding of the pathogenesis. Alternatively, rare genetic defects in other molecules involved in biliary copper excretion may account for the similar presentation.

Diagnosis of Wilson's Disease

No single clinical finding or combination of clinical findings is sufficient, in itself, to diagnose Wilson's disease. None of the symptoms or signs of liver, neurologic or psychiatric disease is unique. The observation of Kayser-Fleischer rings is helpful in the setting of neuropsychiatric illness. However, in other cholestatic liver diseases K-F rings can occasionally be observed.[64, 65] Furthermore, even in neuropsychiatric disease, they are not invariably present (see above). Presymptomatic individuals, almost by definition, will not be detected

Clinical Diagnosis of Wilson's Disease

Characteristic	Wilson's Disease	False Negative	False Positive
Liver disease	hepatic or "mixed" presentation only	neurologic presentation (inactive)	other liver disease
Psychiatric disease	incidence increases with age	"pure" hepatic presentation; presymptomatic	other psychiatric disease
Neurologic disease	incidence increases with age	"pure" hepatic presentation; presymptomatic	other neurologic disease
K-F rings	>>95% neurologic <90% hepatic <50% presymptomatic	"pure" hepatic presentation; presymptomatic	cholestatic liver disease

Laboratory Diagnosis of Wilson's Disease

Assay	Wilson's Disease	False Negative	False Positive
↓ Ceruloplasmin	<95%	>5% "acute phase response"	20% heterozygotes low protein states severe liver disease Menkes' disease hereditary
↑ Urine copper	>95%	presymptomatic	cholestatic liver disease
↑ Hepatic copper	>95%	variability in tissue technical error	chronic cholestasis Cu-associated cirrhosis hereditary cholestasis
↓ Cu into ceruloplasmin	>>95%	"acute phase response"	heterozygotes low ceruloplasmin

by clinical means, although some will display K-F rings.[81, 82] The diagnosis of Wilson's disease thus relies on a series of investigations that test copper metabolism in the individual.

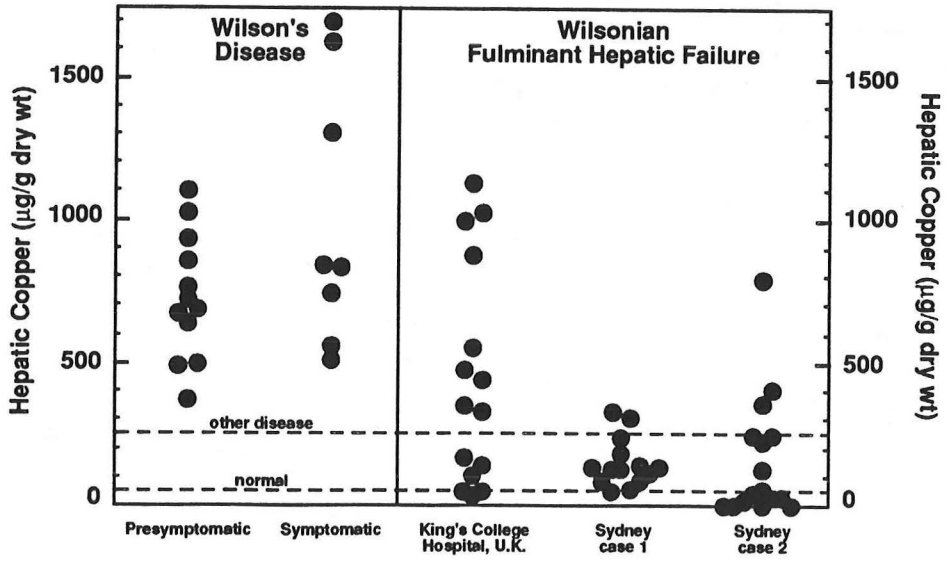
The mainstays of diagnosis are the measurements of ceruloplasmin, urine copper and hepatic copper. In some special circumstances in the past, the kinetics of detection of orally ingested radiolabeled copper in serum was utilized to diagnose Wilson's disease in individuals with normal ceruloplasmin and to distinguish heterozygotes with low ceruloplasmin. This assay was never readily available and will likely be replaced by genetic techniques.

1. **Ceruloplasmin:** A low level is found in ~95% of patients with Wilson's disease. However, as a screening test it has a number of drawbacks. First, 20% of true heterozygotes have a low ceruloplasmin but no disease.[1] Secondly, other non-specific causes of a low level such as protein deficiency states and protein-losing disorders are much more common in the population than is Wilson's disease. Finally, changes in ceruloplasmin levels as part of the acute phase response or in response to estrogens may interfere with detection.

2. **Urine copper:** Elevated levels of copper are probably observed in all symptomatic patients with Wilson's disease if the collection is complete and there are no technical errors. Unfortunately, the height of the elevation may not always be sufficiently different from that observed in cholestatic liver disease to separate diagnoses completely.[83] With that as a caveat, this is probably the most cost-effective way of screening cooperative individuals by non-invasive means.

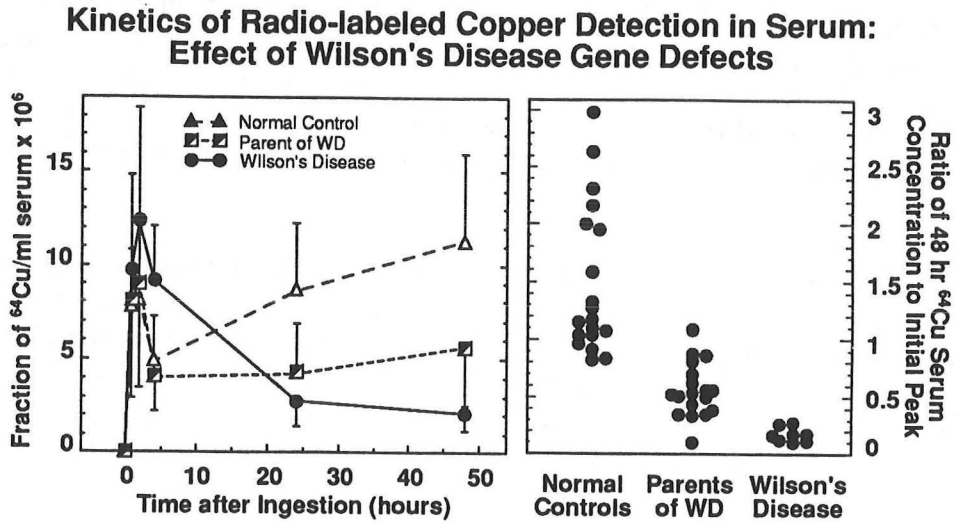
3. **Hepatic copper:** This measurement, when taken together with the non-invasive assays of urine copper and ceruloplasmin, allows a definite diagnosis to be made in most patients with Wilson's disease. Histopathologic findings are of interest but are never diagnostic and staining for copper may be misleading to the naive. Problems arise with technical errors (saline in the biopsy collection method can leach copper - dextrose does not) and with sampling, particularly in fulminant disease where necrosis and collapse can decrease copper

Elevated Hepatic Copper Levels in Wilson's Disease: Variability in Fulminant Hepatic Failure

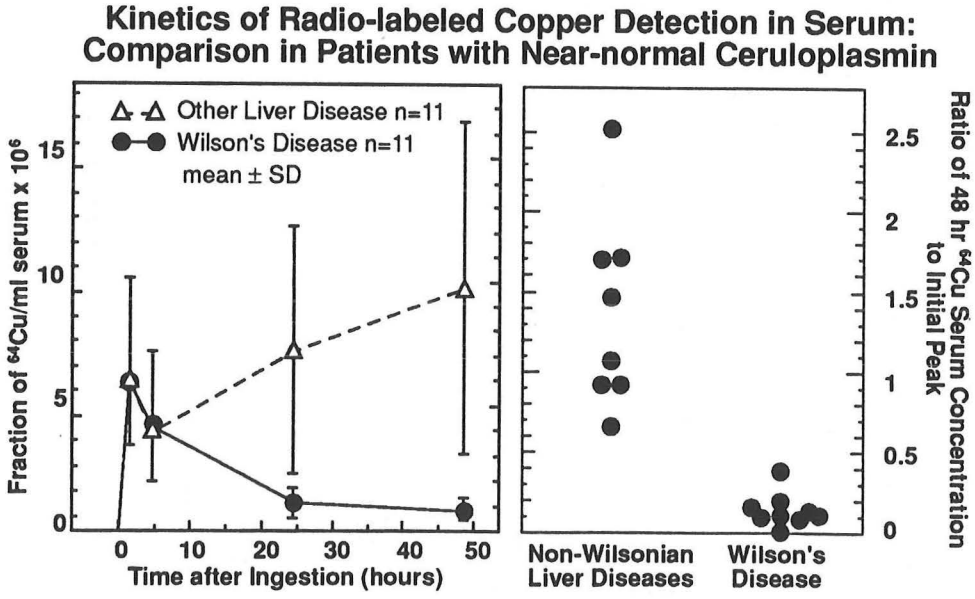


content. In two cases of Wilsonian fulminant hepatic failure that underwent liver transplantation, multiple samples were obtained from the explanted liver. In only 2/14 and 3/16 samples were copper levels diagnostic.[84] Similarly, in 21 patients transplanted at King's College Hospital for Wilsonian fulminant hepatic failure, levels of hepatic copper were not diagnostic in 6 patients and varied widely when more than one sample was obtained in others.[53] Causes of elevated hepatic copper, including chronic cholestatic liver disease in adults[83] and other hereditary forms of chronic cholestasis in children,[85] need to be excluded. In individual cases, particularly without a family history, this can be difficult and in future may be solved by direct genetic testing.

4. **Radiocopper detection in serum:** This test provided the first means of directly assaying hepatic copper metabolism.[86, 87] After oral ingestion of radiolabeled copper (^{64}Cu or ^{67}Cu), blood is collected at 1-2, 4, 24 and 48 hours for measurement of radioactivity in serum. There is prompt appearance of radioactivity after absorption, followed by hepatic clearance



Data from: Sternlieb *et al*, *J Clin Invest* **40:** 707-715, 1961



Data from: Sternlieb & Scheinberg, *Gastroenterology* **77:** 138-142, 1979

and then later re-appearance (as copper in ceruloplasmin). In patients with Wilson's disease, the radioactivity did not reappear. Heterozygotes were distinguished from homozygotes by a slow, lower level re-appearance of radioactivity rather than continued fall in radioactivity but there was overlap.[86] A ratio was applied, using the level at 48 hours to the initial peak level to counter some of the differences in absorption that occurred regardless of the presence or absence of disease. Some overlap still persisted between heterozygotes and patients with Wilson's disease but normals and Wilson's disease patients were clearly distinguished. When patients with Wilson's disease and normal or near-normal ceruloplasmin levels were compared with patients with non-Wilsonian liver disease, a distinction was still apparent.[87]

5. **Genetic diagnosis:** In family studies, linkage analysis has been used in presymptomatic testing.[82] By using highly polymorphic microsatellite DNA markers that closely flank the gene, a correct diagnosis can be obtained in most families if tissue from the proband is available.[88] Individual studies are more problematic since linkage analysis is not possible without informative family members. Atypical patients may be diagnosed in the future but preliminary studies indicate that the array of mutations to be screened is likely to be large. Consequently, detection of single strand conformational polymorphisms or heteroduplex analysis will probably be necessary. Silent, inconsequential polymorphisms in the general population may be a major problem in this approach. The multiplicity of mutations also currently precludes the use of a genetic technique in screening studies.

6. **Pre-symptomatic patients:** Two series of patients with diagnosis of Wilson's disease in pre-symptomatic siblings have been reported.[81, 82] The findings are similar to those in patients with active disease, hepatic copper being the most useful. In these individuals, there is no possibility of another liver disease to explain the elevated hepatic copper levels. Non-invasive testing was not able to detect all cases.

7. **"Atypical" patients:** In two case reports, the diagnosis of Wilson's disease was excluded on the basis of absence of K-F rings and/or lack of cirrhosis on biopsy.[43, 89] As discussed above, the literature contains other cases where a diagnosis of Wilson's disease has been made despite an "atypical" presentation. I consider that both cases may represent Wilson's disease.

Diagnosis of Pre-symptomatic Wilson's Disease*

Series	K-F rings (present)	Cerulo-plasmin (low)	Free Copper (high)	Urine Copper (high)	Liver Copper (high)
U.S. (Brewer 1991)	4/13	12/13 4/12 intermediate	5/13 >25µg/dl	8/13 >100µg/24 hrs	13/13 >225µg/g dry wt
U.K. (Walshe 1988)	7/21	21/21	21/21 >10µg/dl	20/21 >30µg/24 hrs	5/5 >50µg/g WET wt

* individuals detected by family screening (distinguished from heterozygotes)

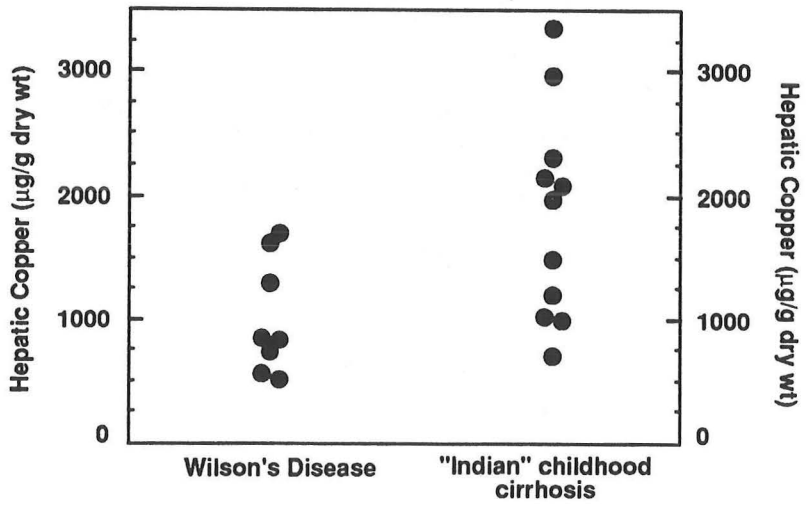
Is the Diagnosis Wilson's Disease?

Finding	Normal Control	Case #1	Case #2
Liver Disease	no	yes age 62	no
Neuropsychiatric Disease	no	yes age 63	yes age 34
K-F rings	no	no	no
Ceruloplasmin	20-40 mg/dl	normal	16.3mg/dl (nl 15-60)
Urine copper	<100µg/d	normal	82µg/d
Hepatic copper	<50µg/g	671µg/g	2500µg/g

Non-Wilsonian Copper-associated Cirrhosis

The disorder, termed Indian childhood cirrhosis or copper-associated cirrhosis since its description in non-Indian infants and children, is of particular interest in relation to Wilson's disease.[3] Originally observed in male infants in the Indian sub-continent, this syndrome is one of rapidly progressive liver disease with fatal outcome in months to a few years after presentation. The liver contains extremely high levels of copper but unlike Wilson's disease there are no neurologic signs and ceruloplasmin levels are not low. Whether this is strictly an environmental disease, resulting from copper-contamination of food from cooking utensils or water, is debated.[90, 91] In cases reported from outside India, those with presentation during infancy often include a potential environmental contaminant whereas cases occur in older children without such exposure.[91] A recent survey of childhood deaths in areas of Massachusetts with high copper content of the water supply failed to document the occurrence of a similar disease.[92] There may be both an environmental component, resulting in presentation during infancy, as well as a familial factor, represented by consanguinity in some cases.

Elevated Hepatic Copper Levels in Wilson's Disease and Copper-associated Cirrhosis



The lack of neurologic findings may be the result of death at an early age before symptoms could develop. Alternatively, a different part of the biliary copper excretion pathway may be involved and/or the copper released from the liver may be in a form that is non-toxic to other organs or cleared rapidly by other means. Further examination of these cases could increase our understanding of the remaining enigma of biliary copper transport.

Management of Wilson's Disease

The major goal of management in Wilson's disease is to decrease the excess copper in the liver and other organs.[1-3] Four measures are use to achieve this goal. First and most important is copper chelation therapy to increase urinary excretion of the excess copper. Secondly, rendering copper non-toxic by complexing with proteins, eg formation of Cu-metallothionein. Thirdly, decreasing dietary copper intake as much as feasible, by avoiding or minimizing consumption of foods high in copper such as shellfish, liver, nuts, chocolate and mushrooms. Finally, decreasing the absorption of copper and thereby diminishing toxic reaccumulation. Additionally, ancillary therapy directed towards specific symptoms may be necessary. Psychotherapy and/or psychotropic medicine may be required for management of psychiatric symptoms. Furthermore, standard measures for management of complications of cirrhosis are often a necessity. Correction of the underlying defect by gene therapy may be part of future management options. Currently, the closest approach to correcting the defect is that of liver transplantation. However, since the projected survival of patients treated with the combination of medical therapies to decrease excess copper is greater than that of transplant recipients, transplantation is generally reserved for those with life-threatening acute or chronic hepatic insufficiency.

Copper Chelation Therapy of Wilson's Disease

In 1948, Cumings reported that there was excess copper in both the liver and brain of patients with Wilson's disease. He followed this observation in 1951 with the first therapy, parenteral injection of 2,3-dimercaptopropanol (British anti-Lewisite). Although potentially effective, the daily parenteral injections were tedious and painful. A major breakthrough came in 1956 with the introduction of oral therapy (D-penicillamine, β,β-dimethylcysteine) by Walshe.[66] Since then, other drugs with chelation properties have been introduced for patients with hypersensitivity reactions to penicillamine. These include triethylene tetramine dihydrochloride (Trientine)[93, 94] and tetrathiomolybdate,[95, 96] however penicillamine remains the mainstay of therapy for most patients.[66]

Penicillamine (1 gram/day in 2 to 4 divided doses for adults) is best absorbed if taken on an empty stomach and most effective if ingestion is separated from dietary copper (1½ hours before or 2 hours after a meal). Penicillamine binds free copper and increases renal excretion. Penicillamine may also induce the synthesis of apo-thionein, thereby increasing the content of non-toxic Cu-metallothionein.[97] Studies *in vitro* suggest that either penicillamine alone or the copper-penicillamine complex may be involved in the process of induction and that penicillamine does not remove copper once bound in Cu-metallothionein. Penicillamine is slow to be effective, with noticeable improvement taking weeks to months. In 20-30% of patients, there may be an initial worsening of symptoms considered secondary to changes in copper distribution in the brain.[98] The deterioration does not always resolve. If severe neurologic impairment was present before therapy, this can leave devastating

D-Penicillamine Therapy of Wilson's Disease
Responses of Neurologic Disease

Response	Residual Deficits	Number	%
Excellent	None (symptom-free)	57/137	42%
Good	Minor	36/137	26%
Poor	Disabled (process arrested)	24/137	17%
None → died	9 virtually untreated 11 despite therapy	20/137	15%
Transient deterioration	Final prognosis unaltered	30/137	22%

residual deficits. Walshe recently reviewed the response of 137 patients with neurologic symptoms to chelation therapy, mostly with D-penicillamine.[98] In two-thirds of patients, there was total or near total recovery. However, in the remaining third, disability persisted or the patient died. In 11/20 patients, no response was apparent and they succumbed despite apparently adequate therapy. Liver copper levels at autopsy in 6 of these patients were decreased to $<100\,\mu g/g$ wet wt (normal $<10\,\mu g$; heterozygotes $<50\,\mu g$). However, basal ganglia copper concentrations were in the toxic range in 4 patients, indicating that chelation therapy had failed to mobilize copper from the brain. Penicillamine does not restore liver copper content to normal[99] but rather converts the remainder to a non-toxic form. The continued presence of penicillamine is apparently necessary to maintain the copper in this state. Consequently, irreversible hepatic failure invariably follows discontinuation of therapy, after a period of months to a few years[1, 100] (a considerably shorter interval than the time taken to develop symptoms from birth).

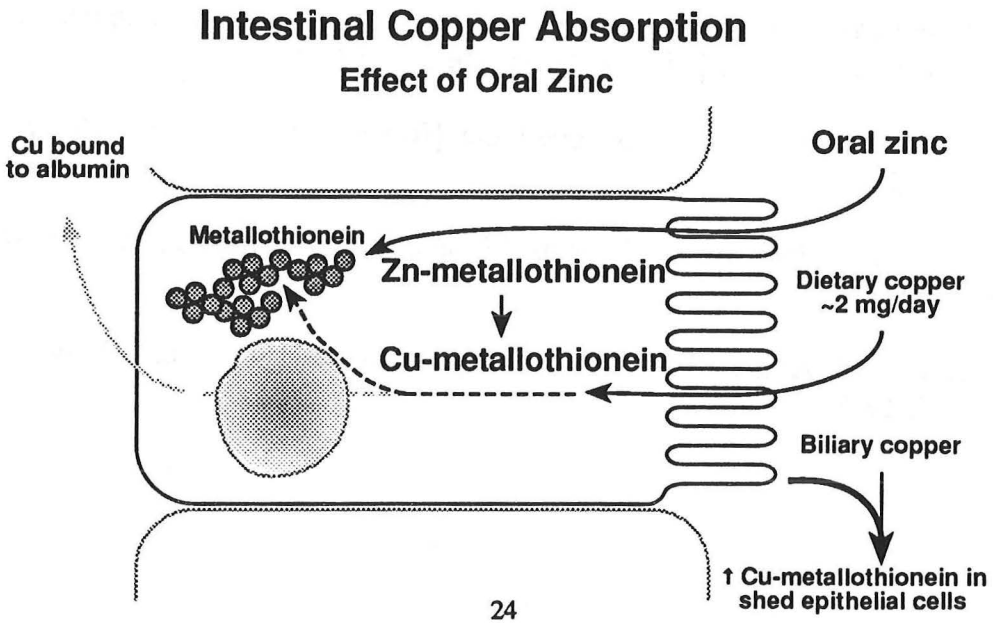
Early side-effects may limit penicillamine therapy in up to 20% of patients. These hypersensitivity reactions include fever, rash, lymphadenopathy and cytopenias. Side-effects are often controlled by dose reduction with or without a short course of corticosteroids in conjunction with the re-introduction of the drug. However, in some patients the severity of the side-effects necessitates changing to the second line therapeutic agent, trientine. Late reactions to penicillamine occur in 5-10% of patients. These include elastosis perforans, aphthous mouth ulceration and nephrotic syndrome. Goodpasture's syndrome, systemic lupus erythematosus, myasthenia gravis, polymyositis, neuromyotonia, cytopenias (red cells, white cells and platelets), decreased IgA levels and cholestasis have been reported.[2] Following urinalyses and complete blood counts is warranted at regular intervals. Side-effects were more common initially, when mixed isomers (D- and L-) were used as compared with the current single isomer, D-penicillamine. D-penicillamine has been used successfully during pregnancy in patients with Wilson's disease. Continuation of therapy, rather than temporary cessation is important, to avoid hepatic decompensation.

Trientine (triethylene tetramine dihydrochloride, trien)[93, 94] was introduced by Walshe in 1969 as an alternative therapy for patients with intolerable side-effects from penicillamine. It may differ in mode of action from penicillamine since serum copper levels rise with trientine but not penicillamine therapy.[2] Like penicillamine, trientine has been successfully

used in pregnancy.[101] Other chelating agents with limited application include unithiol (2,3-dimercaptopropane-1-sulphonate) derived from BAL, 2,3,2-tetramine and N,N´-bis-(2-aminoethyl)-1,3-propanediamine. Currently, these agents are limited to experimental protocols.

Tetrathiomolybdate MoS_4 was first reported of potential benefit in the 1980's by Walshe. It blocks absorption of copper and also forms non-toxic complexes with copper and plasma proteins after absorption or when given intravenously.[95] Unlike penicillamine, tetrathiomolybdate removes copper from Cu-metallothionein *in vitro* and *in vivo* is an extremely effective chelator. It may be useful in rapidly removing copper redistributed/released early in penicillamine therapy when deterioration ensues.[95] Long-term therapy with tetrathiomolybdate may be complicated by bone marrow suppression and skeletal growth disturbances, limiting its usefulness.

Zinc salts (acetate or sulfate) for treatment of Wilson's disease are advocated by a number of investigators. The rationale for their use is the capacity of zinc to induce apothionein transcription and synthesis, in intestinal epithelial cells and other tissues including liver. Copper then displaces zinc, forming a non-toxic Cu-metallothionein compound. In the gastrointestinal tract, the copper is excreted when epithelial cells are shed. A major effect of zinc therapy is therefore to block absorption of dietary copper. In the liver, the copper is complexed and non-toxic, an additional effect over and above that of preventing absorption. Zinc is relatively innocuous when compared with D-penicillamine, however, some patients are troubled by gastrointestinal symptoms, particularly with zinc sulfate but also to a lesser extent with the acetate salt. The place for zinc in the therapeutic armamentarium may be in individuals who have been successfully "de-coppered" by chelating agents and as a temporary measure during pregnancy. Proponents of zinc therapy, however, report that there is no worsening of neurologic symptoms during initial therapy unlike treatment with penicillamine. There outlook may be naive if insufficient patients with neurologic symptoms have been treated initially with zinc. This possibility is suggested by a recent report of fatal deterioration in a patient with neurologic symptoms treated with zinc after earlier discontinuation of penicillamine. The known record of penicillamine is preferable to the unknown possibilities attendant upon initial therapy with zinc.



Liver Transplantation

When inexorable hepatic insufficiency ensues, either with the acute onset of fulminant hepatic failure or as a complication of long-standing cirrhosis (with or without discontinuation of chelation therapy), liver transplantation is the only management option available.[100] Of 55 patients with Wilson's disease undergoing transplantation world-wide, 21 (38%) presented with fulminant hepatic failure.[2] In most of these patients, penicillamine therapy is not an option because of the delay in beneficial effects. Tetrathiomolybdate has also been used, because of its potency in chelating copper, but to no avail. The severity of elevated levels of bilirubin and aminotransferases, together with prolongation of the prothrombin time, are potentially useful in predicting poor prognosis without transplantation. Transplantation was also used in 32 patients with cirrhosis (6 having discontinued medical therapy) and hepatic failure. In some (but not all) patients with residual neurologic deficits at the time of transplant, there is improvement in symptoms.[2, 102] Transplantation was also used to manage recurrent variceal hemorrhage in one patient, historically portal-systemic shunt surgery is complicated by development of neurologic symptoms. Since the major defect in Wilson's disease is failure of hepatic transport of copper, transplantation resolves most or all of the genetic defect. However, medical therapy remains superior to transplant in most patients.

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