Challenges and Recent Advances in Diagnosing Wilson Disease

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Wilson disease (WD) is a rare autosomal recessive disorder caused by ATP7B gene mutations, leading to pathological copper accumulation that primarily affects the liver, brain, and eyes. Diagnosing WD remains a significant challenge due to its highly variable clinical presentation, which ranges from asymptomatic biochemical abnormalities to acute liver failure and severe neuropsychiatric manifestations. Traditional diagnostic markers, such as serum ceruloplasmin, urinary copper excretion, and liver biopsy, lack sufficient specificity and sensitivity, often leading to delays in diagnosis and misclassification. Additionally, the absence of a single gold-standard test and the overlap with other hepatic and neurological disorders further complicate early detection.

Recent advances in diagnostic techniques offer promising solutions to overcome these limitations. Novel biomarkers, including relative exchangeable copper (REC) and ATP7B protein quantification in dried blood spots have demonstrated improved accuracy in distinguishing WD from other conditions. Advanced imaging modalities, such as anterior segment optical coherence tomography (AS-OCT), quantitative susceptibility mapping (QSM), and copper-64 positron emission tomography imaging provide noninvasive tools for detecting early disease-related changes. Furthermore, next-generation sequencing (NGS) enhances genetic screening, facilitating earlier diagnosis, and family screening.

A comprehensive approach integrating conventional and emerging diagnostic methodologies is essential for improving early detection and patient outcomes. Greater awareness of the limitations of traditional tests and the incorporation of novel biomarkers and imaging techniques into clinical practice can help refine diagnostic accuracy, reduce delays, and optimize treatment strategies for WD. (J Clin Exp Hepatol 2025;15:102531)

Wilson disease (WD) is an autosomal recessive disorder in which copper accumulates in the body's vital organs, particularly the liver, brain, and eyes. In this disease, there is a defect in the biliary excretion of copper due to the ATP7B gene mutation on chromosome 13.[1,2] The estimated prevalence of the disease is 1:30000 to 1:50000 worldwide, and it may occur at any age.[3] The clinical presentations of WD are variable, including hepatic presentation, which ranges from an asymptomatic elevation of liver enzymes to acute or chronic hepatitis, compensated or decompensated liver cirrhosis, and acute liver cell failure. In addition, the neuropsychiatric disturbances are variable, including tremors, ataxia, dystonia dysarthria, abnormal behavior, personality changes, and depression.[3] The Kayser–Fleisher (KF) ring is the clinical hallmark eye sign of the disease that occurs in 95% of patients with neurological manifestations; however, it may be absent in children with hepatic insult. This sign occurs due to copper deposition in the corneal Descement's membrane.[3] The copper may also be deposited in the center of the lens, causing sunflower cataract.[4] Because of these ophthalmic involvements, the slit-lamp examination by an experienced doctor is used to detect these signs. Sometimes, marked pallor due to coombs–negative hemolytic anemia may be the first presentation of the disease, whereas renal affection, myopathy, cardiomyopathy, osteoarthritis, and infertility are less common manifestations. However, some patients may be asymptomatic, usually discovered during a family disease screening.[3] Once the disease is diagnosed, lifelong therapy is needed as the mortality is very high in untreated patients. The patient's survival is improved with early diagnosis and the introduction of chelation therapy.[3] However, diagnosing WD is complex and challenging as it enters the differential diagnosis list

Keywords: Wilson disease, diagnostic challenges, ceruloplasmin, copper, Kayser–Fleischer rings

Received: 20.10.2023; *Accepted:* 18.2.2025; *Available online 22 February 2025*

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Abbreviations: AIH: autoimmune hepatitis; ALF: acute liver failure; ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate transaminase; EM: electron microscopy; KF: Kayser–Fleisher; NAFLD: nonalcoholic fatty liver disease; NGS: next-generation sequencing; PCR: polymerase chain reaction; WD: Wilson disease

https://doi.org/10.1016/j.jceh.2025.102531

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of many diseases, such as acute hepatitis, acute liver cell failure, liver cirrhosis of unknown cause, Hemolysis, Elevated Liver enzyme levels, and Low Platelet (HELLP) syndrome, puberty behavior problems, and juvenile parkinsonism.[5–9] Consequently, a combination of clinical, laboratory, and genetic tests, alongside imaging and advanced new modalities, are needed not only for early disease diagnosis and treatment but also to avoid fatal complications and to prolong patient survival.

CLINICAL DIAGNOSTIC CHALLENGES OF WD

Diagnosing WD can be challenging due to several factors:

Varied Clinical Presentation

WD can manifest with a wide range of symptoms depending on the affected organs. Liver involvement can present as fatigue, jaundice, abdominal pain, or even acute liver failure. Neurological symptoms like tremors, clumsiness, speech difficulties, or psychiatric issues can be present. Kayser–Fleischer rings (copper deposits in the cornea) are a classic but not always present in ocular sign. This variability makes it difficult to suspect WD based solely on clinical features.[3]

Lack of Specific Biomarkers

No single laboratory test is definitive for WD. Commonly used tests like serum ceruloplasmin, serum copper, and 24-h urinary copper excretion all have limitations (discussed later).[4]

Overlap With Other Diseases

Several other medical conditions can mimic WD symptoms, including autoimmune hepatitis, chronic hepatitis, drug-induced liver injury, movement disorders like Parkinson's disease, and psychiatric illnesses.[6]

Age of Onset

WD can present at any age, making it difficult to distinguish from other age-related conditions.[1]

Incomplete Penetrance

Not everyone with mutations in the ATP7B gene (responsible for WD) develops the disease. This can lead to missed diagnoses in carriers with no symptoms.[2]

KAYSER–FLEISCHER RINGS IN WD

KF rings hold a unique position in diagnosing WD. While their presence can be a valuable clue, their limitations require a nuanced understanding. KF ring detection in WD is vital in early diagnosis and treatment initiation without delay.[3] Nearly 90% of patients with neurological WD, 40–50% with hepatic WD, and 20–30% of pre-symptomatic WD patients have KF ring.[10] A KF ring is formed by copper deposits within the Descemet membrane's interior corneal layer, giving it a golden, brown, or green color.[11] The superior region of the anterior chamber is first affected by copper deposits, which may be related to the direction of aqueous humor circulation there. The inferior chamber is affected after that.[12] In WD, early copper deposits in the anterior chamber angle within the Descemet membrane, standard slit-lamp cannot detect KF ring, as the angle view is obscured by the corneal limbus that requires an ophthalmological exam with an advanced modality like gonioscope.[13] Both slit lamp examination and gonioscopy require experienced operators.[14] KF rings were considered pathognomonic of WD until Fleming *et al.* noticed pigmented rings in patients with primary biliary cirrhosis.[15] Jones noted another similar corneal ring in patients with intrahepatic cholestasis.[16] These patients had no other evidence of WD, and the correlation between elevated bilirubin levels and pigmented rings was established.[17] So, early detection of copper deposits in the cornea and differentiation of non-Wilsonian eye rings are the most prominent obstacles in WD diagnosis. These findings suggest the need for other techniques that might be used to detect early copper deposits in the cornea.[18] An illustration of the KF ring is shown in Figure 1.

LABORATORY DIAGNOSIS OF WD AND THEIR DIAGNOSTIC CHALLENGES

While clinical history offers valuable clues, definitively diagnosing WD relies heavily on laboratory investigations. Establishing the diagnosis always depends on laboratory tests like liver enzymes, serum ceruloplasmin, urine copper excretion, and genetic testing (if feasible), but none of those laboratory markers are 100% specific for WD.[19] Therefore, to reach a confirmatory diagnosis for WD, there is a need for a combination of clinical presentation, biochemical parameters, and genetic analysis, which has set the basis for the development of the Leipzig score, which was developed at the 8th International Meeting on WD in Leipzig, hence the name. According to the Leipzig score, a patient with a score of ≥4 points is confirmatory of WD.[3] The scoring system for diagnosing WD has been updated to reflect a more comprehensive and patient-centric approach. Previously, the system relied heavily on tests that could be misleading or invasive. This is the breakdown of the changes:

- **Serum Ceruloplasmin:** This test was removed entirely from the scoring system. While low ceruloplasmin levels were previously indicative of WD, other conditions like inflammation or liver damage can also cause low levels, making it an unreliable marker.[20]

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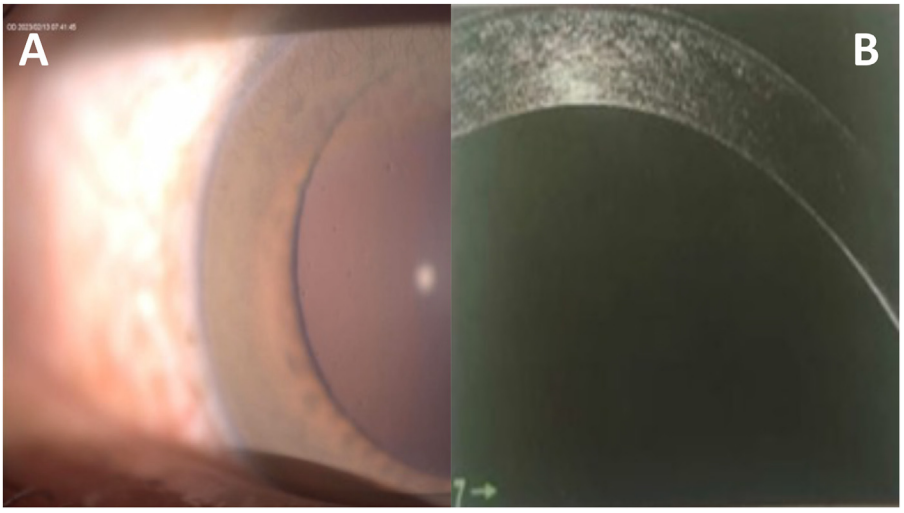

Figure 1 Corneal copper deposits in a patient with Wilson disease. (A) Kayser–Fleischer ring seen in a slit lamp. (B) Copper deposits on Descemet membrane in the same patient seen in AS-OCT (clearly distinguishable hyper-reflective copper deposits at the level of Schwalbe's line. AS-OCT, anterior segment optical coherence tomography.

- d-**penicillamine Challenge for Urinary Copper:** This test involved measuring copper excretion in urine after a dose of a medication called penicillamine. However, its accuracy could be affected by how well the patient followed instructions and the timing of sample collection. The test has been removed from the new scoring system.[20,21]
- **Liver Copper Content:** Previously, points were assigned based on elevated copper levels found during a liver biopsy. This invasive procedure is now optional because alternative, noninvasive methods for assessing liver copper are being developed.[21]
- **Family History:** The new scoring system now includes a point for a positive family history of WD. Recognizing that the disease is genetic, a family history adds valuable information to the diagnosis.[20,21]

Overall, these changes aim to improve the accuracy and flexibility of diagnosing WD. The new system acknowledges the limitations of individual tests and emphasizes a more comprehensive evaluation that considers a combination of clinical symptoms, various lab tests, genetic analysis (when necessary), and now, family history. This shift ensures a more nuanced approach that prioritizes patient comfort and minimizes reliance on invasive procedures.[22]

The biochemical tests commonly diagnose WD and assess adherence and therapy compliance (Tables 1–3) details the criteria for WD diagnosis included in the Leipzig score. This section delves into the essential laboratory workhorses used for WD diagnosis, acknowledging their strengths and challenges.

Liver Functions Tests

Almost 50% of WD patients have hepatic dysfunction as a typical feature; hence, careful monitoring of the liver enzyme levels is indispensable for all WD patients. When WD is suspected, a comprehensive panel of diagnostic tests should be conducted, considering a wide range of clinical scenarios.[5] Hepatocellular injury usually presents with an elevation in liver enzymes [alanine transaminase (ALT) and aspartate transaminase (AST)] and bilirubin levels. Disturbance of the coagulation profile with decreased blood albumin level is developed with end-stage liver disease.[23] Some reports demonstrated that the increased AST-to-ALT ratio with low serum alkaline phosphatase (ALP) is commonly associated with fulminant WD.[24] Moreover, the prediction of acute liver failure (ALF) in addition to WD is 100% diagnostically sensitive and specific when the ALP: total bilirubin ratio is below four and the AST: ALT ratio is above 2.2.[25]

Challenges

These tests are not specific to WD and can be elevated in various liver diseases.[5]

Serum Ceruloplasmin

Ceruloplasmin is an acute phase reactant, and any inflammatory illness may cause its level to rise to normal levels, giving false-negative test results. Ceruloplasmin levels in the blood can indicate WD when they are below 0.2 g/L (the normal range is 0.2–0.5 g/L) or 50% of the lower limit of the normal range.[5]

Challenges

Similar circumstances can arise from estrogen treatment or hyperestrogenism during pregnancy. Low levels of ceruloplasmin can also be found in other liver diseases, such as autoimmune hepatitis, severe liver failure from an unrelated cause, and familial aceruloplasminemia,

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Table 1 Criteria for WD Diagnosis Included in the Leipzig Score.[3]

Kayser–Fleischer rings		**Liver copper (in the absence of cholestasis)**	
Present	2	>5 × ULN (>4 μmol/g)	2
Absent	**0**	0.8–4 μmol/g	1
		Normal (<0.8 μmol/g)	−1
		Rhodanine-positive granules *	1
Neurologic symptoms or typical abnormalities of brain MRI		**24 h urinary copper (in the absence of acute hepatitis)**	
Severe	2	Normal	0
Mild	1	1–2 × ULN	1
Absent	0	>2 × ULN	2
		Normal but >5 × ULN after D-penicillamine	**2**
Serum ceruloplasmin (g/L)		**Mutation analysis**	
Normal (>0.2)	0	Mutations detected on both chromosomes	4
0.1–0.2	1	Mutations detected on one chromosome	1
<0.1	2	Mutations absent	**0**
Coombs-negative hemolytic anemia			
Present	1		
Absent	0		
TOTAL SCORE			
Diagnosis established	4 or more		
Diagnosis possible, more tests needed	3		
Diagnosis very unlikely			

WD: Wilson disease.

Table 2 Modified Leipzig Scoring System for Diagnosis of Wilson's Disease.[17]

Features	Score
Kayser–Fleischer corneal rings	Present = 2; Absent = 0
Serum ceruloplasmin	Normal (>20 mg/dl) (0–5 mg/dl 3, 6–11 mg/dl 2, 11–20 mg/dl)
24-h urinary copper (in absence of acute hepatitis)	>100 mcg/day = 2; 40–100 mcg/day = 1; <40 mcg/day = 0
Coomb's negative hemolytic anemia with liver disorder	Present = 1; Absent = 0
Genetic mutation	Detected on both chromosome = 4, one chromosome = 1
Liver biopsy	Not detected/test not done = 0
Neurobehavioral symptoms	Orcein- or rhodamine-positive granules = 1
MRI brain	Present = 2; Absent = 0
Family history of WD	Typical features suggestive of WD present = 1; absent = 0 Sibling death from liver or neurological features
Total score	4 or more = Definitive diagnosis of WD 3 = Possible WD and further evaluation needed 2 or less = WD unlikely

MRI: magnetic resonance imaging; WD: Wilson disease.

as well as in illnesses that cause protein loss in the kidneys or the intestines and malabsorption disorders like celiac disease.[3,8,26] Patients with neurologic WD often have lower serum ceruloplasmin levels, although around half of those with active liver WD may have levels within the low normal range. Serum ceruloplasmin levels are reduced in about 20% of heterozygous carriers of ATP7B mutations who do not show copper accumulation in their body.[27] A prospective study on serum ceruloplasmin, as a screening test for WD in patients with liver disease, showed that subnormal ceruloplasmin had a positive predictive value of only 6%. In children with WD, 15–36% had ceruloplasmin in the normal range.[28,29]

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Table 3 Changes to the Leipzig Score for Wilson's Disease Diagnosis.

Feature	Original Scoring	Modified Scoring	Reason for Change
Serum ceruloplasmin	Points assigned for levels <20 mg/dl	Removed entirely	Unreliable due to inflammation, liver damage, or analytical variability
D-penicillamine challenge	Increased urinary copper excretion after challenge supports diagnosis	Removed	Accuracy affected by patient compliance and timing
Liver copper content	Points assigned for elevated levels through liver biopsy	Not required	Invasive procedure with sampling errors; new noninvasive methods emerging
Family history	Not included	1 point for positive family history	Recognizes genetic nature of Wilson's disease

Serum Copper

The two types of copper that comprise total serum copper are free copper and ceruloplasmin-bound copper. Since it does not reflect tissue concentrations, the total serum level is a subpar diagnostic tool. The assessment of the free copper [i.e. non-ceruloplasmin-bound copper (NCC)], which is toxic, and tissue deposits would be more appropriate for the WD diagnosis because 90% of the copper in the blood is bound to ceruloplasmin. However, NCC measures are prone to precision errors in serum copper and non-ceruloplasmin-bound copper testing.[30,31]

Challenges

Serum copper is not helpful as a test for diagnosing WD. Despite being a disease of copper overload, WD typically has decreased levels of total serum copper (which includes copper incorporated in ceruloplasmin) in proportion to the decreased levels of ceruloplasmin in the blood. Whether serum ceruloplasmin levels are high or low, blood copper may be within the normal range in patients with severe liver injury. Due to the abrupt release of the metal from liver tissue reserves, serum copper levels may even be noticeably raised in cases of acute liver failure caused by WD.[3] When ceruloplasmin levels drop, normal or elevated serum copper levels signal an increase in the amount of copper in the blood that is not linked to ceruloplasmin (NCC). As a WD diagnostic test, the serum NCC concentration has been proposed.[32] In cases of chronic cholestasis, acute liver failure of any origin, and copper poisoning, the serum NCC content may be high.[33] Also, precision errors: Analytical variability during testing can lead to falsely elevated or depressed ceruloplasmin values. This can be due to instrument calibration, reagent batches, or sample handling. Similar to ceruloplasmin, analytical variability can lead to inaccurate serum copper measurements as it is affected by precision errors. The main issue with using NCC as a WD diagnostic test depends on accurate ceruloplasmin and serum copper measures. It is more helpful in monitoring medication than for WD diagnosis.[34]

Urinary Copper

The 24-h urinary copper excretion test is another screening tool for diagnosing WD and is also essential for planning the proper treatment.[35] The existence of WD is confirmed by high values, which often surpass 100 mcg/24 h in adults and 40 mcg/24 h in children.[5] Urinary copper excretion typically rises to more than 100 mcg/24 h in patients with neuropsychiatric aspects of the disease (normal range: 0–50 mcg/24 h). Elevated urine copper levels in asymptomatic heterozygotes are occasionally seen but typically do not reach above 40 mcg/24 h (Table 3).[36]

Challenges

The precise urine volume and total creatinine excretion per 24 h are crucial for accurately assessing urinary copper excretion. The test is inappropriate if there is a renal failure.[5] Incomplete urine collections and, on the other hand, copper contamination of the collection equipment are issues that affect measurements of 24-h copper excretion (this being less problematic with the advent of disposable containers). It cannot be easy to interpret 24-h urine copper excretion because the results overlap with those from other liver diseases (e.g. autoimmune hepatitis, chronic active liver disease, cholestasis, and, particularly, during acute hepatic failure of any origin). Additionally, heterozygotes may excrete more copper than their healthy counterparts; however, they rarely exceed normal range levels.[37] The penicillamine challenge test in children was reevaluated, and it was discovered to be inaccurate, with too-low sensitivity to rule out WD in asymptomatic children.[36] The challenge test is not advised for adults with WD either because of the ambiguity of the findings from clinical trials.[5]

Coombs-negative Hemolytic Anemia

Patients with WD who have Coombs-negative hemolytic anemia appear to have higher oxidative stress from copper accumulation in red blood cells, seen in up to 15% of WD patients.[38]

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Challenge

Coombs-negative hemolytic anemia in WD poses a challenge to diagnosis for several reasons. First, it is a nonspecific finding**:** Coombs-negative hemolytic anemia can occur in various conditions unrelated to WD, such as G6PD deficiency or infections. This lack of specificity makes it difficult to rely solely on this finding for diagnosing WD.[39] Second, healthcare professionals may not be aware of the association between Coombs-negative hemolytic anemia and WD, leading to missed diagnoses or delays in identifying the underlying cause.[40]

Genetic Analysis for ATP7B Gene

The ATP7B protein is mainly expressed in the liver and brain. Significant levels of ATP7B protein expression have also been found in the kidneys, placenta, and lungs. The genetic testing modalities of WD include the detection of common point mutations by polymerase chain reaction (PCR), next-generation sequencing (NGS), whole genome sequencing, and multiplex ligation-dependent probe amplification.[35] In order to establish the diagnosis and begin screening a patient's first- and second-degree relatives, the guidelines recommend testing for the ATP7B gene mutation in each suspected patient with WD.[5] The patient's clinical and biochemical characteristics (low ceruloplasmin, increased 24-h urinary copper excretion, and hepatic copper concentration) must also be present for the ATP7B gene variations to be considered confirmatory of the disease.

Challenges

There are no clear genotype-phenotype correlations.[41] There is a continuous increase in the number of mutations affecting the ATP7B gene, with more than 900 variants reported.[35] H1069Q missense mutation is the most common variant in European patients.[35,42,43] The current clinical criteria and genetic testing have significant limitations for diagnosing WD, often creating ambiguities in patient identification and leading to delayed diagnosis and poor management.[35] One of the significant obstacles in understanding WD and genetic testing is the need for widespread access to and interpretations of genetic testing as many of these techniques may not be available and need an expert to interpret the results.[35] Molecular and genetic diagnostics in WD are expensive and complex because of the numerous rare mutations and because most patients are heterozygotes.[32,33] The guidelines recommend testing for the ATP7B gene mutation whenever WD is suspected to confirm the diagnosis and to start screening of first- and second-degree relatives of WD patients.[34] However, the lack of an evident genotype-phenotype association in the WD course forces us to develop advanced molecular techniques and search for further investigation of alternative mechanisms, such as epigenetic regulation of gene expression and modifier genes, to explain the pleiotropic effects of WD.[37] Recently, advanced techniques such as whole genome sequencing, demonstrated that the genetic prevalence of WD is much higher than the clinical prevalence. This may be due to incomplete penetrance or unknown modifier genes.[35,44] The apparent discrepancy could also be attributed to missed diagnoses because of a lack of clinical diagnostic gold standards.[35] Although the genetic basis of WD has been fully understood, these genetics cannot predict the phenotypic variability of WD. Recent studies demonstrated a lack of correlation between the genotype and phenotype of WD and no evidence that WD phenotype could be predicted based on genotype.[45] This discrepancy comes from the interaction between epigenetics and metabolic factors, incomplete penetrance, and missed diagnosis.[35,41] Whenever a WD diagnosis is confirmed, a screening test for first-degree relatives is recommended because the likelihood of homozygous mutation in children affected by the disease is 0.5%, and in siblings, it is 25%. Genetic counseling is recommended for parents of affected children before attempting subsequent pregnancies.[35]

IMAGING MODALITIES FOR DIAGNOSIS OF WD

It is estimated that about 18–68% of WD patients presented neurological symptoms without evident liver disease.[46] Imaging plays a vital role in diagnosing WD and monitoring patients during therapy; magnetic resonance imaging (MRI) is more sensitive to revealing WD abnormalities than other neuroimaging methods.[47] MRI brain has recently been included in the modified Leipzig scoring system (Table 2).[30] These MRI abnormalities are brought about by neuronal loss, gliosis, fiber degeneration, and vacuolization linked to high water content in the brain in WD patients. The severity of signal abnormalities varies with the disease stage and can be treated in the early stages to reverse them.[31] Asymmetric or symmetrical hyperintensities in T2-weighted imaging of the basal ganglia, thalamus, midbrain, and pontine regions are the most typical findings in brain MRIs of WD patients.[8,36] Furthermore, images of the brain stem's deep grey matter (DGM) nuclei and white matter obtained using fluid-attenuation inversion recovery (FLAIR)[38] showed that cortical regions, corticospinal tracts, cerebellum, and white matter could also be affected.[8]

The "face of the giant panda" in the midbrain is the most recognizable MRI sign of WD, and it occurs in 14–20% of WD patients with neurological manifestations. T2-weighted images occasionally display hypointensity in the basal ganglia region due to iron deposition in exchange for copper after chelation.[12,13] In patients with severe liver failure or portosystemic shunting, hyperintensities in T1-weighted imaging can be seen in the substantia nigra

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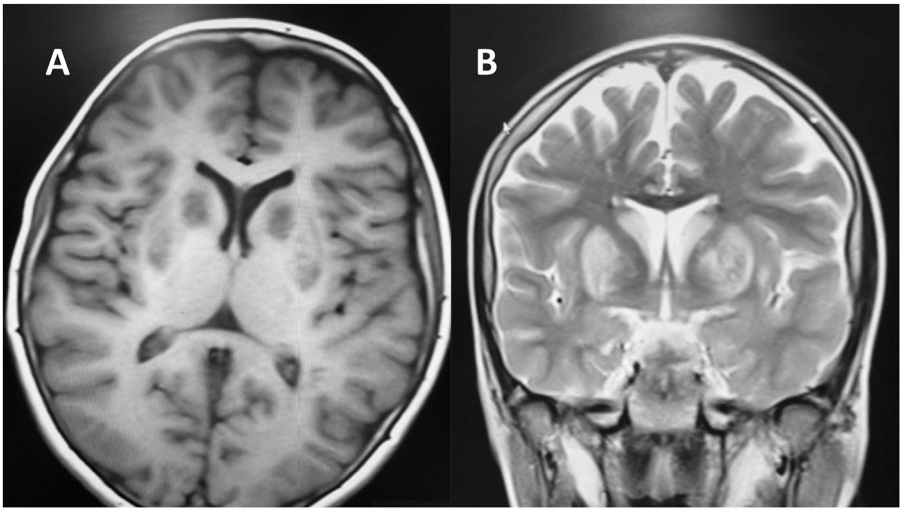

Figure 2 Wilson disease in a 13-year old boy with bilateral abnormal signal intensity seen involving basal ganglia. (A) T1 weighted axial MRI shows hypo signal intensity in basal ganglia. (B) T2W coronal MRI shows higher intensity l on both putamen and globus pallidus.

and globus pallidum, which may be caused by the buildup of manganese.[14] Dusek and his colleagues created a novel brain MRI rating scale for WD that may semi-quantitatively evaluate the severity of neurological abnormalities in MRI. This scale, which offers a system for categorizing radiological severity, contains the acute toxicity score (calculated by T2 or T2-FLAIR hyperintensities) and the chronic damage score (calculated by T2 hypointensity and brain atrophy). Both at baseline and 24 months into treatment, the MRI rating scale scores were highly linked with the severity of the condition.[15] However, subsequent investigations still need to demonstrate the value of the MRI rating scale and its use in determining disease severity.[15] The mobility of water molecules, namely the proton in H2O in cells, is assessed using diffusion-weighted imaging (DWI). In WD, copper toxicity results in cell swelling and inflammation, which restricts diffusion.[16] Figure 2 shows an image of the MRI brain in WD.

Challenges

Conventional brain MRI cannot find abnormalities in 10% of WD patients with neurological disease.[48] Conventional MRI sequences make it difficult to detect early brain lesions in WD, especially in preclinical patients.[49] Moreover, many neurological disorders, including Leigh disease, hypoxic-ischemic encephalopathy, methyl alcohol poisoning, Japanese B encephalitis, and selective extra pontine myelinolysis caused by osmotic disequilibrium syndrome, all could have MRI abnormalities that are comparable to those observed in WD. Correlating imaging with clinical characteristics and WD biochemical markers is crucial.[50] Although excessive copper deposits in the brain are the hallmark of WD, iron deposits may also be present. This copper and iron deposition alters brain tissue's magnetic sensitivity.[51] Although T2*-weighted images and susceptibility-weighted imaging (SWI) have been utilized to examine the distribution of metal deposition in WD patients because they are more effective than traditional MRI sequences, both T2*-weighted images and SWI technique could not quantify metal deposits in the brain 50,52.

LIVER BIOPSY

Liver biopsy in WD is indicated only when the clinical presentation and/or the results of noninvasive tests cannot confirm the final diagnosis of the disease and/or when other concurrent liver diseases are suspected.[53] Both the liver pathology and the clinical signs and symptoms are frequently nonspecific, and they can resemble other common liver diseases such as autoimmune hepatitis (AIH), alcoholic hepatitis, and nonalcoholic fatty liver disease

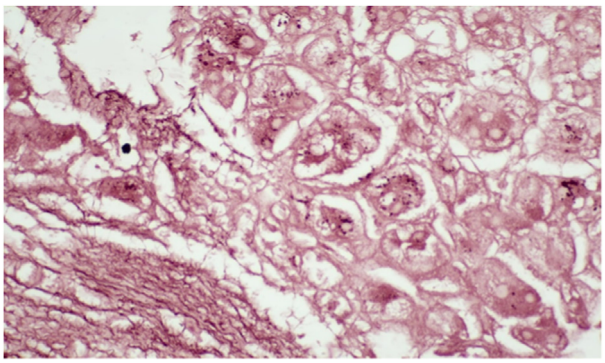

Figure 3 Histopathological evaluation for 10 years old male with Wilson disease. Orcein stain highlighting copper-associated protein by the cytoplasmic coarsely granular dark brown -colored granules.

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(NAFLD).[54] In the early stages of WD, steatosis might be the only histopathological feature, although this is often seen alongside portal inflammation and fibrosis. The diagnosis of WD may be supported by examination of quantitative copper content in the liver parenchyma.[55] Another less accurate histopathological diagnostic method is to stain the liver tissue with the orcein stain to detect the copper-binding protein. This protein appears coarsely granular and black-brown (orcein) or purple (Victoria blue). However, orcein-positive granules can be seen in chronic cholestatic disorders and are not specific to WD.[56] Rubeanic acid or rhodanine can also be used to demonstrate the elemental copper. Rubeanic acid gives a greenish-black color to copper, while rhodanine stain gives a red to orange-red cytoplasmic granular positivity to elemental copper.[56] An example of the histopathological picture seen in WD is shown in Figure 3.

Challenges

Even though the high copper concentration in dry liver tissue was considered the best diagnostic method for WD, the possibility of sampling error should be considered. The most important source of sampling error is the uneven distribution of copper within the liver parenchyma in advanced disease. Therefore, normal intrahepatic copper levels do not exclude the diagnosis of WD.[53] Additionally, hepatic copper overload may also be seen in chronic cholestatic diseases such as PBC (Primary Biliary Cholangitis), PSC (Primary sclerosing cholangitis), cirrhosis, and primary liver tumors (most commonly fibrolamellar hepatocellular carcinoma).[57]

Moreover, histological features can be similar to those of chronic hepatitis of any other etiology, including viral or autoimmune hepatitis. These histopathological characteristics include portal and periportal inflammation, comprised of lymphocytes and plasma cells destroying the limiting plate, as well as parenchymal necrosis and bridging fibrosis.[58] On the other hand, In cases where fulminant hepatitis or ALF is present, the role of liver biopsy in diagnosing WD is not helpful as hepatic damage with massive or submassive necrosis, hepatic copper is shifted to Kupffer cells and portal macrophages.[55] Table 4 illustrates false negative and positive WD Investigations.

Treatment of WD

Once WD diagnosis is established, treatments are initiated by continuously administering either D-penicillamine or trientine hydrochloride. These drugs help eliminate copper from tissues. Alternatively, zinc acetate can be prescribed to hinder the absorption of copper in the intestines and facilitate its excretion.[3] After eliminating the excess copper from the body, a maintenance phase follows, where the disease is managed by gradually reducing the dose of chelating agents to prevent copper deficiency. Additionally, supplementation with vitamin B6 and adherence to a diet low in copper is advised.[59] The treatment efficacy is assessed through clinical and biochemical progress and by

Table 4 False Negatives and Positives of WD Investigations.

Investigation	False Negative	False Positive	Reason
Liver function tests	- Can be normal in early WD or with predominant neurological presentation	- Can be elevated in various other liver diseases	Not specific for WD
Serum ceruloplasmin	- Inflammatory conditions can elevate levels	- Other liver diseases and hormonal changes can cause low levels	Not specific for WD, affected by other factors
Serum copper	- Can be normal in severe liver damage	- Acute liver failure due to WD can cause high levels	Doesn't reflect tissue copper concentration
24-Hour urinary copper excretion	- Incomplete collections, inaccurate volume measurement	- Severe liver failure from any cause	Requires strict collection protocol, can be elevated in other conditions
Genetic testing (ATP7B gene)	- Mutations might not be detected by current methods	- Rare: Variants of uncertain significance	Not a standalone diagnostic tool, incomplete understanding of genotype-phenotype correlation
Kayser–Fleischer rings	- Early deposits in Descemet's membrane undetectable by slit lamp	- Similar rings observed in other conditions (primary biliary cirrhosis, intrahepatic cholestasis)	Requires specialized examination (gonioscopy) for early detection, not exclusive to WD
Coombs-negative hemolytic anemia	- Not a specific finding for WD	- Can occur in various other conditions (G6PD deficiency, infections)	Low awareness among healthcare professionals of association with WD

WD: Wilson disease.

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evaluating the 24-h urinary copper excretion during treatment. The highest levels of urinary copper excretion are typically observed right after initiating the treatment.[59]

TREATMENT CHALLENGES OF WD

Medication Side Effects: Chelating agents, e.g. D-penicillamine and trientine hydrochloride, the mainstays of treatment, can cause a variety of side effects, including skin rash, bone marrow suppression, and kidney problems. These side effects can necessitate dose adjustments or even discontinuation of the medication in some cases, potentially hindering long-term copper removal.[60]

Treatment Adherence

The long-term nature of WD treatment, often lifelong, requires significant patient commitment. Strict adherence to medication regimens, dietary restrictions, and regular follow-up appointments is crucial but can be challenging for some patients.[60]

Monitoring and Dosage Adjustments

Close monitoring of clinical symptoms, laboratory tests (including liver function and 24-h urinary copper excretion), and potential side effects is essential. Regular dose adjustments of chelating agents may be required based on individual response and copper levels, adding complexity to the management process.[61]

Liver Transplantation

In severe cases with advanced liver damage, liver transplantation may be the only option. However, lifelong immunosuppression post-transplantation carries its own set of challenges.[61]

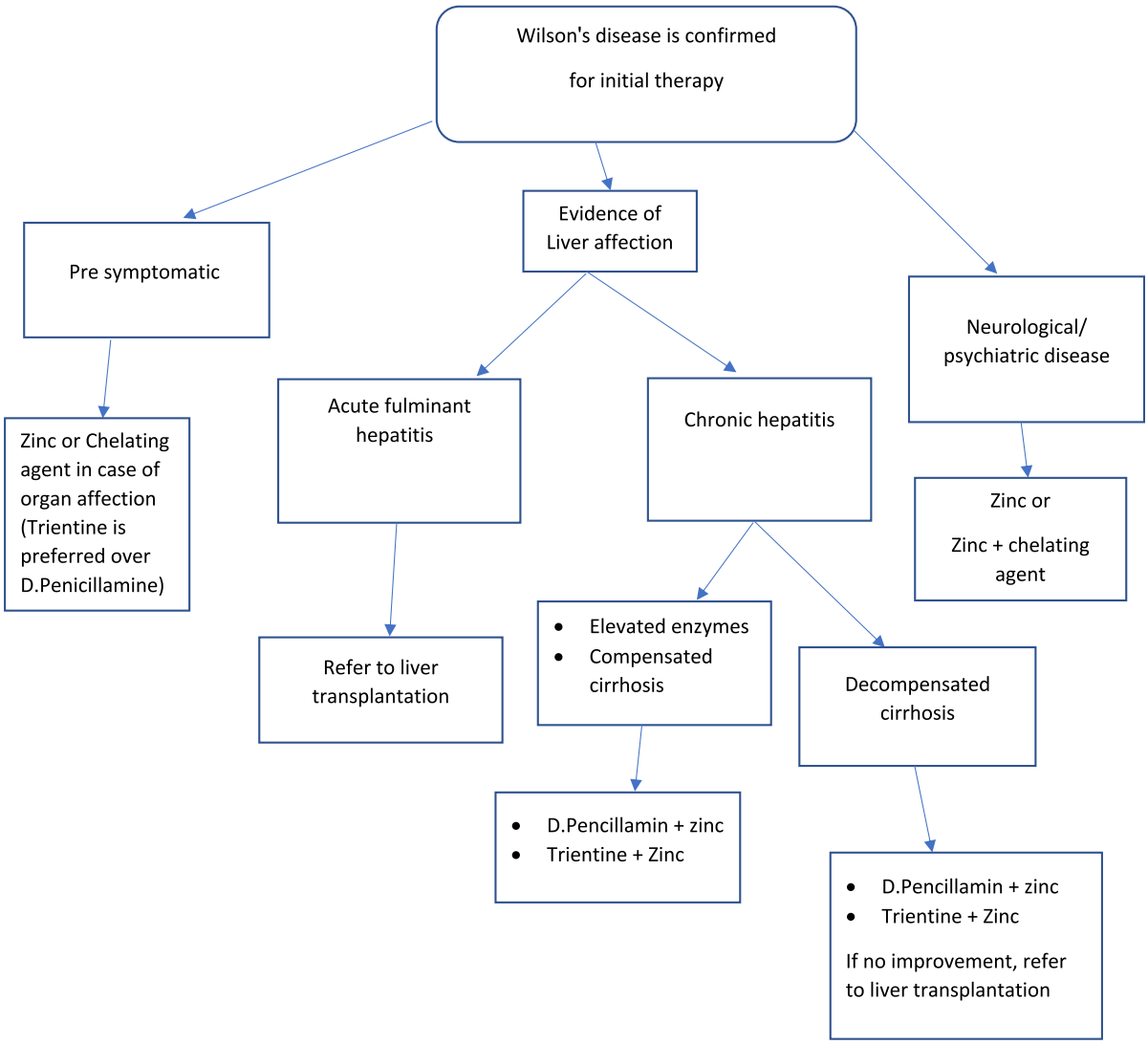

Figure 4 Simplified algorithm for treating Wilson disease.

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Copper Deficiency

During the maintenance phase, copper deficiency can occur when chelating agents are used at lower doses. This necessitates monitoring and potential copper supplementation to maintain a balance.[61]

Limited Treatment Options

While D-penicillamine and trientine are the primary treatment options, some patients may not tolerate either medication due to side effects. In such cases, alternative therapies like tetrathiomolybdate are still under investigation and may not be widely available.[62] Figure 4 illustrates a simplifies algorithm for managing WD.

RECENT ADVANCES TO OVERCOME THE DIAGNOSTIC DILEMMA OF WD

Relative Exchangeable Copper, the Ratio of CuEXC to Total Serum Copper

Relative exchangeable copper (REC) is the exchangeable (CuEXC) ratio to total serum copper. REC represents the toxic fraction of copper in the blood.[56] Recent reports stated that REC is a specific, sensitive, noninvasive tool for WD diagnosis.[63–65] El Balkhi S *et al.* studied sixteen new WD patients diagnosed in their institution between January 2009 and May 2011. They reported that the biological tests used for WD diagnosis demonstrated lower sensitivity and specificity than REC. In that study, REC was an excellent tool for diagnosing WD with 100% sensitivity and 100% specificity.[66] Moreover, REC is a promising tool for family screening in WD, especially for heterozygous ATP7B carriers who could present with slight biological abnormalities, particularly for patients with low levels of ceruloplasmin or copper anomalies.[64,65]

REC is an essential tool in evaluating toxic blood copper. It can evaluate non-ceruloplasmin-bound copper to confirm WD diagnosis in family screening. REC can significantly discriminate people without WD (Htz and NoM) [acronyms need explanation] from WD patients with a cutoff of 15%. REC may help avoid liver biopsy in a few specific cases when biological abnormalities with no or one mutation are observed.[64] REC demonstrated a good discriminative performance in non-Wilsonian liver disease patients (REC >18.5%, sensitivity, and specificity of 100%).[64,65] Although accurate and reliable, the REC technique has some limitations, including complicated techniques, unavailability in many laboratories, and requiring specific and costly equipment.[65]

Quantification of ATP7B Protein in Dried Blood Spots by Peptide Immuno-SRM

Newborn screening for WD and early interventions can improve results by preventing irreversible neurological disability or liver cirrhosis.[67] Measuring ceruloplasmin (CP) alone in infants or children is reported to be insufficient to meet the universal screening for WD. WD develops due to mutations that cause absence or markedly diminished levels of ATP7B. Therefore, quantifying the ATP7B protein concentration in dried blood spots (DBSs) may serve as an adjunctive test and marker for WD screening and diagnosis.[67,68] Collins CJ *et al.* reported that the ATP7B protein concentration in dried blood spot samples might provide primary evidence of WD, using immunoaffinity enrichment mass spectrometry for measurements. Results showed that the test effectively identified WD patients in 92.1% of presented cases and reduced ambiguities resulting from ceruloplasmin and genetic analysis.[68] Collins *et al.*'s study concluded that quantifying ATP7B protein in DBSs by peptide immuno-SRM (Selected Reaction Monitoring) had clarified patients with ambiguous genetic results, significantly aiding in noninvasive diagnosis. They recommended establishing a diagnostic score and algorithm containing ATP7B peptide concentrations that can be rapidly diagnostic and supplemental to the current Leipzig scoring systems.[68]

Another study by Jung S *et al.* reported a method to quantify ATP7B protein in DBS by peptide immuno-SRM as a potential screen for WD[67] and demonstrated that the immuno SRM platform is highly sensitive and can quantify ATP7B in DBS in the picomolar range. In addition, the study showed that the assay could significantly differentiate between affected cases from normal controls with an assay precision of <10% CV (Coefficient of Variation), and the protein stability in DBS at room temperature was one week. This data provided a promising proof-of-concept for screening WD in newborns using proteins as biomarkers in DBS.[67]

Sequencing and Mutation Detection Techniques

The genetic testing of WD includes the detection of possible mutations or sequencing of the entire ATP7B gene. Sanger sequencing can detect point mutations, minor deletions, and insertions. Multiplex-ligation probe amplification (MLPA) and promoter sequencing must be used to look for any potential significant deletions or insertions if no diagnosis was made. Next-generation sequencing (NGS) is a much better alternative to MLPA and Sanger sequencing as it enables the discovery of substantial copy number variants (CNVs).[69] Moreover, it allows for a time- and money-saving technology for comprehensive sequencing of the entire ATP7B gene.[70,71] Diagnostic challenges in Wilson disease diagnosis and the recent advances in modalities that might overcome these challenges are shown in Figure 5.

Recent Advances in Imaging Modalities for the Diagnosis of WD

Anterior Segment Optical Coherence Tomography and Quantification of KF Ring

Anterior segment optical coherence tomography (AS-OCT) is a noninvasive investigative technique that does

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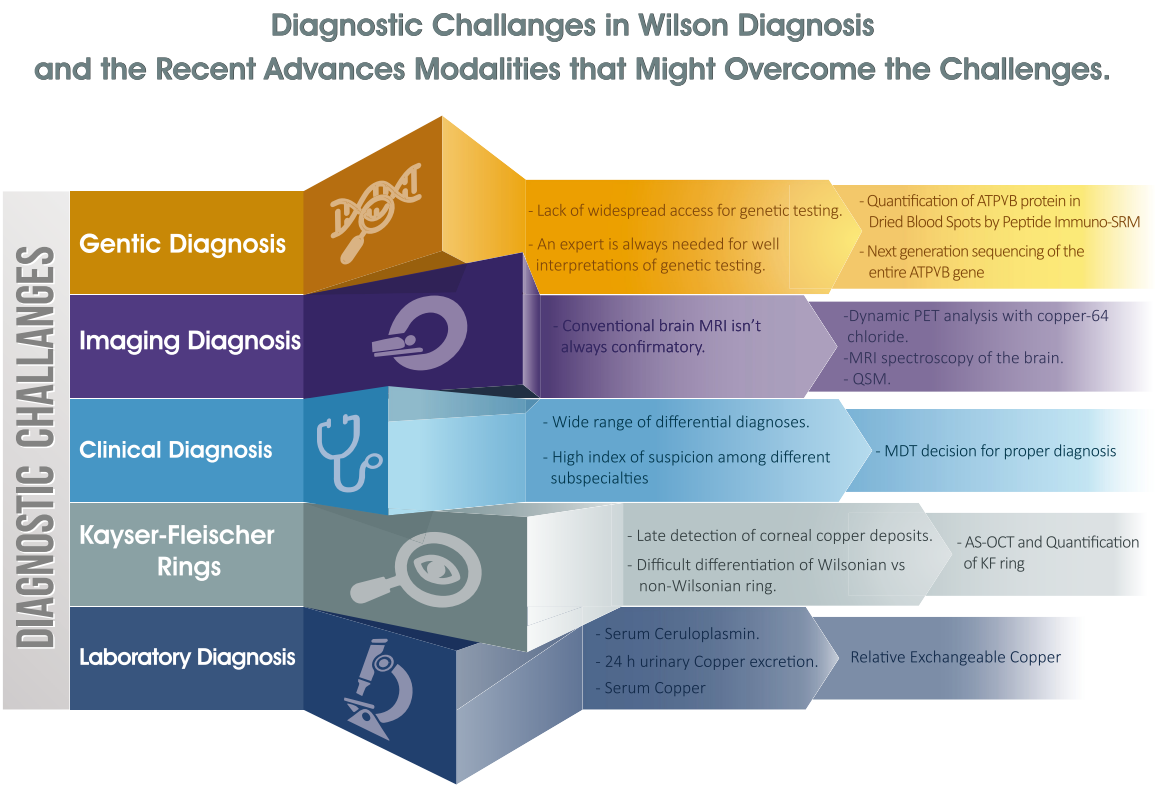

Figure 5 Diagnostic challenges in Wilson disease diagnosis and the recent advances in modalities that might overcome these challenges.

not require eye anesthesia and scans each eye in less than 20 s.[72] It can determine the density of copper deposits in the KF ring and assist the clinician in determining the severity of the disease. Moreover, it also enables imaging of lesions that a less experienced ophthalmologist cannot see with a slit lamp.[14,73]

In AS-OCT, the KF ring is a hyper-reflective layer at the level of Descemet's membrane in the cornea's periphery; on a color scale, the rings are visible as a yellow-orange band. In patients with hepatic and neurological WD symptoms, reports hypothesized that AS-OCT is a more accurate diagnostic method that might identify significantly more cases of KF ring than the slit-lamp examination.[14] Using the AS-OCT technique helps in the early detection of KF ring copper deposits and allows the determination of disease severity. Moreover, nonexperimental ophthalmologists and other practitioners can easily use it[12,74] and apply it to noncooperative children.[75] Further research is required to determine whether AS-OCT can differentiate KF ring from pigmented corneal rings in non-Wilsonian liver disease or arcus senilis, as well as whether repeated AS-OCT can be used to evaluate the effectiveness of chelator therapies in WD.[12]

Dynamic Positron Emission Tomography Analysis With Copper-64 Chloride

Previously, radioactive copper isotopes were used to investigate copper metabolism in both normal and WD patients; however, these clinical studies have been discontinued due to the limited spatial resolution of primitive nuclear scintillation cameras at that time.[76] Positron emission tomography (PET) is a promising tool with high sensitivity and much better spatial resolution for in vivo assessment of hepatic copper handling, using ^{64}Cu as a tracer.[77,78] Dynamic PET analysis in ATP7B knockout mice using copper-64 chloride as a tracer detected increased accumulation and reduced clearance of copper from the liver.[79] Thomas and colleagues recently discriminated WD patients from heterozygote and healthy subjects using ^{a} ^{64}Cu PET/CT study. They show that marked retention of ^{64}Cu was reported in the WD patient group as a promising single gold standard test to diagnose both symptomatic and presymptomatic WD patients depending on retention of ^{64}Cu in the liver as a pathognomonic hallmark in WD diagnosis; however, further studies, including a large number of patients are required.[80]

MRI Spectroscopy of the Brain for Detection and Quantification of Early Neurological Change of WD

A noninvasive diagnostic procedure called magnetic resonance (MR) spectroscopy is used in conjunction with conventional MRI to measure biochemical changes in brain tissue that occur before pathological structural changes, such as changes in energy metabolism, neuronal integrity, and cell proliferation.[81,82]

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The ability to describe the metabolite composition of brain tissue makes MR spectroscopy useful in certain neurometabolic disorders.[83] The frequency of these metabolites is measured in units called parts per million (ppm) and plotted on a graph as peaks of varying height. In the case of single-voxel spectroscopy, spectra are obtained from a single selected brain region, but in the case of MR spectroscopic imaging, spectra are collected from several selected brain regions.[84] MR spectroscopy can help identify and characterize mitochondrial diseases.[85] Lactate in MR spectra from suspicious areas in the basal ganglia, thalamus, and brainstem strengthens and improves diagnosis susceptibility of Leigh syndrome, which is one of the mitochondrial disorders. In WD, reduced N-acetyl aspartate (NAA) creatine and choline: creatine ratios are hallmark diagnostic changes in MR spectroscopy. However, this decrease may be partially reversible with chelation therapy.[86,87] A prospective study that included 26 children with WD and 26 healthy controls revealed that magnetic resonance spectroscopy (MRS) could confirm early neurological changes even with a normal MRI since it detects metabolite abnormalities before cerebral structural changes are seen on MRI.[49] There are few studies on MRS changes in WD with contradictory findings, and the clinical significance is yet to be fully defined.[81,88,89]

Quantitative Susceptibility Mapping

The key to improving the prognosis and lowering the handicap of WD is early diagnosis and therapy. Early brain lesions in WD are challenging to identify with standard MRI sequences, particularly in preclinical patients.[49] A recently created magnetic resonance postprocessing method called quantitative susceptibility mapping (QSM), which is extremely sensitive to magnetic metal deposition in the brain, is preferably applied.[90] In WD, basal ganglia metal deposition can be quantitatively analyzed using QSM, which allows for early diagnosis and condition evaluation of WD.[91] Doganay *et al.* have shown that even when no signal changes are detected in T1-weighted and T2-weighted MRI images, the QSM technique shows increased susceptibility in the basal ganglia and brainstem of patients with WD, which helps in early diagnosis and starting the treatment process on time.[92]

Ultrastructure Evaluation of Hepatic Tissue by Electron Microscopic Examination

In WD, copper overload results in cellular damage caused by producing free radicals and oxidative stress.[93] Mitochondria are highly sensitive to oxidative stress, and mitochondrial changes on electron microscopy (EM) can be detected earlier than other histological changes. A mere 1 mm of liver biopsy tissue is needed for EM, which can be easily obtained from the core collected for light microscopy. However, it is crucial to ensure that the biopsy core is not placed in formaldehyde before extracting the tissue for EM.[10,53] Various ultrastructural characteristics of mitochondrial damage in WD have been documented through EM. These include morphological variations, duplication of membranes, expansion of cristae tips, and a condensed matrix.[10] WD histopathology often shows steatosis, which can be mistaken for other liver conditions.[94] EM examination for mitochondrial abnormalities is important to distinguish WD from NAFLD and AIH and could be considered in the diagnostic workup of pediatric liver biopsies.[53]

WD is prone to long-term misdiagnosis due to varying clinical presentation, heterogeneity of biochemical data, and molecular variations. Novel diagnostic approaches, including the REC test, NGS, and quantification of the ATP7BB protein, could limit this misdiagnosis. In addition, using newer MRI-based imaging modalities could provide valuable diagnostic tools for this aspect. Clear guidelines for diagnosis are mandatory to limit missing or late diagnoses and to allow early management.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization, H.S.A. and M.E.K.; data curation and writing original draft preparation, M.K.H., N.F.I, A.M.A., E.G.A. A.E., H.F.H.; writing review and editing, H.S.A., M.E., N.E.D. and M.E.K.; supervision, H.S.A. and M.E.K. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

This is a non-funded study.

REFERENCES

1. Tao TY, Gitlin JD. Hepatic copper metabolism: insights from genetic disease. *Hepatology*. 2003;37:1241–1247.
2. Gitlin JD. Wilson disease. *Gastroenterology*. 2003;125:1868–1877.
3. Ovchinnikova EV, Garbuz MM, Ovchinnikova AA, Kumeiko VV. Epidemiology of Wilson's disease and pathogenic variants of the *ATP7B* gene leading to diversified protein disfunctions. *Int J Mol Sci*. 2024;25:2402.
4. Cairns JE, Williams HP, Walshe JM. "Sunflower cataract" in Wilson's disease. *Br Med J*. 1969;3:95–96.
5. Więcek S, Paprocka J. Disorders of copper metabolism in children problem too rarely recognized. *Metabolites*. 2024;14:38.
6. Brewer GJ. Neurologically presenting Wilson's disease: epidemiology, pathophysiology and treatment. *CNS Drugs*. 2005;19:185–192.

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7. Svetel M, Potrebić A, Pekmezović T, et al. Neuropsychiatric aspects of treated Wilson's disease. *Parkinsonism Relat Disorders*. 2009;15:772–775.
8. Członkowska A, Litwin T, Dusek P, et al. Wilson disease. *Nat Rev Dis Primers*. 2018;4:1–20.
9. Garrido I, Marques M, Liberal R, Cardoso H, Lopes S, Macedo G. Wilson disease in Northern Portugal: a long-term follow-up study. *Orphanet J Rare Dis*. 2022;17:82.
10. Schilsky ML, Roberts EA, Bronstein JM, et al. A multidisciplinary approach to the diagnosis and management of Wilson disease: executive summary of the 2022 practice guidance on Wilson disease from the American association for the study of liver diseases. *Hepatology*. 2023;77:1428–1455.
11. Harry J, Tripathi R. Kayser-Fleischer ring. A pathological study. *Br J Ophthalmol*. 1970;54:794–800.
12. Sridhar MS. Advantages of anterior segment optical coherence tomography evaluation of the kayser-Fleischer ring in Wilson disease. *Cornea*. 2017;36:343–346.
13. Fenu M, Liggi M, Demelia E, Sorbello O, Civolani A, Demelia L. Kayser-Fleischer ring in Wilson's disease: a cohort study. *Eur J Intern Med*. 2012;23:e150–e156.
14. Broniek-Kowalik K, Dzieżyc K, Litwin T, Członkowska A, Szaflik JP. Anterior segment optical coherence tomography (AS-OCT) as a new method of detecting copper deposits forming the Kayser-Fleischer ring in patients with Wilson disease. *Acta Ophthalmol*. 2019;97:e757–e760.
15. Fleming CR, Dickson ER, Wahner HW, Hollenhorst RW, McCall JT. Pigmented corneal rings in non-Wilsonian liver disease. *Ann Intern Med*. 1977;86:285–288.
16. Jones EA, Rabin L, Buckley CH, Webster GK, Owens D. Progressive intrahepatic cholestasis of infancy and childhood. A clinicopathological study of patient surviving to the age of 18 years. *Gastroenterology*. 1976;71:675–682.
17. Jawairia M, Subhani M, Siddiqui G, et al. Unexplained findings of kayser-fleischer-like rings in a patient with cryptogenic cirrhosis. *Case Rep Gastrointest Med*. 2012;2012438525.
18. Izatt JA, Hee MR, Swanson EA, et al. Micrometer-scale resolution imaging of the anterior eye in vivo with optical coherence tomography. *Arch Ophthalmol*. 1994;112:1584–1589.
19. European Association For The Study Of The Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol*. 2009;50:227–242.
20. Kasztelan-Szczerbinska B, Cichoz-Lach H. Wilson's disease: an update on the diagnostic workup and management. *J Clin Med*. 2021;10:5097.
21. Martínez-Morillo E, Bauça JM. Biochemical diagnosis of Wilson's disease: an update. *Adv Lab Med*. 2022;3:103–125.
22. Sánchez-Monteagudo A, Ripollés E, Berenguer M, Espinós C. Wilson's disease: facing the challenge of diagnosing a rare disease. *Biomedicines*. 2021;9:1100.
23. Poujois A, Woimant F. Challenges in the diagnosis of Wilson disease. *Ann Transl Med*. 2019;7(suppl 2):S67.
24. Shaver WA, Bhatt H, Combes B. Low serum alkaline phosphatase activity in Wilson's disease. *Hepatology*. 1986;6:859–863.
25. Korman JD, Volenberg I, Balko J, et al. Screening for Wilson disease in acute liver failure: a comparison of currently available diagnostic tests. *Hepatology*. 2008;48:1167–1174.
26. Mohr I, Weiss KH. Biochemical markers for the diagnosis and monitoring of Wilson disease. *Clin Biochem Rev*. 2019;40:59–77.
27. Gromadzka G, Chabik G, Mendel T, Wierzchowska A, Rudnicka M, Czlonkowska A. Middle-aged heterozygous carriers of Wilson's disease do not present with significant phenotypic deviations related to copper metabolism. *J Genet*. 2010;89:463–467.
28. Sánchez-Albisua I, Garde T, Hierro L, et al. A high index of suspicion: the key to an early diagnosis of Wilson's disease in childhood. *J Pediatr Gastroenterol Nutr*. 1999;28:186–190.
29. Perman JA, Werlin SL, Grand RJ, Watkins JB. Laboratory measures of copper metabolism in the differentiation of chronic active hepatitis and Wilson disease in children. *J Pediatr*. 1979;94:564–568.
30. Nagral A, Sarma MS, Matthai J, et al. Wilson's disease: clinical practice guidelines of the Indian national association for study of the liver, the Indian society of pediatric gastroenterology. *Hepatology and Nutrition, and the Movement Disorders Society of India*. 2019;9:74–98 [published correction appears in J Clin Exp Hepatol. 2020 Jan-Feb;10(1):99. J Clin Exp Hepatol.
31. Litwin T, Dzieżyc K, Poniatowska R, Członkowska A. Effect of liver transplantation on brain magnetic resonance imaging pathology in Wilson disease: a case report. *Neurol Neurochir Pol*. 2013;47:393–397.
32. Poujois A, Woimant F. Challenges in the diagnosis of Wilson disease. *Ann Transl Med*. 2019;7(suppl 2):S67.
33. Chang IJ, Hahn SH. The genetics of Wilson disease. *Handb Clin Neurol*. 2017;142:19–34.
34. European Association for Study of Liver. EASL clinical practice guidelines: Wilson's disease. *J Hepatol*. 2012;56:671–685.
35. Leung M, Aronowitz PB, Medici V. The present and future challenges of Wilson's disease diagnosis and treatment. *Clin Liver Dis*. 2021;17:267–270.
36. King AD, Walshe JM, Kendall BE, et al. Cranial MR imaging in Wilson's disease. *AJR Am J Roentgenol*. 1996;167:1579–1584.
37. Kieffer DA, Medici V. Wilson disease: at the crossroads between genetics and epigenetics-A review of the evidence. *Liver Res*. 2017;1:121–130.
38. van Wassenaer-van Hall HN, van den Heuvel AG, Jansen GH, Hoogenraad TU, Mali WP. Cranial MR in Wilson disease: abnormal white matter in extrapyramidal and pyramidal tracts. *AJNR Am J Neuroradiol*. 1995;16:2021–2027.
39. Yang X. *Zhonghua Gan Zang Bing Za Zhi*. 2017;25:881–885.
40. Liu J, Luan J, Zhou X, Cui Y, Han J. Epidemiology, diagnosis, and treatment of Wilson's disease. *Intractable Rare Dis Res*. 2017;6:249–255.
41. Mordaunt CE, Kieffer DA, Shibata NM, et al. Epigenomic signatures in liver and blood of Wilson disease patients include hypermethylation of liver-specific enhancers. *Epigenetics Chromatin*. 2019;12:10.
42. Rodriguez-Granillo A, Sedlak E, Wittung-Stafshede P. Stability and ATP binding of the nucleotide-binding domain of the Wilson disease protein: effect of the common H1069Q mutation. *J Mol Biol*. 2008;383:1097–1111.
43. Huster D, Hoppert M, Lutsenko S, et al. Defective cellular localization of mutant ATP7B in Wilson's disease patients and hepatoma cell lines. *Gastroenterology*. 2003;124:335–345.
44. Coffey AJ, Durkie M, Hague S, et al. A genetic study of Wilson's disease in the United Kingdom. *Brain*. 2013;136(Pt 5):1476–1487.
45. Ferenci P, Stremmel W, Członkowska A, et al. Age and sex but not ATP7B genotype effectively influence the clinical phenotype of Wilson disease. *Hepatology*. 2019;69:1464–1476.
46. Lorincz MT. Neurologic Wilson's disease. *Ann N Y Acad Sci*. 2010;1184:173–187.
47. Page R, Davie C, MacManus D, et al. Clinical correlation of brain MRI and MRS abnormalities in patients with Wilson disease. *Neurology*. 2004;63:638–643.
48. Litwin T, Gromadzka G, Członkowska A, Gołębiowski M, Poniatowska R. The effect of gender on brain MRI pathology in Wilson's disease. *Metab Brain Dis*. 2013;28:69–75.
49. Basha MAA, Refaat R, Ahmed AF, et al. Brain magnetic resonance spectroscopy (MRS) as a diagnostic tool for detecting early

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neurological changes in children with Wilson's disease. *Eur J Radiol*. 2019;111:41–46.
50. Kluska A, Kulecka M, Litwin T, et al. Whole-exome sequencing identifies novel pathogenic variants across the ATP7B gene and some modifiers of Wilson's disease phenotype. *Liver Int*. 2019;39:177–186.
51. Dusek P, Bahn E, Litwin T, et al. Brain iron accumulation in Wilson disease: a post mortem 7 Tesla MRI - histopathological study. *Neuropathol Appl Neurobiol*. 2017;43:514–532.
52. Kocabay G, Tutuncu Y, Yilmaz H, Demir K. Impact of apolipoprotein E genotypes on phenotypic expression in Turkish patients with Wilson's disease. *Scand J Gastroenterol*. 2009;44:1270–1271.
53. Gerosa C, Fanni D, Congiu T, et al. Liver pathology in Wilson's disease: from copper overload to cirrhosis. *J Inorg Biochem*. 2019;193:106–111.
54. Santos RG, Alissa F, Reyes J, Teot L, Ameen N. Fulminant hepatic failure: Wilson's disease or autoimmune hepatitis? Implications for transplantation. *Pediatr Transplant*. 2005;9:112–116.
55. Guindi M. Wilson disease. *Semin Diagn Pathol*. 2019;36:415–422.
56. Clark I, Torbenson MS. Immunohistochemistry and special stains in medical liver pathology. *Adv Anat Pathol*. 2017;24:99–109.
57. Członkowska A, Litwin T, Dusek P, et al. Wilson disease. *Nat Rev Dis Primers*. 2018;4:21.
58. Madakshira MG, Das A, Umairv M, Dutta U. Liver histology and histochemistry in Wilson disease. *Autops Case Rep*. 2018;8e2018026.
59. Alkhouri N, Gonzalez-Peralta RP, Medici V. Wilson disease: a summary of the updated AASLD Practice Guidance. *Hepatol Commun*. 2023;7e0150.
60. Leung M, Aronowitz PB, Medici V. The present and future challenges of Wilson's disease diagnosis and treatment. *Clin Liver Dis*. 2021;17:267–270.
61. Lankarani KB. Challenging issues in the management of Wilson's disease. *Clin Res Hepatol Gastroenterol*. 2018;42:e95–e96.
62. Ghosh U, Sen Sarma M, Samanta A. Challenges and dilemmas in pediatric hepatic Wilson's disease. *World J Hepatol*. 2023;15:1109–1126.
63. Woimant F, Djebrani-Oussedik N, Poujois A. New tools for Wilson's disease diagnosis: exchangeable copper fraction. *Ann Transl Med*. 2019;7(suppl 2):S70.
64. Trocello JM, El Balkhi S, Woimant F, et al. Relative exchangeable copper: a promising tool for family screening in Wilson disease. *Mov Disord*. 2014;29:558–562.
65. Guillaud O, Brunet AS, Mallet I, et al. Relative exchangeable copper: a valuable tool for the diagnosis of Wilson disease. *Liver Int*. 2018;38:350–357.
66. El Balkhi S, Trocello J-M, Poupon J, et al. Relative exchangeable copper: a new highly sensitive and highly specific biomarker for Wilson's disease diagnosis. *Clin Chim Acta*. 2011;412:2254–2260.
67. Jung S, Whiteaker JR, Zhao L, Yoo HW, Paulovich AG, Hahn SH. Quantification of ATP7B protein in dried blood spots by peptide immuno-SRM as a potential screen for Wilson's disease. *J Proteome Res*. 2017;16:862–871.
68. Collins CJ, Yi F, Dayuha R, et al. Direct measurement of ATP7B peptides is highly effective in the diagnosis of Wilson disease. *Gastroenterology*. 2021;160:2367–23682.e1.
69. Gromadzka G, Bendykowska M, Przybyłkowski A. Wilson's disease-genetic puzzles with diagnostic implications. *Diagnostics*. 2023;13:1287.
70. Öz G, Alger JR, Barker PB, et al. Clinical proton MR spectroscopy in central nervous system disorders. *Radiology*. 2014;270:658–679.
71. Sitter B, Sjùbakk TE, Larsson HBW, Kvistad KA. Clinical MR spectroscopy of the brain. Klinisk MR-spektroskopi av hjernen. *Tidsskr Nor Laegeforen*. 2019;139 https://doi.org/10.4045/tidsskr.17.1099.
72. Ramos JL, Li Y, Huang D. Clinical and research applications of anterior segment optical coherence tomography - a review. *Clin Exp Ophthalmol*. 2009;37:81–89.
73. Telinius N, Ott P, Hjortdal J. Detection of Kayser-Fleischer ring using Scheimpflug imaging. *Acta Ophthalmol*. 2017;95:e248–e249.
74. Sridhar MS, Pineda R. Anterior segment optical coherence tomography to look for Kayser-Fleischer rings. *Pract Neurol*. 2017;17:222–223.
75. Rathi A, Takkar B, Gaur N, Maharana PK. Optical coherence tomography of the Kayser-Fleischer ring: an ancillary diagnostic tool for Wilson's disease in children. *BMJ Case Rep*. 2017;2017 bcr2017220007.
76. Walshe JM, Potter G. The pattern of the whole body distribution of radioactive copper (67Cu, 64Cu) in Wilson's Disease and various control groups. *Q J Med*. 1977;46:445–462.
77. Peng F. Positron emission tomography for measurement of copper fluxes in live organisms. *Ann N Y Acad Sci*. 2014;1314:24–31.
78. Kjærgaard K, Sandahl TD, Frisch K, et al. Intravenous and oral copper kinetics, biodistribution and dosimetry in healthy humans studied by [^{64}Cu]copper PET/CT. *EJNMMI Radiopharm Chem*. 2020;5:15.
79. Peng F, Lutsenko S, Sun X, Muzik O. Imaging copper metabolism imbalance in Atp7b (-/-) knockout mouse model of Wilson's disease with PET-CT and orally administered 64CuCl2. *Mol Imag Biol*. 2012;14:600–607.
80. Sandahl TD, Gormsen LC, Kjærgaard K, et al. The pathophysiology of Wilson's disease visualized: a human ^{64} Cu PET study. *Hepatology*. 2022;75:1461–1470.
81. Alanen A, Komu M, Penttinen M, Leino R. Magnetic resonance imaging and proton MR spectroscopy in Wilson's disease. *Br J Radiol*. 1999;72:749–756.
82. Merle U, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. *Gut*. 2007;56:115–120.
83. Saneto RP, Friedman SD, Shaw DW. Neuroimaging of mitochondrial disease. *Mitochondrion*. 2008;8:396–413.
84. Lucato LT, Otaduy MCG, Barbosa ER, et al. Proton MR spectroscopy in Wilson disease: analysis of 36 cases. *AJNR Am J Neuroradiol*. 2005;26:1066–1071.
85. De Stefano N, Matthews PM, Arnold DL. Reversible decreases in N-acetylaspartate after acute brain injury. *Magn Reson Med*. 1995;34:721–727.
86. Pulai S, Biswas A, Roy A, et al. Clinical features, MRI brain, and MRS abnormalities of drug-naöve neurologic Wilson's disease. *Neurol India*. 2014;62:153–158.
87. Jayasundar R, Sahani AK, Gaikwad S, Singh S, Behari M. Proton MR spectroscopy of basal ganglia in Wilson's disease: case report and review of literature. *Magn Reson Imaging*. 2002;20:131–135.
88. Hagemeier J, Zivadinov R, Dwyer MG, et al. Changes of deep gray matter magnetic susceptibility over 2 years in multiple sclerosis and healthy control brain. *Neuroimage Clin*. 2017;18:1007–1016.
89. Nikparast F, Ganji Z, Zare H. Early differentiation of neurodegenerative diseases using the novel QSM technique: what is the biomarker of each disorder? *BMC Neurosci*. 2022;23:48.
90. Doganay S, Gumus K, Koc G, et al. Magnetic susceptibility changes in the basal ganglia and brain stem of patients with Wilson's disease: evaluation with quantitative susceptibility mapping. *Magn Reson Med Sci*. 2018;17:73–79.
91. Sternlieb I. Mitochondrial and fatty changes in hepatocytes of patients with Wilson's disease. *Gastroenterology*. 1968;55:354–367.

Please cite this article as: Aboalam et al., Challenges and Recent Advances in Diagnosing Wilson Disease, Journal of Clinical and Experimental Hepatology, https://doi.org/10.1016/j.jceh.2025.102531

92. Alqahtani SA, Chami R, Abuquteish D, et al. Hepatic ultrastructural features distinguish paediatric Wilson disease from NAFLD and autoimmune hepatitis. *Liver Int*. 2022;42:2482–2491.
93. Sternlieb I. Fraternal concordance of types of abnormal hepatocellular mitochondria in Wilson's disease. *Hepatology*. 1992;16:728–732.
94. Hassan MK, Alswat K, El-Kassas M. Shedding lights on diagnostic challenges in Wilson's disease: the positive impact of introducing steatotic liver disease new nomenclature umbrella. *Liver Int*. 2024;44:1267–1268.

Please cite this article as: Aboalam et al., Challenges and Recent Advances in Diagnosing Wilson Disease, Journal of Clinical and Experimental Hepatology, https://doi.org/10.1016/j.jceh.2025.102531