MINISTRY OF HEALTH OF THE REPUBLIC OF MOLDOVA

PUBLIC INSTITUTION
STATE UNIVERSITY OF MEDICINE AND PHARMACY
NICOLAE TESTEMITANU

Adela TURCANU Elina BERLIBA

WILSON'S DISEASE. BASIC FACTS

Guidelines for students

CHISINAU
2014

MINISTRY OF HEALTH OF THE REPUBLIC OF MOLDOVA

PUBLIC INSTITUTION
STATE UNIVERSITY OF MEDICINE AND PHARMACY
NICOLAE TESTEMITANU

Department of Internal Medicine
Gastroenterology and Hepatology

Adela TURCANU Elina BERLIBA

WILSON'S DISEASE. BASIC FACTS

Guidelines for students

CHISINAU
CEP *Medicina*
2014

CZU [616.36-002+616.411]-056.7(075.8)
Ţ 94

Recommended for publishing by Central Methodical Council of the PI State University of Medicine and Pharmacy *Nicolae Testemiţanu*, protocol no 3 from 06.03.2014

Authors:
Adela Turcanu, MD, associate professor
Elina Berliba, MD, associate professor

Referenced:
Valentin Calancea, MD, associate professor
Iurie Moscalu, MD, associate professor

Ilustrated by: *Adela Turcanu*
Redactor: *Nadejda Şamşurina*
Typesetting: *Iulia Don*

DESCRIEREA CIP A CAMEREI NAŢIONALE A CĂRŢII

Turcanu, Adela.
Wilson's disease. Basic facts: Guidelines for students / Adela Ţurcanu, Elina Berliba; Publ. Inst. State Univ. of Medicine and Pharmacy *Nicolae Testemiţanu*, Dep. of Internal Medicine Gastroenterology and Hepatology. – Chişinău: CEP *Medicina*, 2014. – 65 p.
Referinţe bibliogr.: p. 59(80 tit.). – 50 ex.
ISBN 978-9975-118-36-1.
[616.36-002+616.411]-056.7(075.8)
Ţ 94

ISBN 978-9975-118-36-1

CONTENTS

PREFACE

The publication of the ***Wilson`sdisease. Basic facts*** combines an important synthesis of clinical and theoretical matters with the major advances in genetics, diagnosis, imaging, and therapeutics.

This book intends to represent the management of Wilson disease based of evidence-based medicine and astute clinical experiences from leading international authors.

Recognition ofWilson's disease is challenge number one facing Wilson's disease patients and their physicians today, and this book is designed to help clinicians recognize the possible (or likely) diagnosis and help these patients. Intended for physicians, these recommendations suggest preferred approaches to diagnostic, therapeutic, and preventive aspects of care. They are intended to be flexible, in contrast to standards of care, which are inflexible policies to be followed in every case.

A significant problem with the literature on Wilson disease is that patients are sufficiently rare to carry out large cohort studies or randomized controlled trials; moreover, most treatment modalities were developed at that time when drug assessment requirements were less strict than at present.

Finally, after the study of there guidelines we hope that you can:

- Diagnose of Wilson disease (WD) in a patient with unexplained liver disease
- Diagnose of WD in a patient with a neurological disorder or psychiatric disease with or without liver disease
- Screen for WD in a sibling or child of a patient with a precise diagnosis of WD

A ***Wilsondisease. Basic facts*** will have a great appeal to students, residents/fellows andinternists interested in gastroenterology.

HISTORICAL FACTS

Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism leading to the accumulation of this metal in different organs and tissues. Hepatic and neurological symptoms are the main clinical features of the disease [4, 31, 40].

Some authors attribute to *FT Frerichs* first case report of Wilson Disease, *in 1860-61.* The patient described by **Frerichs** [74] was *a 9-year-old boy who developed neurological symptoms characterized at the beginning by speech changes and difficulty to control the movements of the limbs. He later developed intention tremor, difficulty in swallowing and died at the age of 10. The autopsy revealed abnormalities compatible with cirrhosis of the liver.*

Westphall et al. in 1883 [77], reported the study of *two cases which had as the main neurological manifestation a tremor similar to that seen in patients with multiple sclerosis but their necropsy did not show the typical white matter lesions of this disease, well-known at that time.* For this reason, Westphall used the term "pseudosclerosis" for this new disease.

Strümpell (1899) reported *three other cases of pseudosclerosis and the pathology of the third one revealed the presence of cirrhosis of the liver.*

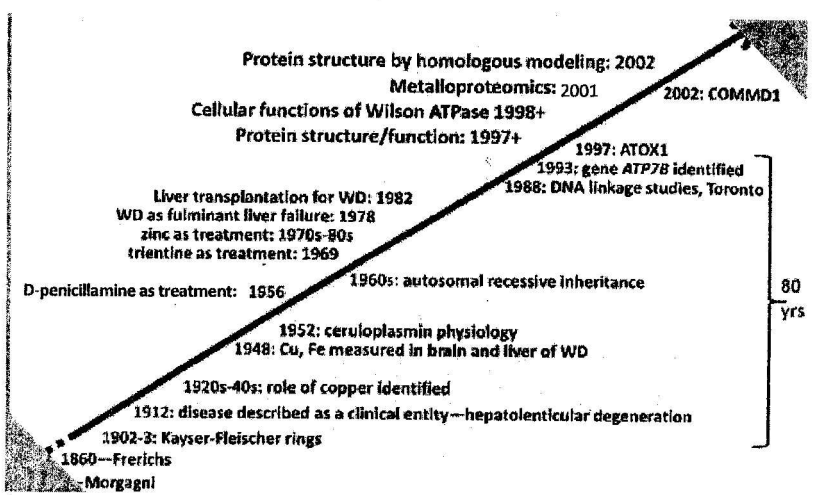


Fig. 1. Historical facts about *Wilson's disease.*

Gowers (1888), described the cases of *a 10 year-old boy and his sister, in which there were a predominance of a kind of movement disorder in the clinical picture that was named by the author as "tetanoid chorea".* In 1906, Gowers, in his new report named *"On tetanoid chorea and its association with cirrhosis of the liver"* described with more details the neurological abnormalities seen in one of the patients as: *"both arms presented slow changing tonic spasm... at times the spasm changed so that the fingers were spasmodically extended; occasionally they were spread out and moved irregularly in a manner resembling athetosis".* This description shows clearly that Gowers referred to what today is called dystonic movements and postures. *The pathological findings of both cases revealed the presence of cirrhosis of the liver while there were no defined lesions in the brain.*

Ormerod (1890) [51], reported a similar case in *a 10 year-old child with rapid-onset of neurological manifestations along 3 to 4 months* and he described them like this: *"weakness of right hand and arm, with cramped position of the fingers, soon followed by difficulty of speech. Drawing of the face, then an unnatural gait. Speech gets worse; he seems silly; has some difficulty in swallowing. Gait much worse. Lastly some affection of left arm and hand". The progression was fast and the death came 8 months after the beginning of the symptoms.* Thenecropsyshowed *necrosis of the external segment of the lentiform nucleus (putamen) and the presence of cirrhosis of the liver. These pathological findings are in agreement with the diagnosis of Wilson' disease.* In this remarkable report, Ormerod emphasized the great similarity between his case and those reported by Gowers two years before with the patients described by Homén in the same year.

Homén' report (1890) [43] included 3 siblings *(two males and a female) at the ages ranging from 12 to 21 at the onset of the disease and neurological manifestations similar to those described by Ormerod, although with a slower progression. Severe damage of lentiform nucleus and cirrhosis of the liver were also observed by Homén in his cases, despite he thought these were related to syphilis.*

In 1902, **Kayser** [45] *reported the presence of a greenish-brown ring around the cornea of a 23-year-old man, thought to have multiple sclerosis. A* year later, **Fleischer** (1903) [33] described the same pigmented ring in a case of "pseudosclerosis" and in another case of multiple sclerosis.

In 1911, **Wilson** [77, 79] presented his monograph describing the *"progressive lenticular degeneration"* that resulted in the publication of a historical paper in Brain "journal, in 1912, with the title *"Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver.*

Brief versions of the same study were published in 1912, in two other journals: the *"Lancet"* and the *"Revue Neurologique"* (Paris). In these seminal reports Wilson described four personal cases (three of them with neuropathological study), two referred (but not studied) by Gowers and Ormerod and six other cases from the literature (the cases described by Gowers; Ormerod and Homén). He emphasized the familial character of some cases and the presence of cirrhosis of the liver, mostly asymptomatic, but claimed that the liver did not contribute to the clinical progression of the disease.

Bramwell (1916) was the first to realize the importance of liver pathology in WD, when he described *a family in which 4 siblings died of "acute fatal cirrhosis" all between 9 and 16 years of age,* and suggested that these cases might have been related to those reported by Wilson 4 years before.

In his first papers Wilson did not see any relationship between his cases and the pseudosclerosis of Westphal-Strümpell. However, two years later, while writing on "progressive lenticular degeneration", Wilson mentioned similarities between the two entities.

In 1920, **Spielmeyer,** contesting the ideas of Von Hoesllin and Alzheimer, concluded that, from the neuropathological point of view, pseudosclerosis and WD were the same disease.

The controversy between the followers of Wilson' ideas about the unity of the two entities and Westphal, Strümpell and Alzheimer' school, who considered them as independent, was definitely over in 1921 with the publication of **Hall'** monograph [41]. This author, based on the review of 64 cases of literature and studies of four personal cases, demonstrated in a conclusive way the identity between the two diseases. Furthermore, Hall pointed out their inheritable character and assembled the two diseases under the name of "hepatolenticular degeneration".

Once established the clinical characterization of the disease, the studies started to be orientated toward its ethiopathogenesis.

Rumpel (1913) [57], already mentioned for the first time the increase of the amount of copper in the liver of a patient with pseudo-sclerosis.

According to Scheinberg and Sternlieb, **Vogt** (1929), **Haurowitz** (1930) and **Glazebrook** (1945) demonstrated the accumulation of copper in the liver and in the basal ganglia of patients with WD [72].

In 1948 **Mandelbrote et al.** studying the copper metabolism in multiple sclerosis noticed that in the control group, that included patients with other neurological diseases, one patient with Wilson' disease presented an increase of urinary excretion of copper. In a two-hour period the patient with WD excreted 41.7 μg of copper, whereas none of the other twelve patients studied excreted more than 18 μg. In the same year **Cumings** [23] definitely proved *the accumulation of copper in the liver and in the brain of patients with WD.*

In the 1950's the investigation of WD concentrated on the study of copper metabolism and on the treatment of the disease aiming to reduce copper accumulation.

In 1952 **Scheinberg and Gitlin** demonstrated the decreased level of ceruloplasmin in patients with WD and, in 1954 **Cartwright** et al. reported the increase of serum free copper, not bound to ceruloplasmin, in patients with WD.

The attempts of treatment with copper chelating agents were unsuccessful until the introduction ofpenicillamine by **Walshe** (1956) [72], what made possible the long term treatment of the disease, so far always fatal.

The efficacy of penicillamine in the treatment of WD was recognized only after several years. To illustrate this fact in the book on WD published by **Boudin and Pepin** on WD (1959), this drug was put in the same plane of others therapeutic options that later revealed completely to be ineffective such as isoniazide and sodium thiomalate [75, 76].

INTRODUCTION

The liver is a major site of metabolism in humans, playing a central role in the metabolism of carbohydrate, protein, lipid, trace elements, and vitamins. In addition, detoxification and biliary excretion of various endogenous and exogenous compounds, especially lipophilic molecules, including most common drugs, and the degradation and elimination of a variety of hormones and hormonal metabolites, are distinct hepatic characteristics. Because of its involvement in so many metabolic pathways, it is not surprising that many inherited diseases of the liver are manifested as inborn errors of metabolism. Disease occurs as a result the lack of the required gene product or accumulation of a toxic metabolite [47, 51, 58].

There are three genetically determined diseases in which the liver may be the principal target organ, with manifestations of acute, subacute, or chronic disease that may become evident in early or later life. These are:

- **Hereditary hemochromatosis** (HH), a major disorder of iron overload.
- **Wilson's disease**, a genetic disorder of copper overload.
- **Alpha1-antitrypsin** (α1-AT) **deficiency**, a disorder in which the normal processing of a liver-produced protein is disturbed within the liver cell.

Table 1

The specific features of inherited liver disease

Inherited diseases of the liver	Specific features
Hereditary hemochromatosis	• Hereditary hemochromatosis (HH) is an autosomal recessive disorder that results from a mutated hemochromatosis (HFE) protein; • Mutations in the HFE gene can lead to impaired regulation of iron storage and are believed to be the most common cause of HH • Hemochromatosis is the clinical expression of iron-induced end-organ injury. Organs involved are the liver, heart, pancreas, pituitary, joints, nerves, and skin. • Diagnosis of HH is based: the serum ferritin concentration and serum transferrin saturation (elevated). Liver biopsy has been performed in patients with iron overload to confirm a diagnosis of HH and to exclude cirrhosis.HFE gene testing may eliminate the need for liver biopsy in many patients. • Phlebotomy is the preferred treatment because it is simple, effective and relatively inexpensive

Alpha1-antitrypsin deficiency	• Alpha-1 antitrypsin (AAT) deficiency is a condition in which the body does not make enough of a protein *"protease inhibitor"*that protects the lungs and liver from damage. • It is caused by a genetic defect. • The condition is the most common among Europeans and North Americans of European descent. • Severe deficiency of alpha-1 antitrypsin (AAT) is associated with early onset pulmonary emphysema and with several forms of liver disease, including cirrhosis, neonatal hepatitis, and hepatocellular carcinoma. • The diagnosis of A1A deficiency is initially made by quantitation of protein levels in serum followed by determination of specific allelic variants by isoelectric focusing.
Wilson's disease	• Rare autosomal recessive disorder that causes defective copper excretion, resulting in toxic accumulation of copper in multiple organs, particularly the liver, brain (primarily basal ganglia and cortex), kidneys, and eyes • The transport of copper by the copper-transporting P-type ATPase is defective in Wilson disease secondary to one of several mutations in the *ATP7B* gene. • Diagnosis is based on: serum and urinary copper; serum cerulopasmine; hepatic copper and genetic testing • The treatment: copper chelating agents; • Family screening of first-degree relatives is mandatory following diagnosis of an index case.

In some cases, the awareness of these conditions is brought about by suspicion based on a specific clinical syndrome. In other cases, these conditions have to be excluded when faced with nonspecific liver disease abnormalities, such as elevated liver enzyme levels, hepatomegaly, or previously undiagnosed portal hypertension [51, 68].

The recognition of *inherited liver disease* is often the process of exclusion of more common causes (e.g., viruses, alcohol, autoimmunity), it is important to emphasize that awareness of the clinical features of these metabolic liver diseases should promote a proactive diagnostic evaluation. The inherited metabolic liver disease may manifest in childhood or may be delayed until adult life and, in some cases, may regress after the childhood or adolescent years, only to reappear later in life. The advent of molecular diagnostic testing, phenotypic assessment of these conditions may be now complemented in certain cases by genotypic evaluation. The availability of effective treatments, there has been a dramatic impact on the prognosis of metabolic liver diseases in

been a dramatic impact on the prognosis of metabolic liver diseases in both childhood and adult life, further emphasizing the importance of early diagnosis. In several conditions (e.g., α1-AT deficiency, Wilson's disease), liver transplantation corrects the primary biochemical abnormality in the liver and effectively cures the disease [43, 67, 76].

Wilson's disease (WD) is a rare autosomal recessive inherited disorder of copper metabolism. The condition is characterized by excessive deposition of copper in the liver, brain, and other tissues [23, 39, 77].

- ***Synonyms of Wilson'sDisease:*** *Hepatolenticular degeneration, progressive degeneration, Kinnier Wilson's Diseases, Westphal Strumpell s pseudosclerosis*

The major physiologic aberration is excessive absorption of copper from the small intestine and decreased excretion of copper by the liver. The genetic defect, localized to arm 13q, has been shown to affect the copper-transporting adenosine triphosphatase (ATPase) gene (***ATP7B***) in the liver [8, 51, 58].

EPIDEMIOLOGY

The term *'prevalence'* of Wilson's disease usually refers to the estimated population of people who are managing Wilson's disease at any given time.

The term *'incidence'* of Wilson's disease refers to the annual diagnosis rate, or the number of new cases of Wilson's disease diagnosed each year.

Wilson's disease is listed as a "rare disease" by the Office of Rare Diseases (ORD) of the National Institutes of Health (NIH) 23, 53, 78].

- Worldwide, the incidence of Wilson's disease is 10-30 million cases, and the heterozygote carrier rate is 1 case per 100 persons, with the genetic mutation frequency varying from 0.3-0.7%. In Japan, the rate is 1 case per 30,000 population, compared with 1 case per 100,000 population in Australia. In the United States, the carrier frequency is 1 per 90 individuals. The prevalence of Wilson disease is 1 per 30,000 individuals.
- The fulminant presentation of Wilson's disease is more common in females than in males.

- For special populations in which consanguineous mating is common, the risk of autosomal recessive traits such as Wilson's disease is higher.
- In the general population, the prevalence of heterozygous gene carriers (defined as the ratio of all individuals with one mutant *ATP7B* allele to the population at risk of harboring one) is estimated to be 1 in 100.

Carriers have one normal and one abnormal gene. 100% of a patient's children will receive the Wilson's disease gene. 50% of a carrier's children will receive the Wilson's disease gene, since the carrier has one normal and one abnormal gen [10, 28, 31, 49].

- **Siblings**of Wilson's disease patients have a 25% chance of having the disease. If both parents are carriers, each of their children has a 1 in 4 chance of getting Wilson's disease, 2 in 4 chance of being a carrier, and a 1 in 4 chance of being normal.
- **Children**of patients have 1 in 200 chance of having the disease. A patient's children have a 100% chance of inheriting one abnormal gene. The other (normal) parent has 1 in 100 chance carrying the gene, and 1/2 the time he or she will pass it on.

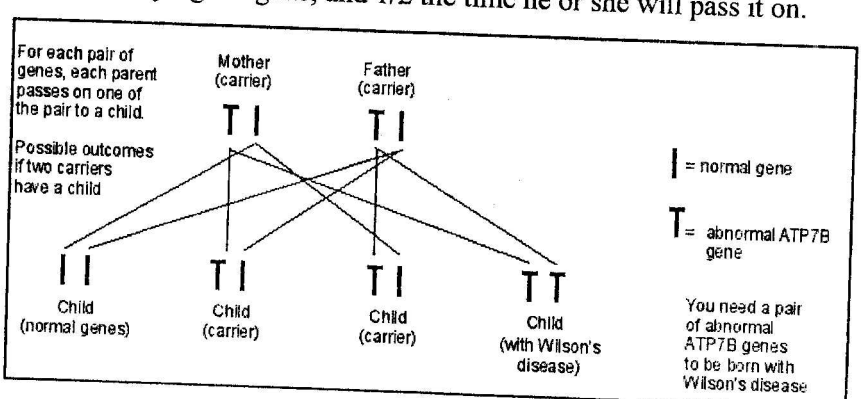


Fig. 2. **The familly screening for Wilson disease.**

- **Grandchildren** of patients have a 1 in 400 chance of having the disease. A patient's grandchildren have a 50% chance of inheriting the gene (1 in 2) since all the patient's children are carriers. From the other parent, a grandchild has a 1 in 200 chance of inheriting the gene (1/2 x[1/2 x 1/100] from the normal spouse = 1/400).

- **Nieces and nephews**of patients with Wilson's disease have a 1 in 600 chance of having the disease. 2 in 3 of unaffected siblings carry the gene. The risk of a couple both being carriers is 2/3 x 1/100 = 1/150, and the risk of each of their children having the disease is 1/4 x 1/150 = 1/600.
- **Cousins**have a 1 in 800 chance of having the disease. 50% of a patient's aunts and uncles arecarriers. Therefore 1/2 x 1/100 = 1/200 couples are both carriers, and 1/4 of their children will be affected = 1/800.

Age-related presentations. A German study of patients with Wilson's disease illustrated that patients presenting earlier show predominantly hepatic symptoms (15.5 year), while those presenting later more often present with neurological symptoms (20.2 year).

Thomas and colleagues reviewed the mutations found in the *ATP7B* gene, and their findings suggested a wide age span in the onset of Wilson's disease, perhaps wider than previously considered typical. Mutations that completely disrupt the gene can produce liver disease in early childhood, at a time when Wilson disease may not be considered in the differential diagnosis.

In general, the upper age limit for considering Wilson'sdisease is 40 years and the lower age limit is 5 years, although the disorder has been detected in children younger than 3 years and in adults older than 70 years.

ETIOLOGY AND PATHOPHYSIOLOGY

The normal estimated total body copper content is 50-100 mg, and the average daily intake 2-5 mg, depending on an individual's intake of legumes, meats, shellfish, and chocolate. Copper is an important component of several metabolic enzymes, including lysyl oxidase, cytochrome "c" oxidase, superoxide dismutase, and dopamine beta-hydroxylase [18, 30, 47].

- Around 50-75% of intestinal copper is absorbed and then transported to the hepatocytes. This pathway is intact in Wilson disease.
- After copper reaches the hepatocyte, it is incorporated into copper-containing enzymes and copper-binding proteins (CBPs), including ceruloplasmin, a serum ferroxidase. Within the liver,

the majority of ininfancy (< 6 mo) CBP granules staining positive may be normal. After 6 months, positive staining of CBPs for copper is almost exclusively found in association with liver diseases such as Wilson disease, chronic biliary disorders (eg, primary biliary cirrhosis, primary sclerosing cholangitis), cirrhosis/extensive fibrosis, and primary liver tumors (most often fibrolamellar hepatocellular carcinoma) [25, 30, 47].

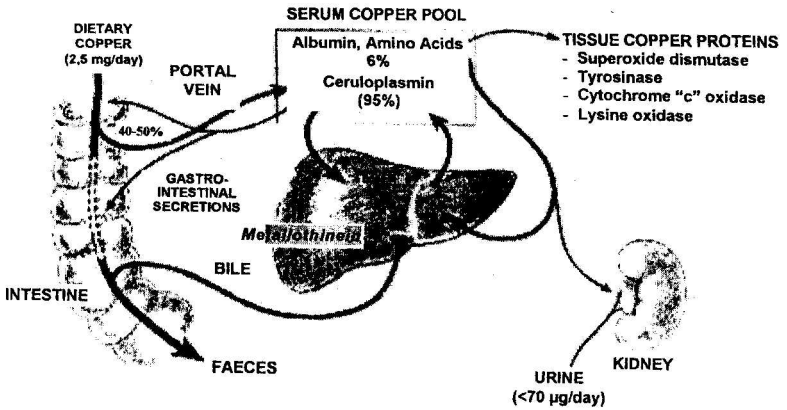


***Fig. 3.* Human copper metabolism.**

- Excess copper may be rendered nontoxic by forming complexes with apo-metallothionein to produce copper-metallothionein, or it may be excreted into bile. Normal copper balance is maintained by regulation of excretion, rather than absorption, and the predominant route of copper excretion (approximately 95%) is hepatobiliary in nature [22, 23, 39].
- *In Wilson's disease*, the processes of incorporation of copper into ceruloplasmin and excretion of excess copper into bile are impaired.
- The transport of copper by the copper-transporting P-type ATPase is defective in *Wilson's disease* secondary to one of several mutations in the *ATP7B* gene. By genetic linkage studies, Bowcock and colleagues narrowed the assignment of the Wilson's disease locus to *13q14-q21*. Many of the gene defects for *ATP7B* are small deletions, insertions, or missense mutations. Most patients carry different mutations on each of their 2 chromosomes.

The wide variation in the clinical phenotype of WD has still to be elucidated and may be related to the ATP7B genotype. Although some authors have tried to establish whether the ATP7B genotype determines the phenotype of the disease, the data are conflicting and no definitive association has been established [10, 28, 37, 60].

- Reasons for the difficulties in assessing genotype-phenotype correlations in WD are the high genetic heterogeneity with the large number of mutations and the rareness of the disease.
- The excess copper resulting from *Wilson's disease* promotes free radical formation that results in oxidation of lipids and proteins. Ultrastructural abnormalities in the earliest stages of hepatocellular injury, involving the endoplasmic reticulum, mitochondria, peroxisomes, and nuclei, have been identified. Initially, the excess copper accumulates in the liver, leading to damage to hepatocytes. Eventually, as liver copper levels increase, it increases in the circulation and is deposited in other organs.

Stuehler et al reported that base pair changes in the *MURR1* gene were associated with an earlier presentation of Wilson disease. *MURR1* had previously been established to cause canine copper toxicosis in Bedlington terriers.

Xin-Hua Li et al. (2011) [68] evaluating genotype-phenotype correlations in WD will help understand the pathogenesis of WD. *Conclusion of this study: the genotype of ATP7B gene may not completely explain the phenotypic variability in WND patients; other factors that affect disease severity may include levels of copper in diet or other geneticfactors*. Xin-Hua Li et al. identified *14 novel mutations* and *found that the spectrum of mutations of ATP7B in China is quite distinct from that of Western countries*. The mutation type plays a role in predicting clinical manifestations. Genetic testing is a valuable tool to detect WND in young children, especially in patients younger than 8 years old. Four exons (8, 12, 13 and 16) and two mutations (p.Arg778Leu, p.Pro992Leu) should be considered high priority for cost-effective testing in China.

HISTORY AND CLINICAL MANIFESTATIONS

The Wilson's disease may manifest in childhood or may be delayed until adult life and, in some cases, may regress after the childhood or adolescent years, only to reappear later in life.

The clinical manifestations are variouses and may be with hepatic signs, neurologic or psihiatric alterations, ophtalmological manifestatations and other uncommon organ involvement: musculoscheletal, kidney, heart.

Table 2

Theclinical features of Wilson's disease

Asymptomatic	Presymptomatic (found by family sereening)
Neurological (40-50%)	Dystonia and Rigidity Tremors (resting Postural, or kinetic unilateral or bilateral) 26 Dysarthria and dysphonia Cerebellar dysfunction (scanning speech, intension tremors, ataxia) Seizures
Hepatic (>50%)	Asymptomatic with only biochemical abnormalities Acute transient hepatitis Chronic active hepatitis Cirrhosis(compensated or decompensated) Fulminant hepatic failure
Psychiatric (20%)	Depression, Personality changes, Neuroses, Psyhosis
Others Systems	Renal disease aminoaciduria nephrolithiasis, hematuria Skeletal disease: arthritis, premature osteoporosis, ostheomalacia Myocardial disease: ardiomyopathy and arrhythmias Hemolytic anemia, skin pigmentation, gynecomastia, weakness, glucose intolerance

HEPATIC MANIFESTATIONS

Since the copper accumulation occurs in the liver initially, the disease often startswith hepatic symptoms; therefore, during childhood and the teenage years it is the mostcommon manifestation [37, 54, 58].

- The youngest case in the literature was a 3-year old girl with severe hepatic presentation: a *3-year-old girl presented with hemolytic anemia, hepatosplenomegaly, ascites, and evidence of decompensated chronic liver disease. Genotypic DNA analysis revealed that the patient was homozygous for a splice site mutation now designated IVS4-:G>C, expected to destroy completely the functional gene product of ATP7B, the gene responsible for Wilson's disease. Wilson's disease should be considered in the differential diagnosis of established liver disease in the preschool-aged child"[80].*
- The liver involvement may range from *mild hepatitis, fulminate hepatic failure to chronic hepatitis and cirrhosis.*
- One fourth of all Wilson patients experienced sometime in their lives an acute episode of hepatitis, presenting with non-specific symptoms (malaise, anorexia, epigastricpain, jaundice, elevated LFT) without viral markers or history of a toxic agent [23, 43, 49].
- It isimportant to rule out Wilson disease in cases with non-viral acute hepatitis, especially if mildhemolysis and low uric acid level are also present.
- Fulminate hepatitis is a relatively rare, severe disease, which is often lethal. It usually occurs during the teenage years or young adulthood with symptoms of rapidly progressing acute hepatitis (deep icterus, encephalopathy, bleeding disorders, terminal renal failure, hepatic coma [36, 37, 49].
- Chronic hepatitis is the most common liver pathology in WD.
- The liver biopsy specimen reveals non-specific changes of chronic inflammation, intranuclear glycogen and periportal steatosis. The staining of the copper associated protein is usually positive. Measurement of the hepatic copper content aids in establishing the definitive diagnosis.

Consider hepatic Wilson disease in the differential diagnosis of any unexplained chronic liver disease, especially in individuals younger than 40 years.

Hepatic insufficiency and cirrhosis may slowly develop and can result in signs of fulminant hepatic failure, including the following:

- Ascites and prominent abdominal veins
- Spider nevi
- Palmar erythema

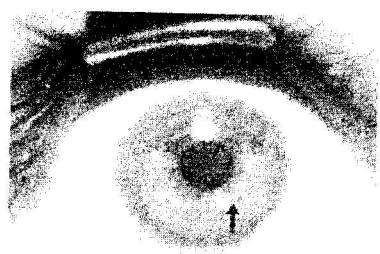
- Digital clubbing
- Hematemesis
- Jaundice

The average age of the patients whose first presenting symptoms of their WD are either neurological or psychiatric, is frequently later than those presenting with hepatic symptoms (18 years versus 11.4 years), although neurological symptoms have been reported as early as age 6 and as late as age 50.8. WD is a disease of motor function, and basal ganglia symptoms are the most common symptoms.

OPHTHALMOLOGIC SYMPTOMS

Kayser-Fleischer rings are formed by the deposition of copper in the Descemet membrane in the limbus of the cornea [32, 44, 54]. The color may range from greenish gold to brown; when well developed, rings may be readily visible to the naked eye or with an ophthalmoscope set at +40. When not visible to the unaided eye, the rings may be identified using slit-lamp examination or gonioscopy.

- **Kayser-Fleischer rings** are observed in up to 90% of individuals with symptomatic Wilson disease and are almost invariably present in those with neurologic manifestations.



Kayser-Fleischer ring (*arrow*)

The rings were first described by **Kayser** in 1902 and then by **Fleischer** in 1903 [32, 44].

The fact that the rings are composed of copper was finally established by Gerlach and Rohsrschneider *as* late as 1949 [29], shortly after Cumings *(1948)* had demonstrated that excess copper was deposited in the brains and livers of patients dying of this disease. That the corneal rings were indeed due to copper deposition was confirmed by Sternlieb *(1966).* Originally, it was believed that the presence of such copper containing rings were diagnostic of Wilson disease but in 1975 Fleming and his associates were able to show that such copper deposits in the cornea could also be found in patients with primary biliary cirrhosis and in 1977 they also recorded the presence of such rings in other forms of chronic liver disease especially those associated with long-term cholestasis.

Sunflower cataract was first described by Seimerlingn and Oloff in 1922 and they noted the similarities between the cataracts seen in their patients with Wilson disease to that produced by an intraocular foreign body containing copper. This was not, in fact a true cataract as the changes were in the lens capsule and did not affect vision. When viewed with the naked eye, the appearance was of a greenish disc in the centre of the pupil, and when the pupil kept dilated the disc was said to increase proportionally in size. Viewed with a slit lamp, the appearance in the anterior capsule of the lens, according to Duke Elder, was of radiating fronds said to correspond to folds in the iris while in the posterior capsule it was uniform and without pattern.

Cairns et al., on the other hand demonstrated that it was the anterior capsule that had the uniform disc and the petal-like fronds were on the posterior capsule and that they had no relationship to the folds in the iris was also noted. They were also able to show that, unlike the claim of Boudin and Pepein, the cataract was only a late manifestation of copper overload in their patient and it was one of the presenting signs

- The natural history of the KF ring is to appear first as a top crescent in the top arc of the cornea from 10 to 2 O'clock, then correspondingly in the inferior crescent eventually joining laterally to form the complete ring. The pigment deposition first appears peripherally, where it is densest, and extending centrally seldom exceeding 5 mm in depth.
- The microscopic appearances were described by Uzman and Jakus. They reported two zones of pigmentation at 2 and 0.6 U from the endothelial surface of Descemet's membrane; some of the pigment granules being as large as 0.35 U. On the other hand, Scheinberg and Sternlieb, using electron microscopy, found three discrete layers of copper containing granules in the same zone as reported by Uzman and Jakus. This was not in keeping with the appearance of the cornea when photographed using a slit lamp and gonioscope).

The reason for the deposition of copper first in the superior and then the inferior crescents, as was suggested by Cairns and Walshe, was due to the vertical flow of aqueous fluid in the anterior chamber of the eye leaving these crescents more freely irrigated by the metal.

Once decoppering treatment was started, copper was removed from both the lens capsule and the corneal rings. The first description of

clearing of a cataract was by Cairns et al. and in their patient the cataract was resolved before the corneal rings.

Removal of copper from the rings starts in reverse order to deposition. First the rings are thinned and then broken laterally after which the top and bottom crescents thin and, in most cases disappear completely but this may take several years. They also showed that as copper is removed from the cornea it leaves pits or scars with the appearance of beaten metal, a valuable sign that a ring is resolving.

False KF rings have been described by Scheinberg and Sternlieb and also by Cairns and Walshe. They also showed that in their patient, the rings, though on superficial inspection were identical to true copper rings, on slit lamp examination were in a different layer of the cornea and were homogeneous and not granular. Careful regular inspection of the status of the rings during treatment is a valuable index of the copper status of the patient and of the efficacy of therapy.

In the experience of Scheinberg and Sternlieb, *corneal rings are always present in neurological Wilson disease but not necessarily in pre-symptomatic and hepatic stages of the disease*. This is also my experience in over 300 cases.

However, there have been some reports of rings being absent in neurological cases. There are two possible explanations of this: early, lightly pigmented rings may have been missed by inexperienced observers not using a slit lamp and gonioscope; or the diagnosis may have been incorrect. A known phenomenon for this disease.

- Although Kayser-Fleischer rings are a useful diagnostic sign, they are no longer considered pathognomonic of Wilson disease unless accompanied by neurologic manifestations. They may also be observed in patients with chronic cholestatic disorders, such as partial biliary atresia, primary biliary cirrhosis, primary sclerosing cholangitis, and cryptogenic cirrhosis.

NEUROPSYCHIATRIC SYMPTOMS

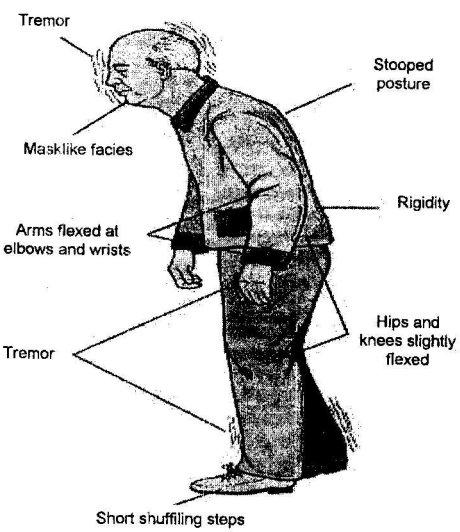
In the CNS copper damages prominently the subcortical nuclei, resulting in dysarthria, movement disorders, choreoathetoid symptoms, tremor [11, 27, 35].

- Patients with neurological symptoms are often misdiagnosed as juvenile Parkinson disease or multiple sclerosis.

- Most patients who present with neuropsychiatric manifestations have cirrhosis.

The earliest presentation of neurological symptoms reported was in a 6 –year-old patient.

Angius A et al. [1] describe *"a patient with Wilson disease who presented at 11 years of age with neurological symptoms and subsequent rapid progression of neurological impairment but absent hepatic manifestations. Molecular analysis showed compound heterozygosity for two frameshift mutations, 2299insC and 214delAT, which most likely result in an absent or inactive protein product. Mutation-phenotypic analysis indicates that this genotype does not explain the severe phenotype, suggesting the presence of modifying factors. Conclusion: Wilson disease may present even in childhood or adolescence with neurological abnormalities in the absence of hepatic manifestations. Early and severe neurological features in a Wilson disease patient compound heterozygous for two frameshift mutations".*



The most common presenting neurologic feature is *asymmetrical tremor*, occurring in approximately half of individuals with Wilson disease. The character of the tremor is variable and may be predominantly *resting, postural, or kinetic.*

Kayser-Fleischer rings are seen in at least 98% of patients with neurological Wilson disease who have not received chelation therapy.

- **Early symptoms** include *difficulty speaking, excessive salivation, ataxia, masklike facies, clumsiness with the hands, and personality changes.*
- **Late manifestations** (now rare because of earlier diagnosis and treatment) include *dystonia, spasticity, grand mal seizures, rigidity, and flexion contractures.*

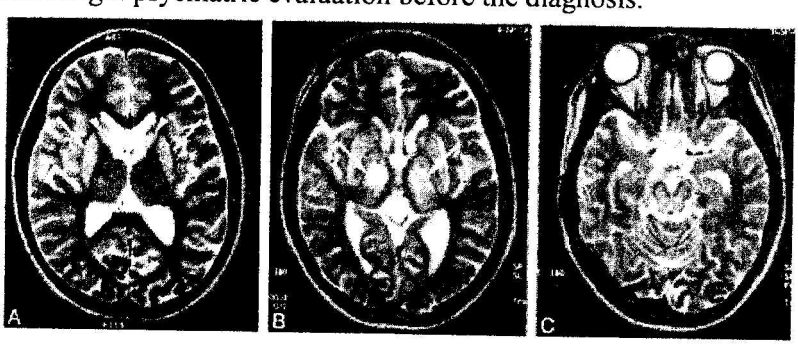
One study described 4 distinct diagnostic categories based on patients' major neurologic findings, as follows:

- patients in the parkinsonian group (45%) – distinguished by paucity of expression and movement;
- patients in the pseudosclerotic group (24%) – had tremor resembling multiple sclerosis;
- patients in the dystonic group (15%) – characterized by hypertonicity associated with abnormal limb movements;
- patients in the choreic group (11%) – predominantly characterized by choreoathetoid abnormal movements associated with dystonia.

Psychiatric features. The reported percentage of patients with psychiatric symptoms as the presenting clinical feature is 10-20% [35, 41, 46, 65]. The range of psychiatric abnormalities associated with Wilson disease has been divided into 4 basic categories, as follows:

1. Behavioral
2. Affective
3. Schizophrenic-like
4. Cognitive

The prevalence of seizures is 10 times higher in patients with WD that in the general population. The psychiatric features of WD are under-appreciated and often misdiagnosed as having primary psychosis or schizophrenia. More than 20 percent patients with WD were found to have sought psychiatric evaluation before the diagnosis.



Wilson disease in a 14-year-old girl with dystonia. *A*. Initial T2-weighted axial MR image shows increased signal intensity in both caudate nuclei (arrowheads) and putamen (arrows). *B* and *C*. Follow-up T2-weighted axial MR images obtained after 3 years show agg...

***Fig. 5.* MRI in Wilson disease.**

MUSCULOSKELETAL SYMPTOMS

Skeletal involvement [3, 32, 43] is a common feature of Wilson disease, with more than half of patients exhibiting osteopenia on conventional radiologic examination.

- Between 1950 and 2000, at least 15 radiological case-series have reported radiological abnormalities in patients with WD. They were open and old studies with small number of patients (between 7 and 42 patients displays reported skeletal radiological studies over 50 years in this rare condition. The main characteristic is bone demineralisation, reported in 24–88% of the patients. Few studies have reported osteomalacia by X-rays examinations (5 series out of 13). Prevalent fractures and their triggering mechanism low or high energy fracture were rarely described.
- Two recent prospective studies have evaluated bone mineral density (BMD) by dual X-ray absorptiometry (DEXA) in patients with WD.They showed a high prevalence of osteoporosis, but small sample size precluded conclusion. In the literature, only one early study evaluated the in vitro effect of copper on the skeletal tissue of chicken embryos. For concentrations of copper between 5 and 20 mg ml/1, thickness of bonematrix and osteoformation were reduced and division and maturation of osteoblasts were interrupted.

Articular manifestations [32] have been described in seven open studies including small samples.

- Additional case-reports of recurrent and unexplained joint complaints (monoarthritis, polyarthritis and arthralgia) in adolescent patients, sometimes before diagnosis of WD, have been published. In the seven uncontrolled studies, joint signs were not always described, and time to onset for joint symptoms in the course of WD was not reported.
- Some patients were diagnosed as having WD after articular presentation, including polyarthritis, recurrent joints complain and low back pain.
- Most often articular symptoms included spontaneous or mechanical-type arthralgias, affecting mainly the large joints, especially the knees (16–77% depending on the series, mean 33%).

- Less frequently stiffness, swelling and perception of "crackles in the joints" were reported in one or more joints.
- Again, the knee joints were the most frequent involved joints, followed, in the order of decreased frequency, by the hip, wrist joints, the hands and the shoulder and ankle joints.
- Rarely, synovial fluid from the knee has been examined and low cell count was observed, without microcrystals.

Interestingly, no relationship was observed between clinical symptoms and radiological changes.

In one study, *joint hypermobility was noted in 9 out of 32 patients (mean age was 37 years), two patients were not treated with D-penicillamine and mean duration of D-penicillamine therapy in the other seven patients was 9.3 years.* [28].

It is therefore difficult to summarise a specific clinical picture of joint involvement in WD.

However, recurrent and unexplained joint complaints, especially with early osteoarthritis (OA) changes on radiographs, in adolescents, should lead to rule out WD.

- The most common featurewas early OA changes, especially on the knee, hip and wrist joints. OA concerned 7–60% of patients depending on the series in young patients (between 25 and 30 years). These unusual degenerative radiological features in a young subject should bring attention to and suggest to rule out underlying metabolic or genetic disorders, including WD.
- Pathogenic mechanisms responsible for joint symptoms (early OA, bone fragmentation and osteochondritis) are unknown.
- The first hypothesis relies on abnormal movements induced by CNS involvement, and responsible for recurrent microtraumatisms, falls and sprains, which can trigger post-traumatic pain.
- The role of treatment can also be discussed.
- A direct toxicity of copper on joint tissues has also been considered.

In vitro, only one study has shown, along with mean concentration of copper (5–40 g ml/1), a significant decrease in the length and weight of cartilage in culture. [*Rest J. 1976*].

Rest et al. have observed a significant correlation between the size of chondrocyte cultures and, on the one hand, copper concentration and, on the other hand, duration of culture. Morphological changes of chon-

drocytes (swollen, rounded and basophilic cells), detachment from their lacunae and reduction of extracellular matrix were disclosed in this early study. This direct effect of copper on articular chondrocytes has to be reevaluated.

HEMATOLOGIC SYMPTOMS

Hemolytic anemia is a recognized, but rare (10-15%), complication of Wilson disease.

- The prodrome to WD is occasionally a severe spherocytic hemolytic anemia.
- Hemolysis in Wilson's disease is due to deficiency of ceruloplasmin, the copper transport protein which results in exessive inorganic copper in the the blood circulation, much of it accumulates in red blood cells. Although exact mechanism is not known, the increased copper accumulation in the RBC'S may damage the cell membrane, accelerate oxidation of hemoglobin and inactivate enzymes of pentose phosphate and glycolytic pathways.
- Coombs-negative acute intravascular hemolysis most often occurs as a consequence of oxidative damage to the erythrocytes by the higher copper concentration. Any patient in whom acute hepatic failure occurs with a Coombs-negative intravascular hemolysis, modest elevations in serum aminotransferases, and a low serum alkaline phosphatase or ratio of alkaline phosphatase to bilirubin of less than 2 must be considered for a diagnosis of Wilson disease.

Acute intravascular hemolysis and acute liver failure associated as first manifestations of WD have been reported earlier by Roche-Sicot et al. *Roche-Sicot J, Benhamou JP* (1977) reported about *three patients, with the first manifestation of Wilson's disease was a syndrome in which acute intravascular hemolysis and acute liver failure were associated. This syndrome developed in three periods; the first, lasting 3 to 14 days, was characterized by fatigue, fever, and jaundice; the second, lasting 1 or 2 days, by severe intravascular hemolysis; and the third, lasting 2 to 6 days, by hepatic encephalopathy. All of the patients died from liver failure 7 to 21 days after the onset of the syndrome. The association of acute intravascular hemolysis and acute liver failure is a characteristic manifestation of Wilson's disease; it is rarely associated with other liver*

diseases. This association might result from hepatic cell necrosis due to accumulation of copper, the consequences being acute liver failure and destruction of erythrocytes by the large amounts of copper released from the necrotic hepatic cells to the plasma.

RENAL SYMPTOMS

- The Wilson disease gene is expressed in kidney tissue; therefore, any renal manifestations may be primary or secondary to release of copper from the liver.
- Clinically, patients may resemble those with Fanconi syndrome, demonstrating defective renal acidification and excess renal losses of amino acids, glucose, fructose, galactose, pentose, uric acid, phosphate, and calcium. The frequency of renal manifestations is variable.
- Urolithiasis, found in up to 16% of patients with Wilson disease, may be the result of hypercalciuria or poor acidification.
- Hematuria and nephrocalcinosis are reported, and proteinuria and peptiduria can occur before treatment as part of the disease process and after therapy as adverse effects of D-penicillamine.

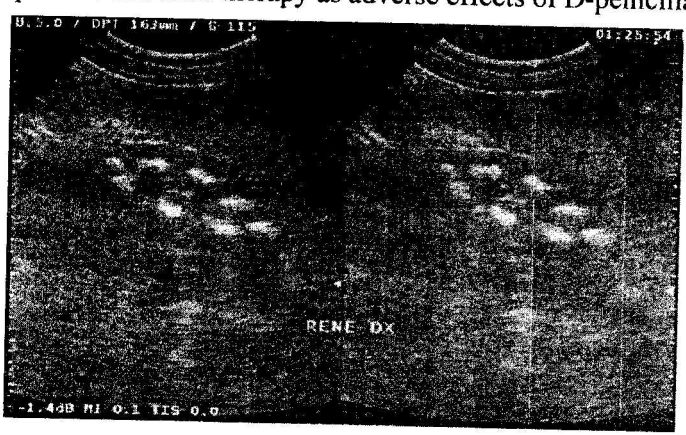


Fig. 8. **Kidney Ultrasonography Showing the Presence of Bilateral Nephrocalcinosis.**

Il Soo Ha et al. (1999, Korean Journal Pediatric) reported *one study that was performed to evaluate the prevalences of the renal problems in Wilson disease and related risk factors.*

***Conclusion**: In Wilson disease, covert renal problems are relatively common. Tubular dysfunctions are the most frequently observed abnormalities followed by microscopic hematuria, proteinuria, and decreased creatinine clearance in order. Hematuria is more common within 3 years after beginning penicillamine treatment, which is also probable for tubular dysfunctionsand proteinuria.*

Cardiac manifestations

Cardiac manifestations [62], such as rhythm abnormalities and increased autonomic tone, have been described in patients with Wilson disease. Cardiac involvement however has received only scantattention in the past.

In 1887 Kuan first reported of 53 consecutive patients with Wilson's disease, electrocardiographic abnormalities were seen in 18 (34%). Two deaths related to cardiac disease were reported, one due to repeated ventricular fibrillation and other due to dilated cardiomyopathy. Four modes of cardiac involvement were indentifided: arrhythmia, cardiomyopathy, cardiac death and autonomic dysfunction.

Autopsy findings have included hypertrophy, small vessel disease, and focal inflammation.

FULMINANT WILSON DISEASE

WD is the identified etiology in 5% of acute liver failure (ALF) patients worldwide [36, 37].

Prior series demonstrate that virtually all patients with WD presenting with ALF will die rapidly without urgent transplantation. Therefore, making the diagnosis quickly and unequivocally is critical for patient management and for family screening as well [35, 38].

- Fulminant hepatic failure with Wilson's disease differed from idiopathic fulminant hepatic failure by the following biochemical findings:
  a. higher copper levels in serum, urine and liver;
  b. less pronounced elevations of transaminase levels;
  c. higher concentrations of total bilirubin;
  d. lower hemoglobin values.
- Serum copper was the most useful biochemical test in diagnosing Wilson's disease before death.

- On autopsy, only hepatic copper concentrations clearly separated the two groups

Serial serum copper levels (antemortem) and quantitative analysis of hepatic copper (after recovery or postmortem) in patients with fulminant hepatic failure should help to exclude Wilson's disease [4, 23, 51].

The diagnosis of WD in the setting of ALF can be difficult to ascertain but is vitally important due to its poor prognosis and implications for family members.

Jessica D. Korman et al [35] identified the best method for diagnosis of ALF (*acute liver failure*) due toWD(ALF-WD), *data and serumwere collected from 140 ALF patients (16 with WD), 29 with other chronic liver diseases and 17 with treated chronic WD. Ceruloplasmin (Cp) wasmeasured by both oxidase activity and nephelometry and serum copper levels by atomic absorption spectroscopy. Conclusion: Conventional WD testing using serum ceruloplasmin and/or serum copper levels are less sensitive and specific in identifying patients with ALF-WD than other available tests. More readily available laboratory tests including alkaline phosphatase, bilirubin and serum aminotransferases.by contrast provide the most rapid and accurate method for diagnosis of ALF due to WD.*

Prognosis in fulminant Wilson disease

Patients with a prognostic index (ie, score) of 7 or greater should be considered for liver transplantation. All patients in the study associated with this prognostic index who exceeded this score died within 2 months of diagnosis, irrespective of the prescription of appropriate medical therapy.

Table 3

Prognostic Index in Fulminant Wilsonian Hepatitis

Score	0	1	2	3	4
Serum bilirubin (reference range, 3-20 mmol/L)	< 100	100-150	151-200	201-300	>300
Serum aspartate transaminase (reference range, 7-40 IU/L)	< 100	100-150	151-200	201-300	>300
Prothrombin time prolongation (seconds)	< 4	4-8	9-12	13-20	>30

Prognosis after liver transplantation is relatively good. In a study involving 55 patients with Wilson disease who underwent hepatic transplantation, a 1-year survival rate was 79% and the overall survival rate was 72% from 3 months to 20 years. Another study of 32 patients reported a 1-year survival of 90.6%, a 5-year survival rate of 83.7%, and a 10-year survival rate of 79.9% after living donor liver transplantation [67].

Important clues for the diagnosis of Wilson disease that a clinician must recognize are a younger patient with hemolytic anemia, impaired hepatic synthetic function, and normal alkaline phosphatase values.

COMPLICATIONS OF WILSON DISEASE

- The major complications in patients with untreated Wilson disease are those associated with: acute liver failure, chronic hepatic dysfunction with either portal hypertension or hepatocellular carcinoma, and sometimes a relentless course to cirrhosis, which is characterized by a progressive lassitude, fatigue, anorexia, jaundice, spider angiomas, splenomegaly, and ascites. Bleeding from varices, hepatic encephalopathy, hepatorenal syndrome, and coagulation abnormalities occur as liver failure ensues.
- Death occurs, generally at the age 30, if emergent liver transplantation is not performed.

Patients with Wilson disease exhibit signs of anemia, presumably due to oxidative injury of the cell membrane caused by excess copper. Skin pigmentation and a bluish discoloration at the base of the fingernails (azure lunulae) have been described in patients with Wilson disease.

DIFFERENTIAL DIAGNOSES in WILSON'S DISEASE

Copper is an essential trace element required by all living organisms. Excess amounts of copper, however, results in cellular damage. Disruptions to normal copper homeostasis are hallmarks of three genetic disorders: Menkes disease, occipital horn syndrome and Wilson's disease [24].

- Menkes disease and occipital horn syndrome are characterized by copper deficiency. Typical features of Menkes disease result from low copper-dependent enzyme activity. Standard treatment involves parenteral administration of copper-histidine.Children with Menkes are often born prematurely. Symptoms usually begin to show within three months after birth and may include: seizures, brain degeneration and developmental delay, hypotonia ("floppy" muscle tone), hypothermia, ostheoporosis. If treatment is initiated before 2 months of age, neurodegeneration can be prevented, while delayed treatment is utterly ineffective. Thus, neonatal mass screening should be implemented. Meanwhile, connective tissue disorders cannot be improved by copper-histidine treatment. Combination therapy with copper-histidine injections and oral administration of disulfiram is being investigated.
- Occipital horn syndrome characterized by connective tissue abnormalities is the mildest form of Menkes disease. Treatment has not been conducted for this syndrome.

Table 4

Diferential diagnosis between Wilson disease and Menkes' Disease

Diseases of Copper Transport	
Menkes' Disease	• X-linked genetic disorder • Defect in the transport of copper from the intestine, leading to copper deficiency intestine, results in death from severe progressive neurodegeneration • Gene product is ATP7A
Wilson disease	• Decreased transport of copper from the liver into bile, leading to copper excess • Genetic defect localizated to chromosome 13 • Gene product is ATP7B

The recognition of Wilson disease is often the process of exclusion of more common causes (e.g., viruses, alcohol, autoimmunity), it is important to emphasize that awareness of the clinical features of these metabolic liver diseases should promote a proactive diagnostic evaluation.

Table 5

Differential diagnoses of Wilson disease in patients with hepatic manifestation

Condition	Differentiating signs/symptoms	Differentiating tests
Viral hepatitis B	Patients with viral hepatitis may have a history of a febrile illness or blood transfusion, but otherwise the symptoms and signs may be identical.	Hepatitis B antigen positive and other serological marchers for HBV
Viral hepatitis C	Patients with viral hepatitis may have a history of a febrile illness or blood transfusion, but otherwise the symptoms and signs may be identical.	Hepatitis C antibody positive
Haemochromatosis	Patients with haemochromatosis may present with other features such as diabetes, skin pigmentation, arthritis, impotence in males, and cardiac enlargement with or without symptoms and signs of heart failure.	Iron parameters and liver biopsy are diagnostic.
Alpha-1 antitrypsin deficiency	Patients with alpha-1 antitrypsin deficiency may have chronic lung disease such as emphysema occurring earlier than expected (in the 40- to 50-year-old age group) as well as liver disease.	Tests show that enzyme is deficient
Autoimmune hepatitis	Patients may have other associated autoimmune conditions and will respond to steroid therapy. However, Wilson's disease should be excluded before this diagnosis is assumed	Patients may have a positive autoantibody screen including ANA, primarily in a homogeneous pattern, anti-smooth muscle antibody, anti-liver-kidney microsomal antibody, and others.
Steatohepatitis	Patients with steatohepatitis tend to be obese with clinical features of hepatitis. Wilson's disease should be excluded before this diagnosis is assumed	Fatty liver and inflammation on biopsy.
Alcoholic cirrhosis	Patients may have a history and signs of alcohol excess. Wilson's disease should be excluded before this diagnosis is assumed, even if the patient drinks.	None.

Haemolytic anaemia	If hepatic bouts are severe in Wilson's disease then haemolysis may occur. Haemolysis in the presence of liver disease in a person aged <40 years should prompt testing for Wilson's disease	Tests for alternative causes of haemolytic anaemia, including Coombs antibody, haemoglobin electrophoresis for HbS, and autoantibody screening for autoimmune diseases, are used to determine the diagnosis.

Table 6

Differential diagnoses of Wilson's disease
(noted usually in patients under 50 years of age)

Liver diseases	Acute hepatitis Acute liver failury Chronic hepatitis Cirrhosis
Dystonia	Dopa-responsive dystonia Idiopatic torsion dystonia Postenchefalitic distonya Lipid storage disease Dystonic cerebral palsy Focal dystonia
Ataxia	Degenerative or metabolic cerebral disease Demyelinating disease Craniovertebral anomaly
Titubation or tremor	Essential tremor Degenerative cerebral disease Demyelinating disease
Chorea	Huntington disease Sydenham chorea Storage disorder Neuroachantocytosis Vasculitis
Psychiatric illness	Major psychosis Personality disorder Mental retardation
Proximal muscle weakness	Muscular dystrophy Metabolic myopathy Inflammatory myopathy
Parkinsonian features	Juvenile Parkinson disease Neurodegeneration with brain iron accumulation

DIAGNOSIS OF WILSON'S DISEASE

Although Wilson disease is present from the first day of life, the disorder usuallyremains unrecognized until the development of clinical symptoms. The symptoms developdue to the toxic effect of copper accumulating mainly in the liver and central nervous system [23, 31, 42, 48].

- Wilson's disease is a rare liver disease, but the diagnosis has a great impact because a specific treatment of proven efficacy exists and because without this treatment the disease is invariably fatal. Early treatment averts severe complications.
- The diagnosis may be difficult because there is no single test with adequate sensitivity and manifestations are not always typical, especially among children, and so it is dependent on a high index of clinical suspicion when presented with a patient with liver and/or neuropsychiatric disease.
- Physicians should have WD in the differential diagnosis when patients under 50 years of agepresent with elevated liver enzymes, and/or parkinsonian symptoms, and/or behavioralchanges. Therefore, it is necessary to screen for WD every patient under 50 withneurological disorder (dysarthria, tremor and other involuntary movements, dystonia,incoordination), hepatic disorders (hepatitis: viral negative acute or chronic; cirrhosis: anypatient under 50 even with history of alcoholism or hepatitis C infection; hepatic failure), andbehavioral disturbances (loss of ability to focus mentally on tasks, loss of control ofemotions, depression, loss of inhibitions, insomnia, anxiety and psychotic manifestations).
- The diagnosis is based on laboratory results such as: low ceruloplasmin and elevated 24-hour urine copper, free copper and copper in hepatic tissue. Observation of KF rings in an ophthalmological examination further supports the diagnosis [39, 43, 53].

Screening methods

The CC1 Conference on Preventive Aspects of Chronic Disease, held in 1951, defined screening as *"the presumptive identification of unrecognized disease or defect by the application of tests, examinations, or other procedures which can be applied rapidly"*. Screening tests sort

out apparently well persons who probably have a disease from those who probably do not. Ascreening test is not intended to be diagnostic.

Persons with positive or suspicious findings must be referred to their physicians for diagnosis and necessary treatment. It should be noted that, by definition, unrecognized symptomatic as well as presymptomatic disease is included; also, physical examination is considered part of the procedure, so long as it can be classed as rapid.

The term "other procedures" may also embrace the use of questionnaires, which are assuming an increasingly important place in screening.

Finally, tests may be "diagnostic", though not necessarily so intended; for example, a gynaecological examination could be covered by this definition providedit were rapidly carried out [42, 58, 59].

Serum ceruloplasmin

This method is helpful if serum level is very low, and usefulonly to increase index of suspicion. Normal serum ceruloplasmin is 0.2-0.6 mg/L [57, 69, 73].

- Although the concentration of the serum copper protein ceruloplasmin is decreased in most patients with Wilson's disease *[Scheinberg, I. H., and D. Gitlin 1952],* there are several objections to the theory that decreased ceruloplasmin synthesis is the underlying defect.
- There are, for instance, several patients with unquestionable Wilson's disease who have normal ceruloplasmin levels.

On the other hand, normal adults unrelated to patients with Wilson's disease, as well as some proven heterozygous persons, have persistently low ceruloplasmin levels without any clinical or chemical manifestations of the disease.

- ForWilson patients the level is often below 0.1 mg/L, however, in about 15% of patients thelevel is normal [20, 68, 59]. Twenty percent of carriers have also low ceruloplasmin levels.
- A concentration less than the lower reference limit (0.20 g/L) has been considered as the conventional diagnostic cutoff for WD [16, 23].

However, the lower reference limit can vary with different measurement methods, standardization, and the age ofsubjects.

Serum ceruloplasmin concentration is age dependent [21].

- It is lower in normal neonates and rises to adult concentrations by the age of 6 months. It increases further to a maximum concentration between 2 and 3 years old, and then falls slowly until the teenageyears, when it reaches the adult concentrations.
- In addition, females who are pregnant or on estrogens have higher concentrations.
- The youngest age suitable for use of serum ceruloplasmin measurement in the diagnosis of WD is 3 years.

Ceruloplasmin may be increased in a variety of circumstances where the test is not used as a clinical tool. However, these conditions can affect the ability of ceruloplasmin to recognize Wilson disease or copper deficiency.

- Ceruloplasmin is an acute phase reactant.
- It is frequently elevated when someone has inflammation, severe infection, tissue damage, and may be increased with some cancers.
- It may be increased during pregnancy and with the use of estrogen, oral contraceptives, and medications such as carbamazepine, phenobarbital, and valproic acid.

Table 7

Differential diagnosis of low ceruloplasmin

• Wilson Disease
• Asymptomatic heterozygote carriers (10-20%)
• Renal protein loss (nephrotic syndrome)
• Protein losing enteropathy
• Severe end stage liver disease of any cause
• Menke's disease (disorder of copper transport)
• Aceruloplasminemia
• Nutrition copper deficiency (eg. inadequate copper in TPN)

The 24-hour urine copper assay

This is a very useful screening tool, which isdiagnostic approximately 75% of the time [16, 23, 30].

- Normal values range between 20 to 50 μg/24 h.
- For symptomatic affected patients this value is usually above 100 μg/24 hr.
- Forpresymptomatic affected patients > 65 μg/24 h.
- For carriers < 100 μg/24 h (< 65 μg/24h in 90% of cases).

High urinary copper is a diagnostic feature of WD linked to liver malfunction; the mechanism behind urinary copper elevation is not fully understood.

How is the test done?The following are directions for collecting a 24-hour urine sample:

- In the morning scheduled to begin the urine collection, urinate in the toilet and flush away the first urine. Write down the date and time. That is the start date and time for the collection.
- The patients have to collect all urine that he pass, day and night, for 24 hours. Use the container given to collect the urine. The urine sample must include the last urine that the patient pass 24 hours after starting the collection.
- Write down the date and time that the last sample is collected.
- The urine sample may need to be kept cool during the 24-hour collection period. If so, keep the closed container in a pan on ice. Do not put ice in the container with the urine.

Laboratory test results may vary depending on the age, gender, health history, the method used for the test, and many other factors.

Results increased in: *chronic active hepatitis,biliary cirrhosis primary sclerosing cholangitis autoimmune hepatitis, proteinuria, rheumatoid arthritis and decreased in protein malnutrition.*

Serumcopper

Serum copper by itself is not very useful and sometimes confusing. Thevalue can be normal, low or high. The measurement of the concentration of total copper in serum may mislead diagnosis, but is valuable in monitoring the response to treatment [18, 21, 25, 47].

- Ceruloplasmin contains 0.3 per cent of bound copper, which is included with free copper in the measurement of total serum copper concentration.

- Since free serum copper is raised in untreated Wilson's disease, total serum copper concentration may be normal.
- Serum copper is influenced by age, acute-phase reactions, pregnancy, many anemias, and medication (oral contraceptives and antiepileptics). Furthermore, ~2% of the population who are heterozygous for P-type ATPase mutations have low copper and ceruloplasmin concentrations

Many tests can be used to investigate patients who may have Wilson disease, including non-ceruloplasmin-bound copper (NCC; also called the "free copper" or copper index), 24-h urine copper, hepatic copper, and genetic mutation testing.

However, the NCC has been advocated as a superior diagnostic tool for Wilson disease (*Walshe JM. 2003*).

This is derived as follows:

$$\text{NCC (μmol/L)} = \text{total copper (μmol/L)} - n\ (\text{μmol/L}) \times \text{ceruloplasmin (mg/L)}$$

where: *NCC* – non-ceruloplasmin-bound copper; *n* – factor for copper bound/mg of ceruloplasmin.

The d-penicillamin provocative test

Penicillamine (500 mg by mouth every 12 hours) is given while 24-hour urinary collections are obtained. It is prudent to obtain three such collections [51, 58]. Although a normal individual may put out 20 times baseline excretion after penicillamine administration, an individual with Wilson's disease will put out considerably more. Mowat and colleagueshave shown that urinary excretion of 25 μmoles of copper per 24 hours (or more) is diagnostic of Wilson's disease and they argue that this test is more reliable than the measurement of hepatic tissue content of copper. Unfortunately, the test is not adequately standardized, and diagnostic values are not established.

Table 8

Tests for the diagnosis of Wilson's disease

Test	Typical findings	Normal range
Hepatic copper	> 250 mg/g dry weight	< 50 μg/g d.w.
24-h urinary copper	> 100 μg/24 h	< 40 μg/24 h
Serum ceruloplasmin	< 20 mg/dL	20-50 mg/dL
Kayser-Fleischer rings	Present	absent
Serum free copper	> 25 μg /dL	< 15 μg /dL

Radiolabeled copper testing directly assays hepatic copper metabolism. Blood is collected at 1, 2, 4, 24, and 48 hours after oral ingestion of radiolabeled copper (^{64}Cu or ^{67}Cu) for radioactivity in serum. In all individuals, radioactivity promptly appears after absorption, followed by hepatic clearance. In healthy people, reappearance of the radioactivity in serum occurs as the labeled copperis incorporated into newly synthesized ceruloplasmin and released into the circulation. Heterozygotes exhibit a slow, lower-level reappearance of radioactivity rather than the continued fall in radioactivity seen in persons with Wilson disease, but there may be considerable overlap between the 2 types of patients.

Patients with Wilson disease, even those with normal ceruloplasmin levels, do not exhibit the secondary rise in radioactivity.

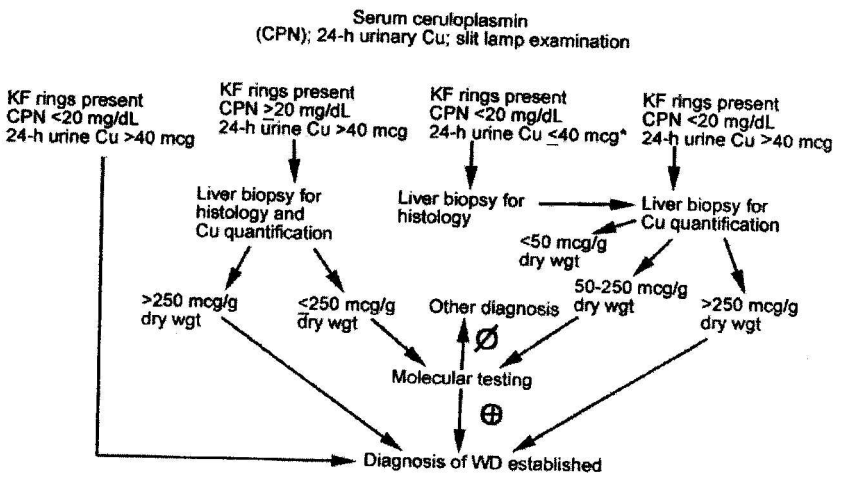


Fig. 8. **Diagnosis Wilson Disease based on ceruloplasmine.**

Cranial CT scanning

The cranial lesions observed on **CT scans** are typically bilateral and are classified into 2 general categories: (1) well-defined, slitlike, low-attenuation foci involving the basal ganglia, particularly the putamen, and (2) larger regions of low attenuation in the basal ganglia, thalamus, or dentate nucleus. Widening of the frontal horns of the lateral ventricles and diffuse cerebral and cerebellar atrophy, which correlate histologically with widespread neuronal loss, have also been described.

The cranial lesions observed on **CT scans** are typically bilateral and are classified into 2 general categories: (1) well-defined, slitlike, low-attenuation foci involving the basal ganglia, particularly the putamen, and (2) larger regions of low attenuation in the basal ganglia, thalamus, or dentate nucleus. Widening of the frontal horns of the lateral ventricles and diffuse cerebral and cerebellar atrophy, which correlate histologically with widespread neuronal loss, have also been described.

BRAIN MRI appears to be more sensitive than CT scanning in detecting early lesions of Wilson disease. MRI studies have identified focal abnormalities in the white matter, pons, and deep cerebellar nuclei. These lesions, measuring 3-15 mm in diameter, are typically bilateral, appearing with low signal intensity on T1-weighted images and with high signal intensity on T2-weighted images, representing cell loss and gliosis. Other studies describe decreased signal intensity in the putamen and other parts of the basal ganglia, which may represent either copper or iron ferritin deposition. A characteristic "face of the giant panda" sign has been described, formed by high signal intensity in the tegmentum (except for the red nucleus), preserved signal intensity of the lateral portion ofthe pars reticulata of the substantia nigra, and hypointensity of the superior colliculus.

Results from a study by *Tarnacka* et al indicated that relative to the thalamus, the basal ganglia are more sensitive to ongoing degenerative changes and portal-systemic encephalopathy in Wilson disease. The authors used proton magnetic resonance spectroscopy (MRS) in 37 patients with newly diagnosed Wilson disease to identify the pathomechanism of the disease's cerebral pathology, specifically looking at the globus pallidus and thalamus to assess cerebral metabolic changes in myoinositol, choline, creatine, N-acetyl-aspartate, lipid, glutamine, and glutamate levels and ratios.The investigators speculated that N-acetyl-aspartate/creatine ratio reductions seen in hepatically and neurologically impaired patients in the study may have indicated an association between neurodegeneration and all presentations of Wilson disease. In addition, they suggested that observed decreases in myoinositol and choline and an increase in neurologic glutamate may have been due to portosystemic shunting.

Genetic testing

Mutation analysis by whole-gene sequencing is possible and should be performed on individuals in whom the diagnosis is difficult to establish by clinical and biochemical testing. Haplotype analysis or specific testing for known mutations can be used for family screening of first-degree relatives of patients with WD. A clinical geneticist may be required to interpret the results.

Genetic testing is not prime time yet. At present, more than 200 mutations of the gene **(ATP7B)** have been identified, but results on phenotype-genotype correlation are not yet conclusive, probably due to the high number of compound heterozygous alleles or the presence of environmental factors that contribute to the phenotypic expression. Detecting disease-causing mutations allows WD to be diagnosed, but a negative result cannot exclude a diagnosis of WD. The use of genetic testing should therefore be limited to screening first-degree relatives of patients with WD.

Although genetic testing may provide definitive proof ofthe diagnosis of Wilson's disease, the multitude of documented mutations identified in Wilson's disease makes commercial genetic testing impractical. Advances and refinements in technology may make this possible in the future, but currently the diagnosis of Wilson's disease still must be made by the judicious employment of a combination of diagnostic tests.

Histologic findings

The earliest changes detectable with light microscopy include glycogen deposition in the nuclei of periportal hepatocytes and moderate fatty infiltration. The lipid droplets, which are composed of triglycerides, progressively increase in number and size, sometimes resembling the steatosis induced by ethanol.

Hepatocyte mitochondria typically exhibit heterogeneity in size and shape, with increased matrix density, separation of the normally apposed inner and outer mitochondrial membranes, widened intercristal spaces, and an array of vacuolated and crystalline inclusions within the matrix. With progression of disease, copper protein is sequestered in lysosomes and is visible as electron-dense pericanalicular granules.

Despite consistently elevated hepatic copper levels in patients with Wilson disease, histochemical staining of liver biopsy specimens for copper is of little diagnostic value. Early in the disease, copper distribu-

tion is primarily cytoplasmic and is not readily apparent with rhodamine or rubeanic acid staining.

The rate of progression of the liver histology from fatty infiltration to cirrhosis is variable, although it tends to occur by 1 of 2 general processes, either with or without hepatic inflammation.

The histologic picture may be histologically indistinguishable from that of chronic active hepatitis. Pathologic features include mononuclear cell infiltrates, which consist mainly of lymphocytes and plasma cells; piecemeal necrosis extending beyond the limiting plate; parenchymal collapse; bridging hepatic necrosis; and fibrosis.

The histologic pattern is one of a macronodular or mixed micro-macronodular cirrhosis, with fibrous septa (containing predominantly types I and III collagen), bile ductule proliferation, and variable septal round cell infiltration. Hepatocytes at the periphery of the nodules frequently contain Mallory hyalin. One proposed mechanism implicates copper as the inducer of fibrogenesis.

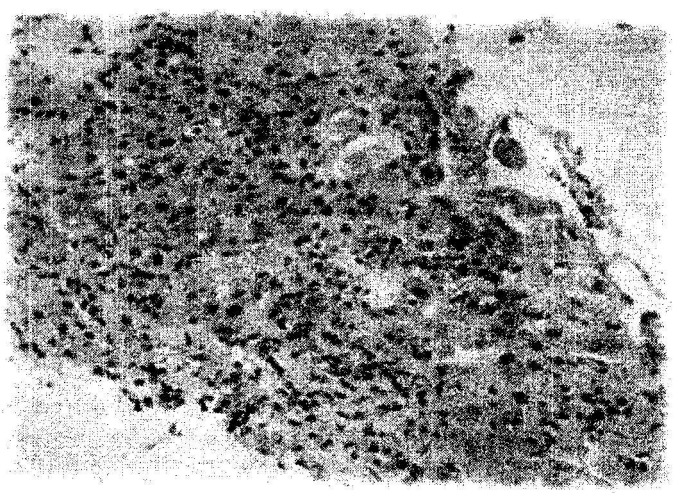


Fig. 9. **The histological findings in hepatic Wilson disease.**

Interestingly, hepatocellular carcinoma is exceedingly rare in patients with Wilson disease compared with patients with hemochromatosis. This may be attributable to the significantly shortened life expectancy in untreated patients with Wilson disease, which does not allow time for carcinoma to develop. An increasing number of case reports suggest that the incidence will likely increase with improved survival. It has

been proposed that the diminished cancer risk is due to the relatively low inflammatory component in the pathogenesis of Wilson disease.

Electron microscopic studies on ultrathin sections reveal numerous electron-dense lysosomes and residual bodies. The elemental analysis in transmission electron microscopy with electron energy loss spectroscopy, and in scanning electron microscopy with energy dispersive x-ray analysis, shows copper-specific signals of electron-dense accumulations inside these dark lysosomes and residual bodies. The electron microscopic detection of copper-containing hepatocytic lysosomes is helpful in the diagnosis of the early stages of Wilson disease, in addition to the quantification of hepatic copper by atomic absorption spectrophotometry.

Neurologic. Observed gross anatomical changes include degeneration and cavitation, primarily involving the putamen, globus pallidus, caudate nucleus, and thalamus. Little correlation has been observed between the degree of neurologic impairment and the neuropathologic findings. The affected areas of the brain do not possess higher copper concentrations than the unaffected portions.

Prognostic scoring systems for WD are a useful tool to direct therapy, identify which patients can be treated medically and which have a high likelihood for death and will require liver transplant. A prognostic index calculated by Nazer et al.

An improvement to the Nazer prognostic index score by Dhawan and associates *(Liver Transpl 2005; 11(4):441–448)* based on serum bilirubin, INR, AST, white blood cell count, and albumin (score range 0–20) was able to predict favorable response to chelation therapy in newly diagnosed pediatric WD patients who scored 11 and less; and those patients who scored >11 died without transplantation.

Not surprisingly, higher prognostic scores will be found in high MELD (Model for End-Stage Liver Disease – scores because both scores share 2 out of 3 parameters of the MELD score, which represents a 3-month survival curve for adults and children 12 and older.

MELD is a prospectively developed and validated chronic liver disease severity scoring system that uses a patient's laboratory values for serum bilirubin, serum creatinine, and the international normalized ratio for prothrombin time (INR) to predict survival.

MELD = 3.8[Ln serum bilirubin (mg/dL)] + 11.2[Ln INR] +
+ 9.6 [Ln serum creatinine (mg/dL)] + 6.4

where: Ln is the natural logarithm. For ease of use, several calculators are readily available for calculating the MELD score.

Table 9

Classification of hepatic failure based upon the prognostic index of Nazer et al.

Laboratory measurement	Normal value	Score (in points)			
		0	1	2	3
Serum bilirubin	0.2–1.2 mg/dl	<5.8	5.8-8.8	8.8–11.7	11.7–17.5
Serum aspartate transferase (AST)	10–35 IU/L	<100	100-150	150-200	201-300
Prolongation of prothrombin time (seconds)	–	<4	4-8	9-12	13-20

The sensitivity and specificity of the Nazer scoring system was 87% and 90%, with a positive predictive value of 72%.

Table 10

Modified Nazer's Wilson Disease Outcome Score

	Bilirubin (μmol/L)	AST IU/l	INR
0	<100		<1.3
1	100–150	100–150	1.3-1.6
2	151-200	151-200	1.6-1.9
3	201-300	201-300	1.9-2.4
4	>300	>300	>2.4

Protrombine Time converted to INR. ≥7 = increased risk of mortality without liver transplant.

Table 11

Scoring system developed at the 8th International Meeting on Wilson's disease, Leipzig 2001

Liver copper (in absence of cholestasis)		Serum ceruloplasmin	
Normal (< 50μ/g)	-1	Normal (> 0,2 g/l)	0
< 5xUNL (50-250 μ/g)	1	0.1-0.2 g/l	1
>5xUNL (250 μ/g)	2	<0.1 g/l	2
Rhodanine stain (in absence of quanti tati ve livercopper determinati on)		Coomb's negati ve haemolytic anemia	
Absent	0	Absent	0

Present	1	Present	1
Mutation analysis		Clinical symptoms and signs	
2 chromosomes mutati ons	4	KF ring	
1 chromosome mutati ons	1	Present	2
no mutation detected	0	Absent	0
Urinary copper Neurological signs (in absence of acute hepati ti s) (or typical abnormaliti es at MRI)			
normal (< 0.9 μmol/day or < 100 mg/day)	0	Severe	2
1-2 x UNL	1	Mild	1
>2 x UNL	2	Absent	0
normal but > 5 x UNL after D-penicillamine	2		

DIAGNOSTIC CONSIDERATIONS IN SPECIFIC TARGET POPULATIONS

"Mimic" Liver Diseases and Acute Liver Failure

- Patients in the pediatric age bracket who present a clinical picture of autoimmune hepatitis should be investigated for WD.
- Adult patients with atypical autoimmune hepatitis or who respond poorly to standard corticosteroid therapy should also be investigated for WD.
- WD should be considered in the differential diagnosis of patients presenting with nonalcoholic fatty liver disease or who have pathologic findings of nonalcoholic steatohepatitis.
- WD should be suspected in any patient presenting with acute hepatic failure with Coombs-negative intravascular hemolysis, modest elevations in serum aminotransferases, or low serum alkaline phosphatase and ratio of alkaline

The clinical presentations of Wilson's disease can mimic AIH (autoimmune hepatitis), especially in younger patients. The differentiation of Wilson's disease from AIH can be supported by the presence of a Kayser Fleischer ring and through urine and serum copper studies in patients with Wilson's disease. Because the onset of fulminant hepatic failure (FHF) may be the first presentation of Wilson's disease (WD) and autoimmune hepatitis (AIH) in previously asymptomatic adolescents, determination of the etiology of FHF is critical as treatment and prognosis differ between these two entities. Patients with AIH may be

salvaged by medical treatment. On the contrary, liver transplantation is currently the only life saving therapeutic option available for patients with WD who present with fulminant liver failure. To establish the diagnosis of WD and AIH in the setting of FHF remains challenging for diagnosticians and decisions regarding liver transplantation may be necessary before a diagnosis is firmly established.

Differential diagnosis of NASH is important, and led to the confirmation of Wilson's disease. Patients with NASH often present with few or no symptoms, though imaging techniques and liver biopsy show fat accumulation in the liver, mostly accompanied by hyperlipidemia. However, evaluation of patients based on fatty liver, hyperlipidemia, and abnormal liver function tests may not be sufficient in detecting the severity of the underlying cause. Therefore, for the adult and even elderly patients with unexplained histologic findings of steatohepatitis, it is reasonable to consider the possibility of Wilson's disease, before starting any treatment regime.

THERAPY OF WILSON DISEASE

Diet

- Patients should generally avoid eating foods with a high copper content, such as liver, chocolate, nuts, mushrooms, legumes, and shellfish (especially lobster). Liver copper is sufficiently high in copper that Wilson's disease patients should not eat liver. Abite of liver pate is acceptable, but no full meals. Shellfish are not as high in copper as liver, and after 6–12 months of treatment, one meal a week containing substantial shellfish is acceptable. Other frequently banned foods, such as chocolate, nuts, mushrooms, etc. are acceptable as a normal part of the diet. Of course, it should be obvious that vitamin/mineral supplements that contain copper should not be taken. If the patient is on enteral tube feedings, it should be noted that most commercial tube feedings are over-supplemented with copper. Tube feedings should be restricted to less than 1.5 mg of copper/day.
- It is good practice to evaluate copper levels in the usual drinking and cooking water consumed by a patient. This may include more than one source, such as home, place of work, or school. While most drinking water is relatively free of copper, occasionally the

combination of copper pipes and relatively low pH will leach copper from the pipes and cause it to be elevated in the drinking water. The reason is practice for patients not to use water with over 0.1 ppm of copper. It is not that this level of copper is unsafe, it is that it indicates that some copper leaching is occurring. With this situation, some samples of water (such as that drawn first in the morning), may have more dangerously elevated levels. 90% of the time, tested water will be well below 0.1 ppm. 3.3.

Physicaltherapy in patients presenting with neurologic disease, physical therapy to maintain range of motion and physical fitness is important.

- If dystonia begins to lead to contracture, approaches to maintaining physiological positioning can be helpful. Since it is expected that the patient will begin improving about 6 months after anticopper therapy initiation, it is important for the patient and caregivers to maintain a positive attitude, and try to retain as much physical capability as possible.

Speech therapy can also be helpful, particularly in teaching the patient speech exercises and strategies.

- Again the initial effort is to maintain and strengthen existing capabilities, so that when neurologic recovery begins, potential outcomes are optimized.
- If the patient is having extreme difficulty communicating, alphabet boards or electronic devises may help relieve frustration.
- As the patient recovery nears the two year point, occupational therapy may be helpful, to assist the patient in getting back into the work force. Often because of residual disability, the original occupation of the patient may no longer be feasible, and the patient may need training for some new area.

Even patients with significant disability can benefit in many ways from having some type of employment. The two-year point is also the time to begin considering rehabilitative surgery in some patients.

- If a hand or foot has undergone contracture, tendon-lengthening surgery may allow a more physiologic position.

Medications in Wilson disease

The mainstay of therapy for Wilson disease is pharmacologic treatment with chelating agents [23, 47].

- Initial treatment for symptomatic patients with WD should include a chelating agent, D-penicillamine or trientine (bettered tolerated than D-penicillamine).
- Zinc is recommended by some experts as a first line therapy in neurological patients. Maintenance therapy of presymptomatic patients or those with neurological symptoms usually requires a chelating agent or zinc.
- Ammonium tetrathiomolybdate shows optimal effect in treating patients presented with neurological symptoms, though not yet commercially available.

The efficacy of copper excretion is measured by the levels of urinary copper.

After the initiation of therapy with a chelating agent, the patient needs to be aware of potential adverse effects of the agents with which he or she is being treated

For instance, some of the concerning adverse effects are those commonly associated with penicillamine use. In addition, a patient must also be aware of the potential to develop worsening of some symptoms when chelation is started; in particular, patients with neurologic signs and symptoms can see worsening of these with chelation, and, in some instances, therapy needs to be reduced or stopped.

Laboratory tests in patients started on penicillamine should include hematology and biochemical monitoring, as well as urinalysis.

D-penicillamine

D-penicillamine chelates copper inside the body. It mobilizes intracellular copper into the circulation and enhances urinary excretion of copper. McArdle et al.found that D-penicillamine increases metallothionein mRNA levels without changing either the rate of copper uptake or the amount of copper within mouse hepatocytes. Metallothionein chelates the excessive copper to form a non-toxic combination [24, 25].

- The initial dose of penicillamine is 750–1500 mg per day in two to four divided doses for adults. Dosing in children is 20 mg/kg/day to the nearest 250 mg, divided in two or three divided doses.
- The treatment is best taken 1 hour before or 2 hours after food.

- Absorption is estimated to be only 50% if it is taken with a meal.
- The use of lower initial doses, 125–250 mg per day, increasing over a few weeks, can enhance tolerance to the agent.
- Pyridoxine (vitamin B6) is added routinely to the treatment regimen in a dosage of 20–50 mg daily, as its deficiency is associated with neurological worsening induced by D-penicillamine [33], which is irreversible in some cases.

The use of D-penicillamine remains controversial for decades due to its adverse effects. Someone suggests that D-penicillamine should not be used as initial therapy in WD [75]. Apart from neurological worsening, they include early reactions such as fever, rash, lymphadenopathy or late reactions such as bone marrow and renal toxicity [36]. Newly reported unfavorable effects consist of ANCA-vasculitis in WD and dermatology toxicity, such as progeric changes in the skin, such as pemphigous or pemphigoid lesions [7].

- Severe adverse effects necessitate discontinuation of D-penicillamine and change of regimen in 20-30% of patients.

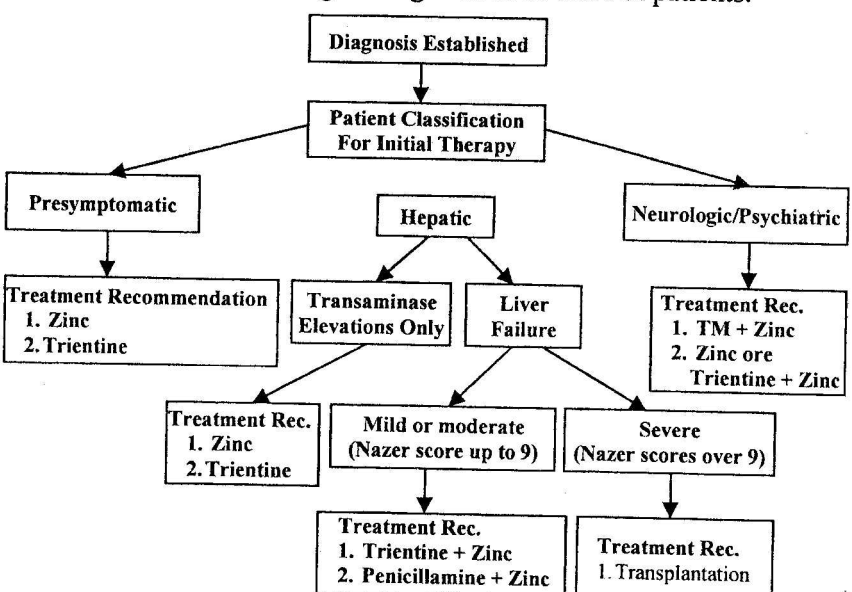


Fig. 10. **The recommendations of therapy in Wilson disease.**

The reasons for the neurological deterioration of the D-penicillamine are still unknown. Depletion of pyridoxine may be one of them.

- Radical therapies that drastically lower serum copper level are found to induce partial status epilepticus as well as severe deterioration of neurological symptoms in one patient, whose conditions improved shortly after discontinuing D-penicillamine and having a high copper diet]. Increased free copper concentrations are found in the serum and brain, and declined protein-bound copper concentrations are found in the brain of toxic milk mice during D-penicillamine administration]. Immunofluorescence staining shows intense staining of ATP7A in the choroid plexus but rare ATP7A and copper transporter 1 (CTR1) on the blood–brain-barrier, which suggests that D-penicillamine mobilizes free copper from the brain parenchyma rather than from the blood.
- During administration of D-penicillamine, periodic clinical, hematological, biochemical and routine urinary parameters are monitored weekly for 1 month, then monthly for 6 months and at 6-month intervals thereafter. It's notable that cessation of penicillamine without replacement treatment causes rapid progression to fatal fulminant hepatitis.

Trientine

Trientine has a polyamine structure, which chelates copper by the formation of stable complexes with the four constituent nitrogens in a planar ring. It is debatable whether the two agents mobilize different pools of copper [20, 25, 42].

- The initial dose is 900–2700 mg per day in two to three divided doses. Maintenance therapy is 900–1500 mg per day.
- Trientine should be given orally 1 hour before or 2 hours after food.
- Trientine is a less potent copper remover than D-penicillamine with its toxic profile similar to that of D-penicillamine, although side effects are less frequent and generally milder. It is believed that neurological deteriorations are less frequent in trientine than in D-penicillamine.

Zinc

Zinc induces intestinal metallothionein, which preferentially binds to copper within the duodenal enterocytes. Thus, copper absorption into the circulation is reduced, and copper is lost when the enterocytes are shed during normal cell turnover. Continuing copper losses in combination with reduced absorption lead to a negative copper balance. Furthermore zinc can induce copper-binding metallothionein in hepatocytes, thereby reducing the damaging effects of free copper [25, 75].

- Zinc has been used successfully in asymptomatic or presymptomatic patients.
- Zinc is effective as a sole therapy and that it has low toxicity based on data from the long-term follow-up of maintenance zinc treatment.
- In patients with severe hepatic disease, maintenance therapy with zinc was effective after an initial period of treatment with trientine and zinc.
- When the patients are given zinc monotherapy, liver functions should be regularly monitored. Among patients with hepatic manifestations, non-response to zinc monotherapy is not uncommon. This is not due to noncompliance. If the level of AST, ALT and γ-GT remains in a high level after the initiation of the zinc therapy, it often suggests a poor response and a change in therapy should be considered as soon as possible [50].
- Patients discontinue zinc therapy mostly due to gastrointestinal discomfort. It is more common with zinc sulfate than zinc acetate. During a long-term follow-up by Bruha et al., with a median follow-up of 12 years, no adverse events were reported with zinc acetate [50].

Ammonium tetrathiomolybdate

Tetrathiomolybdate has a unique mechanism of action. Its four sulfur groups allow it to form a stable tripartite complex with copper and protein [51, 52]. Ammonium tetrathiomolybdate is well-absorbed with or without food. It forms a complex with copper in the food. When it is given away from food, it forms a complex with available copper and albumin so that it can't be up-taken or utilized for intracellular process [7].

- Although not yet commercially available, its high efficacy and rare neurological deterioration compared with trientine makes it a potential cure for WD patients with neurological symptoms.
- Unlike trientine, tetrathiomolybdate stably lowers the serum "free copper" level, which may explain uncommon neurological deterioration. Its recommended dose is 120 mg daily as 20 mg with meals and 20 mg between meals for 2 weeks, and then 60 mg daily as 10 mg 3 times daily with meals and 10 mg 3 times daily between meals.

Therapy of the symptomatic patient

Despite the generally well response to chelation therapy with zinc, additional treatments to control the disturbing symptoms are still necessary for some patients.

Neurological symptoms are usually more refractory than hepatic damages. Neurological symptoms can be categorized into Parkinsonism, dysarthria, dystonia, tremor, pseudosclerosis [35, 41]. Parkinsonism can be treated with L-dopa, dopamine agonists [54] and anticholinergics (trihexyphenidyl) [46]. Essential tremor-like tremor is treated with beta-blocker (propranolol) or barbituate (primidone). Dystonia can be treated with trihexyphenidyl, baclofen, valium or dopamine antagonist (tiapride) and botulinem injection [56, 57]. Proxysmal dystonic movement can be treated with oxcarbazepine. Gabapentine can be used when dealing with penicillamine-induced dystonia when other options fail. However, there are only a small number of case reports regarding symptomatic treatments and the conclusions are inconsistent.

Table 12

Wilson's disease pharmacological treatment

Drug	Dose	Indication	Side effects
DPCA	0.75-1.5 g/day	Hepatic WD	Hypersensitivity reactions, bone marrow depression, late reaction involving skin, joints and immune system. Neurological worsening
Trientine	1 g/day	Hepatic and Neurologic WD (in case of DPCA intolerance)	Lupus-like syndrome, sideroblastic anemia.

Zinc	150-200 mg/day	Hepatic and neurologic WD: pre-symp-tomatic and/or maintenance; pregnancy	Gastric discomfort
TTM	120 mg/day	Neurologic WD	Bone marrow depression, transient increase of liver tests

DPCA – D-penicillamine, *WD* – Wilson diseases, *TTM* – Tetrathiomolybdate

Therapy of the presymptomatic patient

- Presymptomatic patients are those that are diagnosed before becoming clinically ill. Usually these will be siblings of an affected patient who are diagnosed as a result of family screening [23, 43].
- Occasionally a presymptomatic patient will be identified when routine ophthalmologic examination reveals Kayser-Fleischer rings, or when routine serum biochemistries reveal elevated serum transaminase enzymes.
- These patients may be viewed as equivalent to symptomatic patients who have received initial therapy and are in the maintenance phase of treatment. Thus, they are treated with 'maintenance phase therapy', that is zinc or trientine from the beginning.

Liver transplantation

There are some reports of successful management of acute liver disease by medical therapy [43, 68].

However, if medical therapy fails to suppress the progression of the disease, orthotopic liver transplantation (OLT) is the only alternative treatment.

- Liver transplantation is indicated for WD patients with acute liver failure when the revised Nazar's score is 11 or higher and patients with decompensated cirrhosis that's unresponsive to chelation therapy.

Liver transplantations of these individuals can be achieved by a cadaveric donor or living donor transplant, even if the donor is a heterozygous carrier [75,76].

OLT generally gives excellent results. The one year survival rate and 5 year survival rate in pediatric patients are 90.1% and 89% in com-

parison with a survival of 88.3% and 86% for adults in a multi-center observational study over 20 years. The OLT also corrects the underlying renal disorder. However, survival is only one aspect of patients' well-being. Neurological complications can be quite common. The immunosuppressive agents would leave the patients vulnerable to infections. Lack of compliance in the immunosuppressive agents would lead to acute hepatic failure. Long-term follow-up would be in need to assess the efficacy of OLT.

- Liver transplantation is not recommended for patients with neurological and psychiatric symptoms. However, some patients choose to undergo transplantation for their neurological impairment and some patients with liver damage show neurological improvement after the surgery. Otherwise, there are risks associated with the procedure of liver transplantation and the following immunosuppressive therapy [67].
- There are few absolute contraindications for liver transplantation. Patients with poor cardiac and pulmonary functions, namely patients with severe hypoxia or right arterial pressure greater than 60 mm Hg rarely survive surgery and the perioperative recovery period. Before transplantation is attempted, patients with extrahepatic malignancies other than squamous cell skin carcinoma should be postponed for at least 2 years after the curative therapy is completed. Significant psychiatric or neurological disorders and ongoing destructive behavior caused by substance abuse must be under effective medical control to make sure that the patient can be compliant after transplantation. Absence of a viable splanchnic venous inflow system is the most common surgical contraindication to liver transplantation. If the entire portal venous system is occluded, the transplantation is rarely successful. Plus, uncontrolled systemic infection is apparently a contraindication to high-dose immunosuppressive therapy.

Are the decoppering treatment and the low copper diet necessary after the transplant? There is no report yet. Long-term results of liver transplantation are still in lack of large cohort studies. The development of better prognostication for neurological progression or improvement of WD by MRI findings or other clinical or biochemical variables will help improve our abilities to make treatment choices.

Molecular adsorbents recirculating system *(MARS).* MARS [64] is an extracorporeal liver support system using a hollow-fiber dialysis module in which the patient's blood is dialyzed across an albumin-impregnated membrane while maintaining a constant flow of albumin-rich (20%) dialysate in the extracapillary compartment. Case reports and very small series have presented a role for this as a bridge to liver transplantation.

Practical guidelines for the treatment of Wilson's disease

- Start therapy as soon as possible: Wilson's disease is a treatable disorder.
- Initial treatment of symptomatic patients who have only hepatic involvement should include a chelating agent (D-PCA or trientine).
- Treatment of presymptomatic patients or maintenance therapy of patients with mainly hepatic involvement can be safely based on zinc salts.
- Patients with mainly neuro-psychiatric involvement should be treated from the beginning with zinc, and never with D-PCA.
- Liver transplantation represents the ultimate treatment for Wilson's disease, when all medical therapies options fail, but neuro-psychiatric disease as the main clinical symptom is a contraindication to orthotopic liver transplantation.

Pregnancy in Wilson's desease

Female WD patients can become pregnant if her copper status optimized prior to pregnancy. Although there is some concern over the teratogenicity of D-penicillamine, withdrawing treatment is more risky than continuing it.

Low risk of teratogenicity is also true for treatment with trientine or zinc [34]. Breast feeding under chelation therapy is not recommended, although there are reports that children breast fed by mothers on D-penicillamine show no abnormalities. It is recommended to lower D-penicillamine during the first trimester and put patients on the lowered dosage for all trimesters with continued monitoring. Others recommend administering chelators at a minimal dose, i.e. 300–600 mg/day in the last trimester to avoid insufficient copper supply to the fetus or insufficient wound healing after Cesarean section or episiotomy.

Therapy of the pediatric patient provides therapy recommendations for the pediatric patient. Therapy for initial presentations, maintenance, or presymptomatic therapy follows adult recommendations with appropriate reduction in dose. Recommended pediatric doses for zinc are given in. Experience is limited below 6 years, and is nonexistent below 3 years [37], so treatment of very young children should involve regular monitoring. Dose adjustments for trientine are 500–750 mg/day in two or three divideddoses for children under 12 years.

Family screening

First-degree relatives must be screened for Wilson's disease. Liver function tests, serum copper and caeruloplasmin concentration, and urinary copper analysis are done for relatives. If necessary, investigations should be extended to test for K-F rings. 24-h urinary copper might be difficult to interpret in Wilson's disease heterozygotes. The diagnosis could remain contentious when individuals without K-F rings have a low ceruloplasmin concentration. These individuals might need a liver biopsy for hepatic copper quantification to eliminate the diagnosis.

Table 13

Screening family members of patients with Wilson's disease

Tests	Symptomatic	Presymptomatic	Heterozygous carrier
Clinical examinations	Hepatic or neurological features	Hepatomegaly in 38% of cases	No abnormality
Slit lamp exams for KF ring	KF ring positive in neuropsychiatric presentation and in 50% of hepatic presentation	KF ring positive in about 1/3 of cases	negative
Serum cerulo-plasmin level	Low in about 85%	Same as symptomatic	Low (<15 mg%) about 15-20%
24-hour urinary copper excretion	High >100 mg	Same asymptomatic	Normal
Genetic analysis	Mutation in both Wilson disease genes	Same asymptomatic	Normal genes

Molecular genetic analysis is becoming more widely available and is useful for families in which both mutations have been detected in the index patient, allowing molecular analysis for the same mutations in siblings.

Haplotype analysis of markers around the *ATP7B* gene on chromosome 13 has been used in families to establish whether siblings of aff ected individuals have inherited the same pair of chromosomes. This approach would be useful when it has not been possible to detect both mutations in the index case by mutation analysis.

Long-term monitoring

- Perform a physical examination, 24-hour urinary copper excretion assay, complete blood count (CBC), urinalysis, serum free copper measurement, and renal and liver function tests on a weekly basis for the first 4-6 weeks following initiation of chelation therapy.
- The best way to monitor efficacy is to measure serum nonceruloplasmin-bound copper. This is measured by the following formula: Total serum copper (mcg/dL) – 3 [ceruloplasmin (mg/dL)]. The reference range is less than 15 mcg/dL.
- An adjunctive way to monitor efficacy is to measure urinary copper excretion. Urinary chelator levels usually measure 200-500 mcg/day. Urinary zinc levels usually measure less than 75 mcg/day.
- Bimonthly evaluations are recommended through the first year, followed by yearly examinations thereafter. In patients with Kayser-Fleischer rings, a yearly slit-lamp examination should document fading or disappearance if patients are being adequately "decoppered."
- Lifelong, uninterrupted chelation therapy is necessary in all patients with Wilson disease.
- Frequent follow-up with patients is necessary, secondary to patient decompensation due to noncompliance. This is one of the major causes of fulminant liver failure.
- Patients must avoid most alcohol consumption and potential hepatotoxic drug therapy.

Recovery

- In patients with hepatic failure treated initially with trientine and zinc, 75% recovery to normal serum albumin took 6.5 months, serum bilirubin 3.7 months, and serum aspartate transferase (AST) 5.1 months [28],

All of these values were normal at 1 year. Of course, histological abnormalities including cirrhosis often remain more or less permanently, although even these may slowly regress over years of zinc therapy. Along with the persistence of cirrhosis, evidence of portal hypertension, such as thrombocytopenia and leukopenia from hypersplenism, may also persist.

- Neurologic patients begin showing clinical improvement 5–6 months after initiation of anticopper therapy, and continue to improve over the succeeding 18 months [27]. Residual abnormalities present after 24 months of adequate anticopper therapy are usually permanent, although occasional patients report continued improvement in speech.

The degree of recovery varies, but most patients show substantial improvement. The greater the initial severity, the more likely that significant disability will be permanent.

Psychiatric and behavioral symptoms usually improve in conjunction with the improvement in neurologic symptoms that is they are improved by 1 year and mostly resolved by 2 years. Interestingly, as a rule of thumb, psychiatric and behavioral problems that antedate the onset of neurologic symptoms by more than 5 year are probably unrelated.

Summary:

Wilson'sdisease is:

- Rare autosomal recessive disorder that causes defective copper excretion, resulting in toxic accumulation of copper in multiple organs, particularly the liver, brain (primarily basal ganglia and cortex), kidneys, and eyes.
- A high degree of clinical suspicion is essential in any patient (particularly if aged younger than 50) with an undetermined cause of liver abnormality, or a combination of hepatic and neuropsychiatric signs and symptoms; prompt diagnosis and copper removal treatment significantly improve the prognosis.
- The treatment paradigm for symptomatic patients remains copper removal with copper chelating agents; zinc is now increasingly used to maintain normal free serum copper levels in symptomatic patients who have undergone adequate chelation, and as first-line therapy in asymptomatic individuals to prevent copper accumulation.

- If patients adhere to lifelong maintenance therapy, the prognosis is generally good; in rare cases, including patients who present with acute liver failure or advanced end-stage liver disease unresponsive to medical therapy, liver transplantation is necessary and highly effective. A liver transplant reverses the underlying copper metabolic defect.
- Family screening of first-degree relatives is mandatory following diagnosis of an index case.
- Wilson disease may present as acute liver failure. These patients require urgent liver transplantation for survival.

REFERENCES

1. Anjali B. Shah, Igor Chernov, Hong Tao Zhang et al. Identification and Analysis of Mutations in the Wilson Disease Gene (ATP7B): Population Frequencies, Genotype-Phenotype Correlation, and Functional Analyses The American Journal of Human Genetics, Volume 61, Issue 2, 1997, 317-328.
2. Abdel Ghaffar T.Y., Elsayed S.M., Elnaghy S., Shadeed A., Elsobky E.S., Schmidt H.: Phenotypic and genetic characterization of a cohort of pediatric Wilson disease patients. BMC Pediatr. 2011, 11:56.
3. Aksoy M., Camli N., Dincol K. et al. Osseous changes in Wilson's disease. A radiologic study of nine patients. Radiology 1972; 102:505–9.
4. Ala A., Walker A.P., Ashkan K., Dooley J.S., Schilsky M.L. Wilson's disease. Lancet 2007, 369(9559):397-408.
5. Autragésilo Filho A. Degeneração mucóide da oligodendroglia em um caso de enfermidade do tipo Wilson. Arq Neuropsiquiatr 1944; 2:170-176.
6. Boudin G., Pepin B. Dégénérescence Hépato-Lenticulaire. Masson, Paris, 1959; 75-87.
7. Brewer G.J., Askari F., Dick R.B., Sitterly J., Fink J.K., and Carlson M. et al. Treatment of Wilson's disease with tetrathiomolybdate: V. control of free copper by tetrathiomolybdate and a comparison with trientine. Transl Res. 2009; 154(2):70-7.
8. Berr F. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003, 23(3):139-142.
9. Bull P.C., Thomas G.R., Rommens J.M., Forbes J.R. and Cox D.W. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat Genet. 1993; 5(4):327-337.
10. Bowcock A.M, Farrer L.A., Hebert J.M., Agger M., Sternlieb I., Scheinberg I.H. et al. Eight closely linked loci place the Wilson disease locus within 13q14-q21. Am J Hum Genet. Nov 1988; 43(5):664-74.
11. Brewer G.J., Terry C.A., Aisen A.M., Hill G.M. Worsening of neurologic syndrome in patients with Wilson's disease with initial penicillamine therapy. Arch Neurol. 1987; 44:490–3.
12. Canelas H.M., De Jorge F.B., Costa-Silva. Contribuição ao estudo do metabolismo do cobre. I: Metodologia da determinação do cobre em materiais biológicos. Rev Paul Med 1962; 61:350-355.
13. Canelas H.M., De Jorge F.B., Spina-França A. Contribuição ao estudo do metabolismo do cobre. II: Valores normais do cobre no sangue, líquido cefalorraqueano e urina. Rev Paul Med 1963; 62:125-128.
14. Canelas H.M., De Jorge F.B. Contribuição ao estudo da ceruloplasmina. I: Valores normais no soro sangüíneo. Arq Neuropsiquiatr 1964;22:271-276

15. Canelas H.M., De Jorge F.B., Dias J.C., Cury L. Studies on cooper metabolism. III: Cooper contents of saliva, pancreas, and salivary glands. Clin Chim Acta (Amsterdam) 1964; 9:148-150.
16. Canelas H.M., De Jorge F.B., Escalante O.D. et al. Hepatolenticular degeneration: clinical and biochemical study. Arq Neuropsiquiatr 1963; 21:229-250.
17. Cançado E.L.R., Rocha M.S., Barbosa E.R. et al. Ultra-sonografia abdominal na degeneração hepatolenticular: estudo de 33 casos. Arq Neuropsiquiatr 1987; 45:131-136.
18. Cartwright G.E., Hodges R.E., Gubler C.J. et al. Studies on copper metabolism. XIII: Hepatolenticular degeneration. J. Clin Invest 1954; 33:1487-1501.
19. Cisse A., Morel Y., Coulibaly M., Souare I.S., Cisse A.F., Nabe A., Camara L.M., Cisse B., Camara S., Bangoura S.A., Sylla A., Dia H., Da Silva L. Wilson's disease: study of 6 neurologic forms observed at the University Hospital in Conakry. *Med Trop (Mars)* 2004, 64(1):53-57.
20. Compston A. Progressive lenticular degeneration: A familial nervous disease associated with cirrhosis of the liver, by S. A. Kinnier Wilson, Brain 2009; 132:1997.
21. Cox D.W. Factors influencing serum ceruloplasmin levels in normal individuals. J Lab Clin Med 1966; 68:893–904.
22. Cumings J.N. The copper and iron of brain and liver in the normal and in hepatolenticular degeneration. Brain 1948; 71:410-415.
23. EASL: Clinical Practice guideline: Wilson's disease Journal of Hepatology 2012; vol. 56, J. 671–685.
24. Da Costa Mdo D., Spitz M., Bacheschi L.A., Leite C.C., Lucato L.T., Barbosa E.R. Wilson's disease: two treatment modalities. Correlations to pretreatment and posttreatment brain MRI. Neuroradiology. Oct. 2009; 51(10):627-33.
25. Dastych M., Prochazkova D., Pokorny A., Zdrazil L. Copper and zinc in the serum, urine, and hair of patients with Wilson's disease treated with penicillamine and zinc. Biol Trace Elem Res. Jun. 27 2009.
26. Denny-Brown D., Porter H. The effect of BAL (2, 3-dimercaptocapropanol) on hepatolenticular degeneration (Wilson' disease). N. Engl J. Med. 1951; 245:917-925.
27. Dening T.R. The neuropsychiatry of Wilson's disease: A review. Int J. Psychiatry Med. 1991; 21:135–48.
28. Golding D.N., Walshe J.M. Arthropathy of Wilson's disease. Study of clinical and radiological features in 32 patients. Annals of the Rheumatic Diseases 1977; 36:99.

29. Gerlach W., Rohrschneider W. Besteht das Pigmet des Kayser-Fleischerschen Hornhautringes aus sliber Klin Wochenschr 1949; 13:48-9.
30. Ferenci P., Caca K., Loudianos G., Mieli-Vergani G. et al. Wilson's disease: the scourge of copper. J Hepatol 1998, 28(4):734-739.
31. Ferenci P., Caca K., Loudianos G., Mieli-Vergani G., Tanner S., Sternlieb I., Schilsky M., Cox D., Berr F. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003, 23(3):139-142.
32. Fleischer B. Zwei weitere Fälle von grünlicher Verfärbung der Kornea. Klin Mbl Augenheilk 1903; 41:489-491.
33. Feller E.R., Schumacher H.R. Osteoarticular changes in Wilson's disease. Arthritis and Rheumatism 1972; 15:259–66.
34. Furman B., Bashiri A., Wiznitzer A., Erez O., Holcberg G., Mazor M. Wilson's disease in pregnancy: five successful consecutive pregnancies of the same woman. Eur J. Obstet Gynecol Reprod Biol. 2001, 96:232-234.
35. Jackson G.H., Meyer A., Lippmann S. Wlson's Disease. Psychiatric Manifestations May be the Clinical Manifestation. J, Gastroenterol Hepatol 1992; 4:370.
36. Jessica D. Korman, Irene Volenberg, Jody Balko et al The Pediatric and Adult Acute Liver Failure Study Groups/ Screening for Wilson Disease in Acute Liver Failure: A Comparison of Currently Available Diagnostic Tests Hepatology 2008; 48:1167-1174.
37. Thomas C.R., Forbes J.R., Roberts E.A. et al. The Wilson Disease Gene: Spectrum of Mutations and their consequences. Nat Genet 1995; 9:210.
38. Steindl P., Ferenci P., Dienes H.P. et al. Wilson's Disease in Patients Presenting with Liver Disease-A Diagnostic Challenge. Gastroenterology 1997; 113:212.
39. Roberts E.A., Schilsky M.L. AASLD guideline: A practice guideline on Wilson Disease. Retrieved on August 19, 2006. www.uptodate.com
40. Hall H.C. La Dégénérescence hépato-lenticulaire. Paris, Masson, 1921.
41. Hitoshi S., Iwata M., Yoshikawa K. Mid-brain pathology of Wilson's disease: MRI analysis of three cases. J Neurol Neurosurg Psychiatry. 1991; 54:624–6.
42. Homén E.A. Eine eigenthümliche Familienkrankheit, unter den form einer progressiven Dementia, mit besbesonderem anatomischen Befund. Neurol Zentralblat 1890; 9:514-518.
43. Huster D. Wilson disease. Best Pract Res Cl Ga 2010, 24(5):531-539.
44. Kayser B. Ueber einen Fall von angeborener grünlicher Verfärfung der Cornea. Klin Mbl Augenheilk 1902; 40:22-25.
45. Longe A.C., Glew R.H., Omene J.A. Wilson's disease. Report of a case in a Nigerian. Arch Neurol .1982, 39(2):129-130.

46. Machado A., Chien H.F., Deguti M.M., Cançado E., Azevedo R.S., Scaff M. et al. Neurological Manifestations in Wilson's disease: Report of 119 Cases. Mov Disord. 2006; 21:2192–6.
47. Mandelbrote B.M., Stanier M.W., Thompson R.H.S. et al. Studies on copper metabolism in demyelinating diseases of the central nervous system. Brain 1948; 71:212-228.
48. Merle U., Schaefer M., Ferenci P., Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut 2007, 56(1):115-120.
49. Nazer H., Ede R.J., Mowat A.P., Williams R. Wilson's disease: clinical presentation and use of prognostic index. Gut 1986; 27:1377–1381.
50. Nicastro E., Ranucci G., Vajro P., Vegnente A., Iorio R. Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. Hepatology 2010, 52(6):1948-1956.
51. Ormerod J.A. Cirrhosis of the liver in a boy, with obscure and fatal nervous symptoms. St Bartholomew' Hosp Rep 1890; 26:56-68.
52. Owen C.A. Wilson' disease: the etiology, clinical aspects, and treatment of inherited copper toxicosis. New Jersey: Neyes, 1981:1-22.
53. Rathbun J.K. Neuropsychological aspects of Wilson's disease. Intern J Neuroscience1996; 85:221-229.
54. Roberts E.A, Schilsky M.L. American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. Hepatology. Jun 2008; 47(6):2089-111.
55. Rodman R., Burnstine M., Esmaeli B., Sugar A., Martonyi C., Johnson V. et al. Wilson's disease: Pre-symptomatic patients and Kayser Fleischer rings. Ophthalmic Genet. 1997; 18:79–85.
56. Rumpel A. Über das Wesen und die Bedeutung. der Leberveränderungen und der Pigmentierungen bei den damit verbunden Fällen von Pseudosklerose, zugleich ein Beitrag zur Lehre von der Pseudosklerose (Westphal-Strümpell). Dtsch Z Nervenkeilk 1913; 49:54-73.
57. Spielmeyer W. Die histopathologische Zuzammengehörigkeit der Wilsonschen Krank heit und der Pseudosklerose. Z Ges Neurol Psychiat 1920;57:312-351.
58. Scheinberg I.H., Gitlin D. Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson' disease). Science 1952;116:484-485.
59. Saito T. Presenting symptoms and natural history of Wilson disease. Eur J. Pediatr. 1987, 146(3):261-265.
60. Scheinberg I.H., Sternlieb I., Smith L.H. Major Problems in Internal Medicine. Philadelphia: WB Saunders; 1984. Wilson's disease; pp. 86–91.

61. Stapelbroek J.M., Bollen C.W., van Amstel J.K. et al. The H1069Q mutation in ATP7B is associated with late and neurologic presentation in Wilson disease: results of a meta-analysis. J Hepatol. Nov 2004; 41(5):758-63.
62. Stuehler B., Reichert J., Stremmel W., Schaefer M. Analysis of the human homologue of the canine copper toxicosis gene MURR1 in Wilson disease patients. *J Mol Med (Berl).* Sep 2004; 82(9):629-34.
63. Soni D., Shukla G., Singh S., Goyal V., Behari M. Cardiovascular and sudomotor autonomic dysfunction in Wilson's disease-Limited correlation with clinical severity. *Auton Neurosci.* Aug. 7 2009; epub ahead of print.
64. Sen S., Felldin M., Steiner C. et al. Albumin dialysis and Molecular Adsorbents Recirculating System (MARS) for acute Wilson's disease. *Liver Transpl.* Oct. 2002; 8(10):962-7.
65. Tarnacka B., Szeszkowski W., Golebiowski M., Czlonkowska A. Metabolic changes in 37 newly diagnosed Wilson's disease patients assessed by magnetic resonance spectroscopy. *Parkinsonism Relat Disord.* Sep 2009; 15(8):582-6
66. Tyler K. Fragments of neurologic history: "Tetanoid chorea" and progressive lenticular degeneration. Neurology 1983; 33:461-463.
67. Uta Merle, Karl Heinz Weiss, Christoph Eisenbach, Sabine Tuma, Peter Ferenci and Wolfgang Stremmel Truncating mutations in the Wilson disease gene ATP7B are associated with very low serum ceruloplasmin oxidase activity and an early onset of Wilson disease *BMC Gastroenterology* 2010, 10:8.
68. Yoshitoshi E.Y., Takada Y., Oike F. et al. Long-term outcomes for 32 cases of Wilson's disease after living-donor liver transplantation. *Transplantation.* Jan 27 2009; 87(2):261-7.
69. Xin-Hua Li, Yi Lu, Yun Ling et al. Clinical and molecular characterization of Wilson's disease in China: identification of 14 novel mutations *BMC Medical Genetics* 2011, 12:6 doi:10.1186/1471-2350-12-6.
70. Valentine J.S., Gralla E.B. Biochemistry - delivering copper inside yeast and human cells. *Science* 1997, 278(5339):817-818.
71. Von Hoesllin C., Alzheimer A. Ein Beitrag zur Klinnik und pathologischen Anatomie der Westphal-Strumpellschen Pseudosklerose. Z Ges Neurol Psychiat 1912;8:183-20.
72. Vulpe C., Levinson B., Whitney S., Packman S., Gitschier J. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nat Genet.* 1993, 3(1):7-13.
73. Walshe J.M. History of Wilson' disease: 1912-2000. Mov Disord 2006; 21:142-147 Walshe J.M. Copper: its role in the pathogenesis of liver disease. *Semin Liver Di* However, *s.* Aug 1984; 4(3):252-63.

74. Walshe J.M. Wilson's disease: the importance of measuring serum caeruloplasmin non-immunologically. *Ann Clin Biochem* 2003; 40:115-121. Walshe J.M. The liver in Wilson's disease. In: Schiff L, Schiff ER, editors. Diseases of the Liver. 6th ed. Philadelphia: Lippincott; 1987. p. 1037–1050.
75. Westphal C. Über eine dem Bilde der cerebrospinalen grauen Degeneration ähnliche Erkrankung des centralen Nervensystems ohne anatomische Befund nebst einigen Bemerkungen über paradoxe Contraction. Arch Psychiat Nervenkrh 1883; 14:87-134.
76. Weiss K.H., Gotthardt D.N., Klemm D., Merle U., Ferenci-Foerster D., Schaefer M. et al. Zinc monotherapy is not as effective as chelating agents in treatment of Wilson disease. *Gastroenterology*. Apr. 2011; 140(4):1189-1198.e1.
77. Weiss K.H., Thurik F., Gotthardt D.N., Schäfer M., Teufel U., Wiegand F. et al. Efficacy and safety of oral chelators in treatment of patients with Wilson disease. *Clin Gastroenterol Hepatol*. Aug. 2013; 11(8):1028-1035.e2.
78. Wilson S.A.K. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain 1912; 34:295-509.
79. Wilson disease mutation database. Available from: http://www.wilsondisease.med.ualberta.ca/database.asp.
80. Wilson D.C., Phillips M.J., Cox D.W., Roberts E.A. Severe hepatic Wilson's diseasein preschool-aged children. J. Pediatr. 2000; 137:719–722.

Review Answers

SELF ASSESSMENT QUIZ (Wilson Disease. Case Based Approach by author Adela Turcanu, p. 20-21))

1. b, d, e
2. a, b, e
3. a, c, e
4. b, c, e
5. b
6. b
7. c
8. b
9. b
10. a

The normal value of some biochemical test

Biochemical parameters	Normal value
ALT: alanine aminotransferase (SGPT)	5-40 U/l
AST: aspartate aminotransferase (SGOT)	8-20 U/l
Alkaline phosphatase	20-70 U/L
G-glutamiltranspeptidase (GGTP)	0-85 U/L men 0-40 U/L women
Bilirubin total • Indirect (unconjugated) • Direct (conjugated)	0.1 - 1.0 mg/dl 0.2-0.8 mg/dl 0.1 - 0.3mg/dl
Albumin	3.5 - 5 g/dL
Total protein	6.4 - 8.3 g/dL
Globulins (alpha, beta, gamma)	2.3-3.5 g/dl
Prothromibin time	14-16 sec
Ammonia in the plasma	3.2 - 4.5 g/dl
Serum ceruloplasmin	0.2-0.4 mg/L
Urinary copper / 24 hours	20 - 50 µg/24h
Serum free copper	< 15 µg /dL
Serum ferritin	30-300 ng/mL (30-300 mcg/L) male 30-200 ng/mL (30-200 mcg/L) female
Total serum iron	76-198 µg/dl male 26-170 µg/dl female
Transferrin	204-360 mg/dl