WILSON’S DISEASE: AN INHERITED, SILENT, COPPER INTOXICATION DISEASE

Uta Merle,1 *Ralf Weiskirchen2

1. Department of Gastroenterology and Hepatology, University Hospital Heidelberg, Heidelberg, Germany
2. Institute of Molecular Pathobiochemistry, Experimental Gene Therapy, and Clinical Chemistry, RWTH University Hospital Aachen, Aachen, Germany
*Correspondence to rweiskirchen@ukaachen.de

Disclosure: The authors have declared no conflicts of interest.
Received: 11.01.16 Accepted: 25.04.16
Citation: EMJ Neurol. 2016;4[1]:74-83.

ABSTRACT

Wilson’s disease is a rare, autosomal recessive, genetic, copper overload disease, which evokes multiple motor or neuropsychiatric symptoms and liver disease. It is the consequence of a variety of different mutations affecting the ATP7B gene. This gene encodes for a class IB, P-type, copper-transporting ATPase, which is located in the trans-Golgi network of the liver and brain, and mediates the excretion of excess copper into the bile. When functionally inactive, the excess copper is deposited in the liver, brain, and other tissues. Free copper induces oxidative stress, lipid peroxidation, and lowers the apoptotic threshold of the cell. The symptoms in affected persons can vary widely and usually appear between the ages of 6 years and 20 years, but there are also cases in which the disease manifests in advanced age. In this review, we discuss the considerations in diagnosis, clinical management, and treatment of Wilson’s disease. In addition, we highlight experimental efforts that address the pathogenesis of Wilson’s disease in ATP7B deficient mice, novel analytical techniques that will improve the diagnosis at an early stage of disease onset, and treatment results with copper-chelating agents.

Keywords: Inherited disease, genetics, liver, brain, metal, copper, clinical management, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) protocols, therapy, ceruloplasmin, X-linked inhibitor of apoptosis protein (XIAP), oxygen stress, trientine dihydrochloride, D-penicillamine, diagnostics.
Figure 1: Clinical and experimental Wilson’s disease.
A) Chromosomal localisation of human ATP7A and ATP7B genes. The genes ATP7A and ATP7B are both crucial copper-transporting ATPases, which are located on the long arms of chromosomes 13 and X, respectively. The schematic overview of the human ideogram was created with the Genome Decoration Page.40 B) ATP7B knockout mouse, a model of experimental Wilson’s disease. By use of mutagenesis both alleles of the murine ATP7B gene that are located on chromosome 8, region D8Mit341 were...

Wild-type mice
ATP7B+/+
Normal liver
Normal brain
Well-defined distribution of Cu in white and grey matter

Knockout mice
ATP7B−/−
Cirrhotic liver
Normal liver
Normal brain

Concentration [Cu] (µM)
0 1 2 3 4 5 6 7 8 9
Age (months)
Decrease during lifespan

Concentration [Cu] (µM)
0 1 2 3 4 5 6 7 8 9
Age (months)
Up to 60 X (after 5 months)

Elevated expression of inflammatory markers (e.g. TNF-α)
Increase of organ damage
Elevated levels of hepatic Fe
Elevated levels of cerebral Cu and Fe
Increased reactive oxygen species
Homogenous distribution of Cu within the tissue
Well-defined distribution of Cu in white and grey matter

Normal lifespan −2 years
2 x wild-type ATP7B alleles
Normal lifespan
2 x mutated ATP7B alleles
Cu deficiency due to reduced Cu in maternal milk
Gradual Cu accumulation in various organs
2–4 days
7 months

Wild-type mice
Knockout mice
Chromosome 8 (D8Mit3)
Mutagenesis (gene disruption)
In humans, approximately 50% of dietary copper (~0.8–2 mg/day) is absorbed in the proximal small intestine. After uptake by hepatocytes, biliary excretion is the main pathway for the elimination of excess copper. In Wilson’s disease, the export of copper into bile is impaired, leading to a pathological copper accumulation, primarily within the liver and subsequently in the brain (particularly in the basal ganglia) and other tissues (e.g. kidneys and cornea). In addition to reduced biliary copper excretion impaired \( \text{ATP7B} \) function leads to abnormalities in the incorporation of copper into ceruloplasmin, which under normal conditions is the major copper-carrying protein in the blood. Failure to incorporate copper during ceruloplasmin biosynthesis results in the secretion of an apoprotein (i.e. apoceruloplasmin) that is devoid of enzymatic activity and degrades rapidly. The resulting decrease in serum ceruloplasmin concentration, which was first described in 1952, is a diagnostic hallmark of Wilson’s disease.\(^6\)

Ceruloplasmin accounts for most of the copper in serum, thus the total serum copper is abnormally low in 80–95% of all Wilson’s disease cases.\(^7\)

The toxicity of copper in Wilson’s disease is evident in a number of different mechanisms. Free copper has the capacity to generate free radicals and direct oxidative stress, resulting in lipid peroxidation of membranes, DNA, and mitochondria.\(^8\) Several independent experimental studies indicate that mitochondrial dysfunction might be the key factor precipitating liver failure due to impairment of mitochondrial energy production.\(^9,10\) Recently it has been suggested that dysregulation of nuclear receptors such as the liver X receptor/retinoid X receptor (LXR/RXR) heterodimer, which critically impact lipid metabolism and the inflammatory response, is one of the major events that triggers the onset and progression of liver pathology in Wilson’s disease.\(^11\) In addition, elevated traces of copper induce a conformational change in the X-linked inhibitor of apoptosis protein (XIAP), decreasing its ability to inhibit caspase-3, thereby lowering the apoptotic threshold and sensitising the cell to apoptosis. This leads to a further reduction in the concentration of XIAP levels.\(^12\)

Typically, hepatic symptoms in affected patients develop in children or young adults. A wide range of clinical presentations may be observed with respect to hepatic symptoms, including clinically asymptomatic states with only biochemical abnormalities, steatosis, splenomegaly, and hepatomegaly.\(^13\) Symptomatic liver disease may manifest itself as fulminant hepatic failure associated with haemolysis, compensated or decompensated cirrhosis, or as acute or chronic hepatitis. Non-genetic diseases that cause repeated episodes of liver failure or chronic liver cirrhosis may cause an acquired ‘non-Wilsonian’ form of hepatocerebral degeneration. These similarities often result in patients being mistakenly diagnosed as Wilson’s disease-affected patients. However, this disorder is most likely the consequence of toxic deposition of manganese, rather than copper.\(^14\)

The neurological symptoms of Wilson’s disease usually develop later than the liver disease, and occur most often in the third decade of life. Common findings are tremor, lack of motoric co-ordination, dysarthria, dystonia, and spasticity. Many of the individuals with neurological symptoms may have concomitant liver disease, which is frequently asymptomatic. The wide range of the disease pattern is not explained by genetic mutations alone. Several small studies have attempted to correlate a particular \( \text{ATP7B} \) mutation with the phenotypic presentations, however no clear phenotype-genotype correlation in Wilson’s disease has been established.\(^15\)

Environmental, epigenetic, and genetic modulators probably play a role in the observed phenotypic variability. This assumption was recently
supported by a trial in which clinical and genetic genotype-phenotype correlations were studied in two large families living in a socio-culturally isolated community. This study revealed an equal influence of presumed other genetic modifiers and environmental factors on clinical presentation and age of onset of Wilson’s disease in patients with a particular genotype.²

Figure 2: Element measurement by laser ablation inductively coupled plasma mass spectrometry protocols in clinical and experimental Wilson’s disease.

Human liver specimen of a Wilson’s disease patient from normal and tumourigenic areas (left panels) and murine brain specimens (right panels) isolated from wild-type and ATP7B deficient mice were prepared.²²,²³ The prepared sections were subjected to LA-ICP-MS measurements and the spatial distribution of iron and copper were measured. The determined element concentrations in µg/g tissue are visualised in a colour scale using the open software ELAI that allows reconstruction of metal distribution maps using Microsoft Excel with the aid of Visual Basic for Applications user-defined functions.⁴² Note that (i) the cerebral concentration and disease-associated accumulation of copper is not uniform and preferentially affects specialised brain areas, and (ii) the selective copper increase in the liver is associated with alterations in spatial concentration and distribution of iron. More details about LA-ICP-MS measurements in experimental and clinical samples of Wilson’s disease patients are given elsewhere.²⁵,²⁶

LA-ICP-MS: laser ablation inductively coupled plasma mass spectrometry.
In most Wilson’s disease patients presenting with neurological signs, copper overload is associated with corneal deposition of copper within the Descemet’s membrane. Typically, these Kayser–Fleischer rings have a golden-brown appearance. Although this discolouration is not considered pathognomonic of Wilson’s disease, it is important in the diagnosis of this copper overload disease. Kayser–Fleischer rings are also found in cases of cryptogenic cirrhosis, without other clinical features of Wilson’s disease. This exemplifies some of the variations within clinical presentation and demonstrates that the correct clinical diagnosis may be difficult, and can be delayed. On the other hand, the characteristic clinical features associated with Wilson’s disease are only a benchmark; alone they are insufficient to make a reliable diagnosis. Moreover, the clinical consequences of Wilson’s disease are variable, from an asymptomatic state to fulminant hepatic failure, chronic liver disease with or without cirrhosis, neurological, and psychiatric manifestations.

In summary the pathophysiological reason why Wilson’s disease exhibits such a broad spectrum of manifestations is unclear. The diverse array of symptoms often makes diagnosis hard to establish, and commonly used diagnostic markers, such as elevated urinary copper excretion, can be found in non-Wilsonian conditions, for example cholestatic syndromes or acute non-Wilsonian liver failure. Low ceruloplasmin is also found in aceruloplasminaemia, an inherited disorder caused by mutations in the ceruloplasmin gene. This extremely rare disease is associated with neurological symptoms and especially with iron overload due to the role of ceruloplasmin in the absorption, storage, and excretion of iron.

**EXPERIMENTAL MODELS FOR STUDYING WILSON’S DISEASE**

The mechanisms underlying the pathophysiology of Wilson’s disease are not well understood. Established animal models include the Long Evans Cinnamon rat, the toxic milk mouse, and the ATP7B-/- mouse, all of which show hepatic copper accumulation. The inbred Long Evans Cinnamon rat model develops hereditary hepatitis and shows spontaneous hepatic copper accumulation, resulting in concentrations 40-times higher than those observed in normal Long Evans Agouti rats, while the serum ceruloplasmin and copper concentrations in these rats is significantly reduced. These alterations are induced by a deletion that removes the ATP binding domains and induces a phenotype that has clinical features similar to those observed in human Wilson’s disease patients. The toxic milk mouse carries a single nucleotide mutation that results in the substitution of an evolutionarily conserved methionine to valine in the eighth transmembrane domain.

In our laboratory, we use ATP7B null mice that were engineered by homologous recombination of a gene disruption that affects chromosome 8, which prevents the normal translation of the ATP7B protein. This genetically modified mouse strain shows an increase in copper concentration in many organs and is associated with a high propensity for the development of morphological abnormalities, which resemble cirrhosis, in animals aged >7 months. Similarly to Wilson’s disease patients, the genetic abnormalities in these mice gradually induce liver pathologies, which develop in defined stages (Figure 1B) progressing from mild necrosis and inflammation to extreme hepatocellular injury, nodular regeneration, and bile duct proliferation. Interestingly, these mice not only displayed copper accumulation but also tend to acquire an elevated hepatic iron content combined with significantly lower concentrations of serum iron, serum transferrin saturation, and blood haemoglobin levels. We have recently demonstrated by use of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) protocols that the disease-associated accumulation of hepatic copper in patients suffering from Wilson’s disease also results in an increase in intrahepatic iron concentrations (Figure 2, left panels). This study also revealed that hepatic zinc homeostasis is critically affected in these patients. The LA-ICP-MS technique is a robust method, which allows the simultaneous quantitative imaging of various metals in tissue samples with high sensitivity, spatial resolution, specificity, and quantification ability. We have previously used this innovative methodology to confirm the time-dependent increase of copper in the brain parenchyma (Figure 2, right panels) within the ATP7B-/- mice model, and to demonstrate that this method is highly appropriate for discovering and diagnosing metal imbalances in fibrotic and cirrhotic liver disorders, and for documenting the drastic time-dependent metal alterations in the livers of ATP7B-deficient mice.
Figure 3: Diagnosis, phenotypic classification, and therapy of Wilson’s disease.

A) Diagnosis of Wilson’s disease. A diagnostic score that is based on clinical symptoms (occurrence of Kayser–Fleischer rings, existence of neurological symptoms, concentration of serum ceruloplasmin, result of Coombs test) and other tests (hepatic copper content, outcome of rhodanine stain, urinary copper content, genetic mutations) was proposed in 2003 and adapted in 2010.\textsuperscript{30,31} Based on the composite of these key parameters, the diagnosis ‘Wilson’s disease’, ‘possible Wilson’s disease’, or ‘Wilson’s Disease is very unlikely’ is made. Further details of scoring and treatment guidelines were established by different international organisations.\textsuperscript{35,36} The structure of human ceruloplasmin was prepared using X-ray co-ordinates with access no. 4ENZ\textsuperscript{43} that are deposited in the RCSB Protein Data Bank\textsuperscript{44} and the software Ribbons XP (version 3.0), and the formula of rhodanine with CAS identity no. 141-84-4 was displayed with Jmol, version 14.2.15.\textsuperscript{45}

ATP7B: gene encoding the ATP7B protein; CP: ceruloplasmin; EDTA: ethylenediaminetetraacetic acid; KF: Kayser–Fleischer; ULN: upper limit of normal; WD: Wilson’s disease; Cu: copper.

B) Metal chelators in the treatment of Wilson’s disease. D-penicillamine (CAS 52-67-5), dimercaprol (CAS 59-52-9), EDTA (CAS 60-00-4), ammonium tetrathiomolybdate (CAS 15060-55-6), triethylenetetramine (tiennentri dihydrochloride, CAS 112-24-3), dimercapto succinate (CAS 2418-14-6), and dimercaptosuccinic acid (CAS 304-55-2) act as potent copper chelators. They primarily develop their therapeutic efficacy by enhancing urinary copper excretion. Zinc salts on the other side act as inducers of metallothioneins, thereby favouring reduced uptake, a negative copper balance, and a reduction of free plasmatic copper. The different images of the compounds were prepared using the open source software Jmol, version 14.2.15.\textsuperscript{45}
However, LA-ICP-MS imaging studies have previously demonstrated that copper was not increased proportionally in the periventricular regions, and that the cerebral copper overload was associated with alterations in cerebral content of other metals (e.g. manganese and zinc); the concentration of other metals (iron) showed only marginal changes. This high cerebral copper accumulation in specific brain areas, and the resulting cognitive alterations during Wilson’s disease, might be the consequence of differential regional susceptibility to copper within the brain. In contrast, the age-dependent accumulation of hepatic copper in respective mice and also in patients suffering from Wilson’s disease is strictly associated with a simultaneous increase in iron and zinc in the liver. All these findings established by LA-ICP-MS show that Wilson’s disease is more than just a simple ‘copper imbalance’ and that treatment strategies using chelators such as D-penicillamine and trientine dihydrochloride (discussed forthwith) must be carefully executed and clinically monitored. The development of an assay allowing the analysis of the regional distribution of copper and other metals in the murine brain allows analysis of currently available and novel treatments for their experimental effect on tissue metal content. This is of great interest as neurological symptoms are often especially hard to treat and do not respond to therapy as reliably as hepatic symptoms.

Recent work that was performed in the ATP7B−/− mouse model showed that an adeno-associated vector-based transfer of a functional ATP7B complementary DNA that was placed under the control of the liver-specific α1-antitrypsin promoter is sufficient to induce a dose-dependent therapeutic effect, which was visible in reduced serum transaminases and urinary copper excretion, normalisation of holoceruloplasmin, and restoration of physiological biliary copper excretion in response to copper overload. This study impressively demonstrates that the mentioned experimental models are definitely helpful in addressing special aspects of the pathogenesis of Wilson’s disease.

**DIAGNOSTICS IN WILSON’S DISEASE**

Diagnosis is currently established based on clinical findings and laboratory abnormalities. Sternlieb’s criteria makes diagnosis straightforward if two or more of the following symptoms are present: Kayser–Fleischer rings, typical neurologic symptoms, low serum ceruloplasmin levels (<20 mg/dL), and increased hepatic copper content (>250 µg/g dry weight). Diagnosis is far more complex in patients presenting with liver disease. In most patients, a combination of various parameters is required to establish a diagnosis as no single finding is adequate for diagnosis of Wilson’s disease. A diagnostic score based on all available tests was proposed by the Working Party at the 8th International Meeting on Wilson’s disease that was held in Leipzig in 2001. The Wilson’s disease scoring system provides a good level of diagnostic accuracy and the diagnostic algorithm that is applied for scoring is shown in Figure 3A.

Kayser–Fleischer rings are seen in most neurological Wilson’s disease patients and in ~70–80% of hepatic patients. Serum ceruloplasmin is typically decreased below 20 mg/dL in patients with Wilson’s disease, and total serum copper concentration below 70 µg/dL. Urinary copper excretion is commonly increased in patients with Wilson’s disease and conventionally, the level required for diagnosis is 100 µg/24 hours (or 1.6 µmol/24 hours). Liver biopsy with determination of hepatic copper content remains the gold standard for diagnosis, and though the normal hepatic copper content in healthy subjects is <40 µg/g dry weight, this typically exceeds 250 µg/g dry weight in Wilson’s disease patients. Based on a recently published prospective study, the optimal cut-off value of liver copper content indicative of Wilson’s disease can potentially be reduced to >210 µg/g dry weight. This concentration achieves the highest sensitivity and specificity results (i.e. >95%) to date.

In regards to liver histology, there is no single pathognomonic feature for the diagnosis of Wilson’s disease. While the pathology can be similar to an ethanol-induced steatohepatitis in some patients, other patients may show histological signs resembling autoimmune hepatitis. Although histological findings are not often helpful for the diagnosis of Wilson’s disease, the exclusion of other aetiologies by liver biopsy may be equally important. De novo diagnosis by molecular studies remains difficult due to the large number of disease-specific mutations and variants (>500) that are scattered across the ATP7B gene and have a different impact on the disease, hampering the interpretation of specific changes. However, depending on the population tested, a few mutations can be prevalent and can
facilitate the otherwise cumbersome diagnostic mutation analysis.

**THERAPY FOR WILSON’S DISEASE**

Copper accumulation is progressive and ultimately fatal without specific therapy; therefore, once a diagnosis of Wilson’s disease is established, lifelong treatment with an appropriate metal chelator is recommended.\(^{35}\) In addition, patients should avoid the intake of foods and water with high concentrations of copper in most cases, especially during the first year of treatment.\(^{36}\) An effective treatment offers patients an excellent long-term survival for this otherwise fatal illness.

Different metal chelating agents and zinc salts are commonly used (Figure 3B). Under treatment, liver function is improved within 6–12 months in most Wilson’s disease patients. D-penicillamine and trientine dihydrochloride are chelating agents that enhance urinary excretion of copper to remove excess. A pioneering study that enrolled 53 Wilson’s disease patients revealed that the medical therapy with D-penicillamine was sufficient to reduce the size and intensity of Kayser–Fleischer rings in 18 out of 20 patients, suggesting that the concentration of serum copper directly correlates to the phenotypic manifestation of this ophthalmic symptom.\(^{37}\)

In contrast to chelating agents, zinc blocks copper absorption in the gastrointestinal tract by inducing metallothionein synthesis in enterocytes. Metallothionein has a high affinity for copper, and the copper is discarded during normal cell turnover. The dose of elemental zinc is 150 mg per day, given in three doses.

According to the practice guidelines from the American Association for the Study of Liver Diseases (AASLD) on Wilson’s disease,\(^{36}\) the initial treatment for symptomatic patients should include a chelating agent. Although a larger body of published evidence exists for D-penicillamine, trientine dihydrochloride seems to have a favourable safety profile, especially in patients with neurological symptoms. Treatment of pre-symptomatic patients and lifelong maintenance therapy of successfully treated symptomatic patients can be accomplished with zinc salts or chelating agents in a reduced dosage. Treatment has to be monitored to ensure both efficacy of the drug and patient compliance, and to identify any adverse events.

While Wilson’s disease is a very treatable condition, in about 5–10% of all patients a liver transplantation is needed. In particular, this procedure is necessary in patients who have developed considerable liver damage by the time of diagnosis. However, liver transplant is rarely indicated in patients presenting with acute liver failure, which is often due to the presence of concurrent liver diseases. As well as current medical therapies, liver transplantation is a promising therapeutic option with satisfactory long-term outcomes in the subset of Wilson’s disease patients with acute liver failure or acute-on-chronic liver failure presenting severe complications. This is well documented in a report that summarised the long-term outcome of 121 Wilson’s disease patients following liver transplantation in France in which the patient survival rates were 87% at 5, 10, and 15 years.\(^{38}\) Similar outcomes after orthotopic liver transplantation combined with improved neuropsychiatric symptoms were also observed in Germany.\(^{39}\)

**FUTURE ASPECTS AND PERSPECTIVE IN MANAGEMENT OF WILSON’S DISEASE**

Wilson’s disease is an inherited copper overload disease related to the *ATP7B* gene. In recent years, rodent models have helped us to understand the mechanisms by which the effects of excess copper concentrations are exerted. The outcome of this disease can be significantly improved by proper treatment with chelating agents and zinc. However, it is essential that the disease is diagnosed as early as possible to prevent hepatic damage and neurological symptoms developing before the onset of any clinical signs. Nowadays, the diagnosis is based on scoring systems that use clinical symptoms (e.g. Kayser–Fleischer rings, neurological symptoms), genetic testing, measurement of serum ceruloplasmin, hepatic/urinary copper, and other diagnostic tests (e.g. Coombs test). However, there are still challenges in diagnosis.

Based on the high number of different mutations in the *ATP7B* gene, genetic testing is difficult. In addition, the diagnosis of Wilson’s disease is challenging because the test parameters overlap with those of other diseases such as cholestasis, and the development of the Kayser–Fleischer rings may also be absent in patients with hepatic Wilson’s disease. The present guidelines for treatment of Wilson’s disease aim to remove excess body copper, reduce the intake of copper-rich
foods, and to treat any damage of the liver and the central nervous system.

However, novel analytical techniques such as LA-ICP-MS that have the capacity to simultaneously measure and image various metals with high sensitivity, spatial resolution, specificity, and quantification ability in experimental and clinical samples have shown that Wilson’s disease is not simply a copper overload disease. The elevated levels of copper are associated with alterations in iron and zinc homeostasis. It will therefore be mandatory to unravel which of the clinical symptoms are associated with which metal. Another important objective in therapy will be the development of drug systems that allow chelators to pass through the blood–brain barrier and deliver chelators to more inaccessible body regions. Such devices might be more useful than the application of undirected chelators, in particular in those patients in whom neurological symptoms are worse than the hepatic disease. These delivery systems might also be favourable to overcome the initial worsening of the neurological conditions, which have often been reported after application of conventional drugs such as D-penicillamine; such therapeutics are experimentally tested and will be evaluated in clinical trials worldwide. Recent encouraging experimental studies have also shown that the administration of functional ATP7B transgenes is sufficient to cure key parameters in models of Wilson’s disease. It will be interesting to see how these findings will translate clinically and how such procedures might be suitable to engender long-term metabolic correction of human Wilson’s disease.

Acknowledgements

Ralf Weiskirchen is supported by the German Research Foundation (DFG, grants SFB/TRR57 P13 and Q3) and the Interdisciplinary Centre for Clinical Research within the Faculty of Medicine at the RWTH Aachen University (IZKF, project E7-6).

REFERENCES

20. Buiakova OI et al. Null mutation of the murine ATP7B (Wilson disease) gene results in intracellular copper