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Wilson Disease

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Wilson disease (WD) is an autosomal recessive inherited disorder of copper metabolism, resulting in pathological accumulation of copper in many organs and tissues. The hallmarks of the disease are the presence of liver disease, neurologic symptoms, and Kayser–Fleischer corneal rings. The leading neurologic symptoms in WD are dysathria, dyspraxia, ataxia, and Parkinsonian-like extrapyramidal signs. Changes in the basal ganglia in brain magnetic resonance imaging (MRI) are characteristic features of the disease. In presence of liver cirrhosis, some features may resemble hepatic encephalopathy. Symptoms and MRI abnormalities may be fully reversible on treatment with zinc or copper chelators. Improvement can be monitored by serial recording of brain-stem-evoked responses. The basic defect is an impaired traficking of copper in hepatocytes. ATP7B is the gene product of the WD gene located on chromosome 13 and resides in hepatocytes in the trans-Golgi network, transporting copper into the secretory pathway for incorporation into apoceruloplasmin and excretion into the bile. While about 40% of patients preset with neurologic symptoms, little is known about the role of copper and ATP7B in the central nervous system. In some brain areas, like in the pineal gland, ATP7B is expressed and functionally active. Increasing evidence supports an important role for metals in neurobiology. Two proteins related to neurodegeneration are copper-binding proteins (1) the amyloid precursor protein (APP), a protein related to Alzheimer's disease, and (2) the Prion protein, related to Creutzfeldt–Jakob disease. A major source of free-radical production in the brain derives from copper. To prevent metal-mediated oxidative stress, cells have evolved complex metal transport systems. APP is a major regulator of neuronal copper homeostasis and has a copper-binding domain (CuBD). The surface location of this site, structural homology of CuBD to copper chaperones, and the role of APP in neuronal copper homeostasis are consistent with the CuBD acting as a neuronal metallotransporter. There are several copper-containing enzymes in the brain, like dopamine beta hydroxylase or Cu/Zn superoxide dismutase (SOD1). Their function may be altered because of copper overload. WD appears to be associated with a dopaminergic deficit. Mutations in the *SOD1* gene cause familial amyotrophic lateral sclerosis. Survival of transgenic mice with a mutant SOD1 which fails to incorporate $Cu(^{2+})$ in its active site was improved by copper depletion. Wilson disease (WD) is an autosomal recessive inherited disorder in which copper pathologically accumulates primarily within the liver and subsequently in the neurologic system and many other organs and tissues. Presence of liver disease, neurologic symptoms, and Kayser–Fleischer corneal rings are the hallmarks of the disease.

Key words: Wilson disease; movement disorders; diagnosis; treatment.

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PHYSIOLOGY AND PATHOPHYSIOLOGY OF HEPATIC COPPER METABOLISM

Copper facilitates electron transfer reactions when incorporated into specific cuproproteins, which are needed for such diverse processes as mitochondrial respiration, melanin biosynthesis, dopamine metabolism, iron homeostasis, antioxidant defense, connective tissue formation, and peptide amidation (Culotta and Gitlin, 2001). Avoiding cellular toxicity, specific pathways allow the intracellular trafficking and compartmentalization of copper, ensuring adequate cuproprotein synthesis. A rapid and efficient clearance of copper from the portal circulation has been shown in studies, in humans, utilizing copper isotopes (Schiefermeier *et al*., 2000). More than 95% of the isotope is removed from the circulation by the liver within 4 h after giving a tracer dose of ^{64}Cu, and 24 h later, 6–8% reappears incorporated into ceruloplasmin, a ferroxidase containing greater than 95% of the copper found in plasma (Hellman and Gitlin, 2002). The only mechanism for copper elimination is biliary excretion. The size of the hepatic copper pool is directly proportional to the amount of copper excreted in the bile. Several transport proteins are responsible for the trafficking of copper in and through the hepatocytes:

Copper transporter 1 (Ctr1), which is expressed in multiple cell types, including hepatocytes and hepatoma-derived cell lines, is an polytopic membrane protein involved in the copper uptake at the hepatocyte plasma membrane. It transports copper with high affinity, in a metal-specific, saturable fashion (Klomp *et al*., 2002; Lee *et al*., 2001).

Metallothioneins (MT) are capable of binding metal ions, including copper, cadmium, and zinc (Palmiter, 1998). MT I and MT II, are ubiquitously expressed in all cell types including hepatocytes, and have a critical role to protect intracellular proteins from copper toxicity (Kelley and Palmiter, 1996).

Metallochaperones mediate the delivery of copper to specific proteins (Huffman and O'Halloran, 2000; Rae *et al*., 1999). They are involved in the transfer of copper from metallothionein to the site of synthesis of copper-containing proteins.

ATP7B is the gene product of the WD gene located on chromosome 13 (Bull *et al*., 1993; Tanzi *et al*., 1993) and resides in hepatocytes in the trans-Golgi network. It is a polytopic membrane protein and similar to other ATP-dependent transporters for heavy metals. ATP7B is involved in transporting copper into the secretory pathway for incorporation into apoceruloplasmin and excretion into the bile (Schaefer *et al*., 1999).

Over 200 distinct disease-causing mutations in this gene were described (see the database maintained at the University of Alberta, www.medgen.med.ualberta.ca). The only better studied one is the H1069Q mutation, which results in a temperature-sensitive instability of ATP7B under high intracellular copper concentrations (Payne *et al*., 1998), whereas folding and stability was maintained under low copper concentration conditions. Thus, this mutation allows synthesis of ceruloplasmin to a certain degree and is associated with late onset neurologic disease. H1069Q is also responsible for proper orientation of ATP in the catalytic site of ATP7B prior to ATP hydrolysis (Tsivkovskii *et al*., 2003).

PATHOGENESIS OF NEUROLOGIC WILSON DISEASE

In Wilsonian liver disease, copper accumulation in hepatocytes initially causes mitochondrial damage with alteration of lipid oxidation, resulting in marked hepatic steatosis.

It is suggested that free-radical formation and oxidative damage, probably mediated via mitochondrial copper accumulation, are important in WD pathogenesis (Gu *et al*., 2000). Accumulation of prooxidant copper within hepatic mitochondria leads to premature oxidative aging of mitochondrial DNA by causing somatic mutations of the mitochondrial genome (Mansouri *et al*., 1997). Further damage of hepatocytes, inflammation, and fibrogenesis is caused by the releasing copper from necrotic hepatocytes.

The pathogenesis of neurologic WD is less clear. Copper is released into the circulation if the capacity of the liver to store copper is exhausted, and taken up by virtually all organs. Since copper is not taken up by neurons (Watt and Hooper, 2000), increased amounts of extracellular copper may explain the mechanism of neuronal damage in WD. Genetic variation of Apolipoprotein E (ApoE) has an important impact on the onset of neurologic symptoms in H1069Q homozygotes. It is known that ApoE is able to bind metal ions with the highest affinity for copper and increases the resistance of cell cultures to oxidative stress. (Miyata and Smith, 1996). However, ApoE isoforms varies in their neuroprotective properties. Patients who carry the wild-type (ApoE $\varepsilon 3$) appear to be protected from copper toxicity to a certain degree (Schiefermeier *et al*., 2000).

Metals play an important role in neurobiology. Copper-binding proteins are able to display oxidant or antioxidant properties, which would impact on neuronal function or in the triggering of neurodegenerative process. A major source of free-radical production in the brain derives from copper. To prevent metal-mediated oxidative stress, cells have evolved complex metal transport systems. In neurodegenerative diseases, two proteins have been described as copper-binding proteins: a protein related to Alzheimer's disease (AD), the amyloid precursor protein (APP), and a protein related to Creutzfeldt–Jakob disease, the Prion protein (PrP). The AD amyloid precursor protein is a major regulator of neuronal copper homeostasis, which has a copper-binding domain (CuBD). The surface location of this site, structural homology of CuBD to copper chaperones, and the role of APP in neuronal copper homeostasis are consistent with the CuBD acting as a neuronal metallotransporter (Barnham *et al*., 2003). In a healthy individual the brain strictly regulates the movement of metals across the blood–brain barrier (BBB), which is relatively impermeable to fluctuations in blood levels. This barrier is relevant in AD because the disease is characterized by the accumulation in the brain, of β-amyloid (Aβ) a copper–zinc metalloprotein that aggregates and becomes redox active in the presence of excessive amounts of these metals. We are only beginning to unravel the age-dependent failure of metal homeostatic mechanisms of the brain that contribute to abnormal Aβ biochemistry in AD (Bush *et al*., 2003). The ingestion of low amounts of copper in drinking water impairs trace conditioning and increases neuronal and brain parenchymal Aβ immunoreactivity in cholesterol-supplemented rabbits (Sparks and Schreurs, 2003). This finding suggests that Aβ metabolism is extraordinarily sensitive to small changes in copper concentrations that might be transduced across the BBB. Clioquinol, an antibiotic and bioavailable Cu/Zn chelator, decreased brain Aβ deposition by 49% in APP2576 transgenic mice treated orally for 9 weeks and imporved their general health and body-weight parameters (Beyreuther *et al*., 2001).

Ceruloplasmin is a ferroxidase that oxidizes toxic ferrous iron to its nontoxic ferric form (Patel *et al*., 2002). If ceruloplasmin is not present, iron concentration may increase.

In addition to the direct toxic effects of copper, in certain brain areas, like in the pineal gland, ATP7B is expressed and functionally active (Borjigin *et al*., 1999). Glucose

metabolism especially in striatal and cerebellar areas is disturbed in patients with WD and correlates with the severity of extrapyramidal motor symptoms. The most severe cases are characterized by the lowest consumption in the striatal area. When there is marked improvement of extrapyramidal motor symptoms, impaired glucose consumption reveals a persistent brain lesion (Hermann *et al*., 2002b).

Furthermore the impaired synthesis of dopamine beta hydroxylase, a copper-containing enzyme, may explain the preferential affection of basal ganglia. By single photon emission CT (SPECT) specific striatal-binding ratios of two tracers ($^{[123I]}2\beta$-carbomethoxy-3β-4$^{[123I]}$iodophenyl tropane $^{[123I]}\beta$a-CIT and $^{[123I]}$iodobenzamide $^{[123I]}$IBZM) were reduced. The concordant bicompartmental dopaminergic deficit in neurologic WD provide in vivo evidence for assigning WD to the group of secondary Parkinsonian syndromes (Barthel *et al*., 2003). Few other neurotransmitter systems have been studied in WD. In vivo neuroimaging studies suggest that depression is associated with central serotonergic deficits (Hermann *et al*., 2002a). $^{[1]}$H spectra demonstrated a reduction of *N*-acetylaspartate and *N*-acetylaspartylglutamate in patients with neurologic WD. Choline was also reduced (Page *et al*., 2004).

CLINICAL PRESENTATIONS

Liver disease and neuropsychiatric disturbances are the most common presentations in the wide spectrum of clinical conditions in WD. None of the clinical signs are typical and diagnostic, and one of the main characteristics of WD is that no two patients, are ever quite alike. A hallmark of the disease are the Kayser–Fleischer rings, which are found in 95% of patients with neurologic symptoms, but only in 50–60% of patients without neurologic symptoms, and in 10% of asymptomatic siblings. Liver disease is the most common presentation of patients with WD. The age of hepatic manifestation usually is between 8 and 18 years, but cirrhosis can already be present in children below 5 years of age. If undiagnosed, chronic liver disease may precede manifestation of neurologic symptoms by many years. All forms of common liver conditions, ranging from asymptomatic transaminasemia, acute or chronic hepatitis, fulminant hepatic failure, and cirrhosis can be observed.

Neurological Presentation

The initial neurological symptoms may be very subtle, such as mild tremor, as well as speech and writing problems. They usually develop in mid-teenage or in the twenties (Oder *et al*., 1991), and are frequently misdiagnosed as behavioral problems associated with puberty. However, presentation much later, between 45 and 70 years, is also possible. Progressive extrapyramidal neurological disorder is the typical sign of neurologic WD. Dysarthria, dysphagia, apraxia, and a tremor-rigidity syndrome are the most common symptoms. Handwriting defects are an early sign of motor impairment in patients with Wilson's disease. There was a significant correlation of putaminal dopaminergic innervation with fine motor ability. Analysis of automated handwriting movements could be useful for therapy monitoring and evaluation of striatal dopaminergic innervation (Hesse *et al*., 2003).

Psychiatric Presentation

Psychiatric abnormalities are initially found in about one third of the patients. Symptoms can include reduced performance in school or at work, depression, very labile mood, sexual exhibitionism, and frank psychosis. The most common psychiatric symptom is depression. Central serotonergic deficits may be associated with depression (Hermann *et al*., 2002a). The Hamilton rating scale for depression shows an inverse correlation with presynaptic serotonin transporters (SERT) availability as measured by $^{[123}I]-2\beta$-carbomethoxy-3β-(iodophenyl)tropane SPECT in the thalamus–hypothalamus region but not in the midbrain–pons (Eggers *et al*., 2003).

DIAGNOSIS

The basis of the diagnosis of WD are typical clinical findings and laboratory abnormalities (see Table 1). If two of the following symptoms are present, diagnosis can be made without any further tests: Kayser–Fleischer rings, typical neurological symptoms, and low serum ceruloplasmin levels (Sternlieb, 1990). A combination of a variety of clinical and biochemical tests is required for the right diagnosis in patients with liver disease I in whom these symptoms may be absent. Recently, a score, based on a variety of tests and clinical symptoms, was proposed by a group of international experts (Ferenci *et al*., 2003). To detect

Table 1. Routine Tests for Diagnosis of Wilson Disease

Test	Typical finding	False "negative"	False "positive"
Serum ceruloplasmin	Decreased	Normal levels in patients with marked hepatic inflammation Overestimation by immunologic assay	Low levels in Malabsorption Aceruloplasminemia Liver insufficiency Heterozygotes
24 h urinary copper	$>100\,\mu$g/d	Normal Incorrect collection Children without liver disease	Increased Hepatocellular necrosis Contamination
Serum "free" copper	$>10\,\mu$g/dL	Normal if ceruloplasmin overestimated imunologic assay	
Hepatic copper	$>250\,\mu$g/g dry weight	Due to regional variation In patients with active liver disease In patients with regenerative nodules active liver disease	Cholestatic syndromes
Kayser–Fleischer rings by slit lamp	Present	In up to 40% of patients with hepatic Wilson disease In most asymptomatic siblings	Primary biliary cirrhosis

neurologic abnormalities, a careful clinical examination by an experienced neurologist is of great importance. Brain magnetic resonance imaging (MRI) is useful to document the extent of changes in the central nervous system (van Wassenaer-van Hall *et al*., 1996). Cerebral lesions were detected by MRI in 60% of the neurologically symptomatic and 19% of the asymptomatic patients with WD (Grimm *et al*., 1991). Focal lesions were found as hypointense spots, most common localized in the lenticular nuclei, ventral or lateral thalamic nuclei, subcortical white matter, lamina tecti, and caudate nuclei (Grimm *et al*., 1991). Atrophy of the hemispheres or brainstem was evident in 68% of patients with neurologic symptoms, but only in 6% of neurologically asymptomatic patients (Grimm *et al*., 1991). A rare but typical MRI finding is "the face of the giant panda" sign (Jacobs *et al*., 2003).

Monotoring of Therapy

The progression of WD can be arrested by chelation therapy. However, neurologic deficits may persist despite adequate treatment. MRI is used to assess patients with WD, but attempts to correlate clinical progression with objective findings are frequently unsuccessful. On treatment, some of the MRI abnormalities are fully reversible.

A very helpful diagnostic tool includes somatosensory evoked and auditory evoked brainstem potentials (Grimm *et al*., 1992). Abnormalities reflect subclinical impairment of central sensory pathways. Auditory I-V and somatosensory N13–N20 interpeak latencies are prolonged in neurologically symptomatic patients. No significant interhemisphere latency and amplitude difference compared to healthy subjects indicates a diffuse rather than focal subclinical involvement of the sensory pathway (Grimm *et al*., 1992). Auditory brainstem and somatosensory evoked potentials (EPs) were more frequently affected than visual EPs, reflecting the involvement of brainstem and vertical neuraxis by copper toxicity (Grimm *et al*., 1992). EPs are also be helpful in assessing the efficacy of de-coppering treatment in WD (Grimm *et al*., 1990). It may be valuable in monitoring a reversible component of the disease that cannot be detected by MRI. The improvement of auditory evoked brainstem potentials by d-penicillamine treatment was more evident than that of somatosensory potentials, suggesting a greater benefit of treatment in the brainstem than in the vertical cervicocortical neuraxis (Grimm *et al*., 1991).

TREATMENT

Today, the mainstay of treatment for WD remains lifelong pharmacologic therapy.

Following the recent American Association for the Study of Liver Diseases (AASLD) practice guideline on WD, the initial treatment for symptomatic patients should include a chelating agent (penicillamine or trientine). Treatment of presymptomatic patients or maintenance therapy of successfully treated symptomatic patients can be accomplished with the chelating agent penicillamine or trientine, or with zinc (Roberts and Schilsky, 2003). The usual dose of penicillamine is 1–1.5 g/day. Once the clinical benefit is established, it is possible to reduce the dosage to 0.5–1 g/day. Trientine at a dose of 1.0–1.25 g/day can be used alternatively (Payne *et al*., 1998). Zinc interferes with the intestinal absorption of copper by two mechanisms. Both metals share the same carrier in enterocytes, and pretreatment with zinc blocks this carrier for copper transport (with a half-life of 11 days). Second, zinc induces metallothionein in enterocytes, which acts as an intracellular ligand,

binding zinc, copper, and other metals. Depending on the type of zinc preparations (zinc sulfate, zinc acetate, zinc histidine) doses of 45–250 mg/day are given (Ferenci, 1997). Tetrathiomolybdate (an experimental drug) appears to be useful as an initial treatment in patients presenting with neurologic symptoms (Brewer *et al*., 1996). Liver transplantation, which corrects the underlying hepatic defect in WD, is the treatment of choice in patients with fulminant disease or with decompensated cirrhosis. The role of liver transplantation in the management of patients with neurological Wilson's disease in the absence of hepatic insufficiency is uncertain (Bax *et al*., 1998; Guarino *et al*., 1995).

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