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Nature Reviews Disease Primers article: Wilson disease

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Abstract

Wilson disease (WD) is a potentially treatable, inherited disorder of copper metabolism characterised by pathological copper accumulation. WD is caused by mutations in the *ATP7B* gene, which encodes a transmembrane copper-transporting ATPase, leading to copper overload in the liver, brain and other organs. The clinical course of WD can vary in severity but progressive liver disease is a common feature. Patients can also present with neurological disorders and psychiatric symptoms. WD is diagnosed based on diagnostic algorithms incorporating clinical symptoms and signs, measures of copper metabolism and DNA analysis. Available treatments include chelators and zinc salts, which reverse copper overload by different mechanisms. Additionally, liver transplantation is indicated in selected cases. New agents, such as tetrathiomolybdate salts, are currently being investigated in clinical trials and genetic therapies are being tested in animal models. With early treatment, the prognosis of disease is good; however, an important issue is diagnosing patients before the onset of serious symptoms. Advances in

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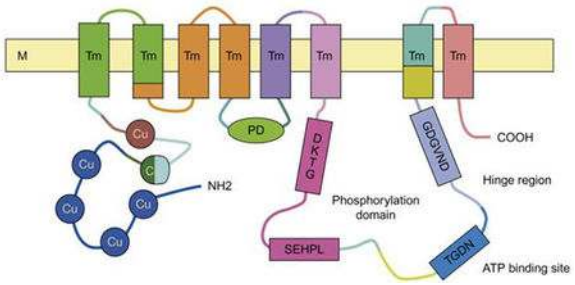
Competing interests

A.C. has served on advisory boards for Wilson Therapeutics, Vivet Therapeutics, GMPO and received speaker fees from EVER, Boehringer Ingelheim and Nutricia; P.F. has served on advisory boards for Wilson Therapeutics, Vivet Therapeutics and Univar and received speaker fees from Univar; V.M. has served as a consultant for Kadmon; K.H.W. is on speakers bureau of Abbvie, Alexion, Bayer, BMS, Chiesi, GMPO, Norgine, Novartis, Univar, Wilson Therapeutics, Vivet Therapeutics and received grants (to the institution) from Alexion, Bayer, BMS, Eisai, GMPO, Novartis, Univar and Wilson Therapeutics; M.L.S. has served on advisory boards for Wilson Therapeutics, Vivet Therapeutics, GMPO and Kadmon, is a speaker for Gilead and is on the Medical Advisory Committee of the Wilson disease association; T.L., P.D., S.L. and J.K.R. declare no competing interests.

screening for WD may therefore bring earlier diagnosis and improvements for patients with this disorder.

Graphical Abstract:



Introduction

Wilson disease (WD) is an inherited disorder of copper metabolism caused by pathological copper accumulation in many organs, particularly the liver and brain, leading to a wide range of symptoms[1] (a timeline of important discoveries is mapped out in Figure 1). The disease is caused by homozygous or compound heterozygous mutations (the presence of two different mutant alleles) in the *ATP7B* gene that encodes a transmembrane copper-transporting ATPase that mediates the excretion of copper into bile and delivers copper for the functional synthesis of ceruloplasmin (the major copper-containing protein in the blood)[2]. In WD, defective ATP7B function leads to copper overload in hepatocytes, with associated liver pathology. Excess copper is also released into the circulation with secondary pathological accumulation in other tissues, particularly the brain, which can lead to neurological symptoms and psychiatric disturbances. Symptoms vary widely and present most commonly between ages 5 and 35 years. WD is rare, with the prevalence of symptomatic disease estimated to be 1 in ~30,000; however, a greater prevalence of genetic WD (based on two alleles with pathogenic mutations) has been observed according to recent molecular studies. WD belongs to just a few genetic disorders which can be successfully managed if diagnosed early and correctly treated[1,3]; however, if left untreated, WD is universally fatal[4–6].

In this Primer, we summarise current knowledge on WD concerning epidemiology, genetics, pathogenesis, clinical manifestations and diagnosis, and discuss existing management as well as future treatment possibilities.

Epidemiology

Epidemiological studies from Germany and Japan in the 1970s indicated a WD prevalence of 29 per 1,000,000 and 33 per 1,000,000, respectively[7,8]. In 1984, the general prevalence of WD was estimated as 1 in 30,000 in non-isolated populations with a mutation carrier frequency of one allele of 1 in 90[9] (almost 1% of the population) and these epidemiological data are still widely cited today. The prevalence of WD is higher in China (58.7 per 1,000,000) and Asian countries than in western countries[10]. Epidemiological studies from isolated communities reported a higher frequency due to consanguinity (for example, Canary Islands: 1 per 2,600; Sardinia 1 per 7,000)[4,11,12]. Furthermore, in a recent study from the

United Kingdom, the calculated frequency of individuals predicted to carry two mutant pathogenic ATP7B alleles was ~1 per 7,000 individuals, with heterozygote mutations found in up to 2.5% of the general population[12]. Epidemiological data from the United Kingdom seem to be currently the most reliable according to WD genetic studies[11,12]; however, further investigations are required. Underestimation of the prevalence of WD may relate to its varying presentation leading to under- and mis-diagnosis, the low sensitivity of certain copper metabolism tests and unknown age-related clinical penetrance of *ATP7B* mutations. With the increasing knowledge of WD among physicians and increasing availability of genetic tests, the number of patients diagnosed with WD appears to be rising. However, new genetic methods including entire *ATP7B* gene sequencing are expensive and not readily available in all countries but are useful when available to define prevalence further.

Mortality data in presymptomatic WD patients compliant with treatment are comparable with the general population[3]. However, in the entire WD population (regardless of adherence, clinical symptoms, initial stage of disease or type of treatment), studies generally show that mortality rates in patients with WD (5–6.1%) are higher than healthy controls[13–15]. The presence of advanced hepatic and neurological disease as well as lack of adherence with treatment impacts on survival.

Mechanisms/pathophysiology

Mutated *ATP7B* and inactivation of the ATP7B transporter results in failure of biliary copper excretion that leads to disturbed copper homeostasis. ATP7B is also responsible for transporting copper for the synthesis of functional ceruloplasmin resulting in its decreased serum level. In WD, levels of total serum copper may be decreased due to low ceruloplasmin formation; however, levels of toxic non-ceruloplasmin-bound copper are often elevated (ref: EASL 2012). Hepatic and systemic overload of toxic copper is the major cause of tissue pathology and clinical symptoms in WD.

Genetics

Mutations in ATP7B.—WD is an autosomal recessive disease caused by mutations affecting the *ATP7B* gene, located on short arm of chromosome 13 and containing 20 introns and 21 exons. More than 700 mutations have been described according to The Human Gene Mutation Database[16,17] and patients can be homozygous for one disease-causing mutation or carry two different disease-causing mutations as compound heterozygotes. Mutations can affect almost all 21 exons and are frequently missense and nonsense. The missense mutation H1069Q in exon 14 is very common. About 50–80% of WD patients from Central, Eastern, and Northern Europe carry at least one allele with the H1069Q mutation[2]. In Southern Europe, other mutations are common, such as the missense mutation M645R in mainland Spain. The R778L in exon 8 is found more frequently in South-eastern Asia where the mutation has an allele frequency of 14 to 49%[2]. Both mutations cause decreased ATP7B protein levels associated with increased degradation of the protein.

Several studies have attempted the challenging task of correlating genotype with phenotype. In vitro experiments demonstrated that different ATP7B variants present different functional

properties with varying copper-transporter activity[18]. Studies in the 2000s suggested that homozygosity of the H1069Q mutation lead to later onset and more frequent neurological presentation than H1069Q compound heterozygotes[19,20] (ref: Gromadzka et al. Mov. Dis. 2006;21:245–248) and frameshift and nonsense mutations led to lower ceruloplasmin levels than those with missense mutations (ref: Nicastro et al. J Hepatol. 2009;50:555–61), which was not confirmed by larger studies[21]. Truncating *ATP7B* mutations have been associated with acute liver failure and earlier age of disease onset[22,23]. However, despite these and other studies in small populations (ref: Usta et al. PLoS One. 2014;9:e109727; Cocoş et al. PLoS One. 2014;9:e98520; Mukherjee et al. Parkinsonism Relat Disord. 2014;20:75–81), other data attempting genotype-to-phenotype correlations have not been conclusive. This is partly due to the poor phenotypic characterisation of patients, late diagnosis, and overlapping neurological, psychiatric, and hepatic signs and symptoms of various severities. Most likely, genetic and environmental factors interact to influence the complex phenotype; however, further studies are required to provide conclusive evidence for specific associations.

Other genes.—Studies have explored the role of proteins and mutated genes other than *ATP7B* as contributors to the WD phenotype. Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is involved in triglyceride metabolism; a PNPLA3 variant most commonly associated with non-alcoholic fatty liver disease (NAFLD) has also been associated with severity of hepatic steatosis in patients with WD[24]. PNPLA3 loss of function has been linked to accumulation of triglycerides in the hepatocytes and stellate cells[25]. In addition, the ApoE ε4 allele of the apolipoprotein E gene (ApoE), which plays a role in lipid metabolism and neurodegenerative diseases, was proposed to be a modifier of WD phenotype[26], but a large study showed that the ApoE ε4 genotype had no association with either the hepatic or neurological phenotype in WD[27]. However, ApoE ε4-positive women tended to present disease onset at a younger age compared to women with ε3/ε3 genotype, particularly when they were also homozygous for the H1069Q mutation. Mutations in the copper metabolism domain containing 1 (COMMD1) gene (formerly MURR1) is the cause of copper accumulation in Bedlington terriers, and one study identified COMMD1 variants in 30% of 63 patients[28]. However, later studies on different populations could not confirm these findings[29,30]. The copper chaperone Atox1 has also garnered much attention given its interaction with ATP7B[31], but correlational studies on patients with WD could not identify a significant role for *ATOX1* mutations[32–34].

Oxidative stress is thought to be the main cause of liver damage associated with copper accumulation. In a group of 435 patients with WD, variants of genes related to antioxidant enzymes, including catalase and manganese superoxide dismutase, were linked to age of onset of WD[35]. Methylenetetrahydrofolate reductase (*MTHFR*) gene variants were studied in a large group of Polish patients with WD. Though the study was criticised for lack of phenotypic detail, the authors identified a correlation between WD phenotype and *MTHFR* polymorphisms[36], as patients with the *MTHFR* 677T allele more frequently exhibited liver disease. Despite the limitations of the study, *MTHFR* represents an interesting gene as mutations can influence folate and methionine metabolism, which has possible downstream effects on epigenetic mechanisms of gene expression and regulation.

Epigenetics

Indirect data from humans and studies in animal models indicate epigenetic mechanisms may be involved in the pathogenesis of WD and its phenotypic presentation. Several case reports describe homozygous twins with WD presenting with different disease phenotypes[37–39]. Furthermore, the potential role of environmental and nutritional factors on epigenetic mechanisms has been explored in animal models of WD. At the interface between gene expression regulation and the environment is methionine metabolism, which has regulatory effects on methylation mechanisms. The enzyme S-adenosylhomocysteine (SAH) hydrolase (SAHH) has a crucial role as it is responsible for metabolising SAH to homocysteine. If the expression or activity of SAHH are decreased, the level of SAH, which acts as an inhibitor of methylation reactions, will increase. Importantly, SAHH activity and gene transcript levels are decreased in the presence of hepatic copper accumulation with consequent downstream changes in methionine metabolism parameters[40,41]. Notably, mouse models of WD showed dysregulation of methionine metabolism and global DNA hypomethylation in hepatocytes[42] with possible effects on the regulation of genes involved in liver damage development. In addition, during embryonic development in this model, the mouse foetal liver, which is a site of major methylation rearrangements, presented major changes in gene transcript levels related to cell cycle and replication compared to control animals. The provision of supplemental methyl-donor choline was able to improve gene expression to control levels indicating that foetal livers are susceptible to nutritional factors with potential lifelong consequences on disease phenotype and progression[43].

Copper homeostasis

Copper is essential for human physiology: copper serves as a cofactor of enzymes that are critically involved in respiration (cytochrome c oxidase), activation of neuroendocrine peptides (peptidyl-α-monooxygenase), pigmentation (tyrosinase), catecholamine synthesis and clearance (dopamine-β-monooxygenase), radical defence (superoxide dismutases [SOD], SOD1 and SOD3) and many other cellular processes. In the blood, ceruloplasmin, the major copper-containing protein, contains six copper atoms per molecule (holoceruloplasmin) but may be present as the protein without the copper (apoceruloplasmin). Normal dietary consumption and absorption of copper, contributed mainly by legumes, potato, nuts and seeds, chocolate, beef, organ meat, and shellfish[44], exceed metabolic demand and appropriate levels are controlled by regulation of the biliary excretion of copper[4,45]. These homeostatic mechanisms are perturbed by in WD.

Copper balance is normally maintained by a network of proteins, which includes transmembrane copper transporters, cytosolic copper carrier proteins, copper-storage molecules (metallothioneins) and copper-requiring enzymes. In addition, proteins that do not bind copper directly, but regulate the abundance or activity of the copper-binding/transporting proteins also contribute to cellular copper homeostasis. This regulatory network includes adaptor proteins, kinases, components of the cellular trafficking machinery, as well as DNA and RNA-binding proteins. The mechanisms regulating copper homeostasis are cell specific and cell types differ significantly in the abundance, distribution, and cellular behaviour of their major copper homeostatic molecules and their regulators. Nevertheless, the same core protein framework regulates copper homeostasis in most cells.

Copper primarily enters cells through the high-affinity copper transporter 1 (CTR1)[46–48] (Figure 2). Copper chaperones, for example, copper chaperon for superoxide dismutase (CCS) and antioxidant protein 1 (Atox1), shuttle copper to specific intracellular targets, SOD1 and ATP7A and ATP7B transporters, respectively. ATP7A and ATP7B transport copper into the trans-Golgi network for subsequent incorporation into copper-dependent enzymes and to the cellular membrane for excretion of excessive copper.

The hepatocytes of the liver are the site of two important physiological process involving copper: first, ATP7B provides copper for incorporation into apoceruloplasmin for the synthesis of functional ceruloplasmin; second, ATP7B facilitates the process of biliary copper excretion (Figure 2). Inactivation of ATP7A (the gene associated with the brain copper deficiency disease, Menkes disease) or ATP7B results in marked copper misbalance, the inactivation of specific copper-dependent enzymes, and manifests clinically as Menkes or WD, respectively. In addition, MEDNIK (mental retardation, enteropathy, deafness, neuropathy, ichthyosis, and keratodermia) syndrome is caused by mutations in the gene for an adapter protein that participates in the intracellular trafficking of ATP7A and ATP7B (Box 1).

Mitochondria use cellular copper for respiration and are also key regulators of the cellular copper balance[49]. It is unclear how copper is distributed between the cytosolic copper proteins and copper-binding proteins in mitochondria. The current model suggests that a gradient of protein–copper binding affinities and, presumably, relative protein abundance govern the partitioning of copper between cytosolic proteins[50]. Inhibitory mutations in SCO1 and SCO2 proteins, which facilitate copper incorporation into cytochrome c oxidase, result in mitochondrial dysfunction and altered cellular copper homeostasis[49]. Knowledge of the overall copper homeostatic network continues to expand, and its link to numerous cellular processes becomes more and more apparent.

Recently, new and intriguing roles for copper have emerged in normal physiological processes and the pathophysiology of disease. For example, it became apparent that copper misbalance is a contributing factor to lipid dyshomeostasis[51,52]. Abnormal lipid metabolism associated with either copper overload or deficiency is commonly observed in such disorders as WD, NAFLD and diabetes[51,53–55]. In addition, important physiological processes, as chylomicron assembly, blood vessel formation, myelination of neurons, wound healing, and immune response depend on copper homeostasis[56–59]. The role of copper in cell proliferation and angiogenesis is finding its first applications in clinical practice as the copper-protein-binding agent, tetrathiomolybdate, is being evaluated in patients with cancer[60].

Pathogenesis

The primary cause of clinical symptoms in WD are pathological tissue changes triggered by the toxic effects of excess copper[61] (Figure 3). Labile non-ceruloplasmin-bound copper (i.e. the pool of ions loosely bound to albumin and other molecules that can easily engage in chemical reactions) present in blood is continuously taken up by virtually all tissues, possibly via CTR1 and divalent metal transporter (DMT) 1. The latter apparently transports

copper ions intracellularly even when in excess (ref: Lin C et al. Metallomics. 2015;7:1285–9).

The toxicity of excess tissue copper is presumably a consequence of its redox activity that leads to oxidative stress and subsequent damage of lipids, proteins, DNA and RNA molecules. Other possible mechanisms of copper toxicity include induction of apoptosis by activation of acid sphingomyelinase, which triggers the release of the apoptotic secondary messenger ceramide[62] as well as direct inhibition of enzymatic activities through its non-specific binding to protein thiol groups[63,64]. At the subcellular level, mitochondria are the most sensitive targets for copper-induced toxicity[65].

Liver.—The liver has the highest tissue expression of the ATP7B copper transporter and is the central organ regulating systemic copper balance. Impairment of copper biliary excretion caused by ATP7B dysfunction leads to hepatic copper accumulation. Liver injury is therefore the earliest and most frequent manifestation of WD. Hepatic copper concentration in WD patients is typically increased by a factor of 5–20 compared to healthy individuals. Copper is not distributed homogeneously within the liver and its cellular localisation also varies during disease progression. In the initial stages, it is present diffusely in the cytoplasm of hepatocytes bound to metallothioneins, cysteine-rich proteins with the ability to bind, store and detoxify heavy metals. In later stages, copper accumulates in lysosomes and may become detectable by stains such as Timm's, rhodanine (Figure 4) and orcein[66]. Severe mitochondrial alterations in hepatocytes can be detected early in the disease course[67]. Mitochondrial damage can result in impaired hepatocytic energy metabolism and the downregulation of genes involved in cholesterol biosynthesis, which both contribute to hepatic steatosis (Figure 4). Chronic hepatocyte injury and cell death ultimately lead to inflammatory changes (hepatitis) and net accumulation of extracellular matrix (fibrosis) within the liver. Notably, apoptosis is an important cause of hepatocyte loss and may be triggered by cytochrome C released from damaged mitochondria or through activation of acid sphingomyelinase and release of ceramide[62,68]. Several types of liver pathology may be observed by light microscopy including glycogenated hepatocytic nuclei (i.e. optically clear intranuclear inclusions due to high glycogen content with accentuated membrane), Mallory-Denk bodies (i.e. cellular inclusions composed of misfolded cytoskeletal elements including keratin and ubiquitin-binding protein p62), portal and lobular inflammation in combination with focal or diffuse hepatocyte steatosis[69]. In general, microscopic findings in WD are not specific; at the initial stage, liver pathology may strikingly resemble NAFLD. With progressive damage, non-specific hepatic fibrosis and subsequently macronodular cirrhosis typically develop.

During disease progression, when the storage capacity of hepatocytes for copper is exhausted, ingested and absorbed copper cannot be further sequestered by the liver and the amount of labile 'non-ceruloplasmin-bound copper increases in the bloodstream[70]. Gradually, released copper from hepatocytes progressively accumulates in other organs, most notably in the brain, eyes, kidneys, bones and heart, exerting extrahepatic toxicity.

Brain.—Brain copper concentration in WD patients may reach values 10–15 times higher compared to control subjects[71,72]. The connection between copper deposits and cerebral

tissue damage was confirmed in a study of brains from 11 patients with WD, which showed a fair degree of correlation between cerebral copper content and neuropathological severity[72]. The toxic effect of copper is first buffered by astrocytes; upon taking up excess copper they increase in numbers (astrogliosis), undergo cellular swelling, and upregulate synthesis of metallothionein to increase their storage capacity for copper[73,74]. Long-term exposure to high copper concentrations ultimately results in damaged astrocytes, dysfunction of the blood-brain barrier and affliction of other brain tissues including neurons and oligodendrocytes.

Different brain regions apparently have distinct susceptibility to copper toxicity. Pathological changes — which include astrogliosis, demyelination, and tissue disintegration (ranging from mild rarefaction to full-blown necrosis) — are most often reported in the basal ganglia, thalamus, cerebellum, and upper brainstem (Figure 5); these abnormalities are depicted as T_2 hyperintense lesions on magnetic resonance imaging (MRI). Demyelination particularly affects bundles passing through basal ganglia and pontine fibres[75,76]. In the basal ganglia, inflammatory changes with accumulation of heavy iron-laden macrophages are frequently present (ref: Dusek 2016). As shown in Figure 5, T_2 hypointense lesions on MRI (Figure 5 a,b) are associated with increased iron deposits (Figure c,f,g). The putamen is the most frequently and severely affected brain region in WD, with lesions linked mainly to dystonia and parkinsonism[77]. Dysfunction of the cortico-striatal pathways may lead to psychiatric symptoms and cognitive deficits mostly affecting the executive domain[78]. The dorsal midbrain, particularly the dentate-rubro-thalamic pathway, is another frequently affected structure and its lesion may be associated with coarse action tremor[79]. Lesions in the cortex and subcortical white matter are only sparsely reported in treated patients. These lesions may be associated with epileptic seizures[80]. Beside copper toxicity, hepatic encephalopathy may contribute to neuropsychiatric symptoms in WD. This is supported by the resemblance of neuropathological abnormalities, such as the presence of abnormal astrocytes referred to as Alzheimer-type glia as well as specific MRI findings, that is bilateral pallidal T_1 hyperintensities, in WD and hepatic encephalopathy. Morphological and functional retinal abnormalities are observed in WD and are associated with the severity of brain pathology detected by MRI and with neurological impairment[81,82].

Other organs.—The pathophysiology of WD in other organs has been less investigated. The rapid release of copper caused by mass hepatocyte necrosis in WD can lead to a dramatic increase of its blood levels within days and therefore may mimic acute copper poisoning. It manifests as Coombs-negative haemolytic anaemia variably accompanied by rhabdomyolysis (the breakdown of skeletal muscle tissue) and renal tubular damage. The mechanism of haemolysis and rhabdomyolysis is not entirely understood. In erythrocytes, copper may theoretically react with membrane lipids as well as inhibit sulfhydryl groups of glucose-6-phosphate dehydrogenase and glutathione reductase; these processes can reduce cellular antioxidant capacity, which may ultimately lead to oxidative damage of haemoglobin and cell membrane[83,84]. Acute rhabdomyolysis may result from copper induced inhibition of Na+/K+-ATPase activity in muscle fibres[85]. Leukopenia and thrombocytopenia are frequent findings in patients with WD, which may be ascribed to splenic sequestration in patients with portal hypertension[86,87].

Non-ceruloplasmin-bound copper in the serum is filtrated by renal tubular epithelium and excreted via urine. Excess copper in renal parenchyma may cause renal tubular dysfunction[88]. In WD, pathological changes to bone structure, such as osteomalacia and osteoporosis, with increased incidence of spontaneous fractures, have been observed[89]. Copper accumulation in the synovial membrane and cartilage has been suggested as the major cause of osteoarthritis and accelerated degenerative changes with deformities affecting particularly larger joints[90]. In addition, myocardial copper accumulation can cause cardiomyopathy and arrhythmias. Pathological cardiac examination has shown interstitial and replacement fibrosis, intramyocardial small vessel sclerosis and focal inflammatory cell infiltration[91].

Diagnosis, screening and prevention

In concordance with the pathogenesis of WD, copper accumulates pathologically in different organs and a wide spectrum of clinical symptoms (Figure 3) is observed over the course of WD, which depend on the organs most affected[1,4,92–94].

Clinical signs and symptoms

Hepatic manifestations.—Liver disease can be the first clinical manifestation (40–60%) of WD but may accompany other symptoms[1,95]. In the absence of an established genotype-phenotype correlation[96], symptoms and disease severity may vary among patients and within families. Accordingly, the clinical presentations regarding liver involvement include a wide spectrum, ranging from asymptomatic subtle morphological changes, simple acute self-limited hepatitis-like illness to autoimmune-like hepatitis, or even recurrent jaundice (in the presence of haemolysis), cirrhosis with or without portal hypertension and even acute liver failure. Age, which affects the duration of untreated copper overload, and gender seem to have a modifying effect, as females present more often with acute liver failure than males and adult patients have a higher likelihood of liver cirrhosis than paediatric patients. The exact reason for the varied clinical course of WD is not clear; however, it seems to be multifactorial and a combination of genetic, epigenetic, hormonal and environmental factors may play a role. Early diagnosis and treatment are crucial to protect from disease progression and the development of cirrhosis or liver failure.

Typically, the first finding in children and young adults WD patients is a fatty liver of mild-to-moderate degree that is evident on liver imaging or on liver biopsy. These findings are associated with abnormal liver function and mildly elevated aminotransferases. In untreated patients, chronic liver disease with portal hypertension, hepatosplenomegaly, ascites, low serum albumin concentration and coagulopathy will develop over time. Thus, WD can present both with acute liver failure or chronic liver disease, which may clinically be indistinguishable from other hepatic conditions.

The most severe form of hepatic presentation, acute liver failure due to WD (formerly ‘fulminant WD’) occurs predominantly in young females (female: male ratio 4:1). This state is often associated with Coombs-negative haemolytic anaemia[83], severe coagulopathy, encephalopathy, and often rapidly progressive renal failure. While bilirubin is highly elevated, serum activity of aminotransferases is only moderately increased, and serum

concentration of alkaline phosphatase is normal or extremely low. The New Wilson Index (modified Nazar Score)(ref: Dhawan 2005) can offer guidance when evaluating the need for urgent liver transplantation.

Like many other hepatic diseases in which there may be cirrhosis, Model for End-Stage Liver Disease[97] and Child-Pugh scores[98] are commonly used to reflect the severity of the liver disease. Presently, abdominal ultrasound (US), CT and MRI are commonly performed at diagnosis and at follow up, if needed. Most common findings are fatty infiltration, contour irregularity and right lobe atrophy. The role and accuracy of non-invasive measurements of liver stiffness (for example, transient elastography) and biochemical fibrosis scores in patients with WD remains unclear as only pilot studies in small cohorts are available[99]. For patients with evidence for chronic liver disease, especially in cirrhotic patients, screening examinations for signs of portal hypertension (oesophagus varices, splenomegaly) and hepatobiliary malignancies[100] should be performed[101] and repeated depending on clinical status, although hepatobiliary malignancies are rare.

Patients with WD and symptomatic hepatic decompensation may present with ascites, jaundice, gastrointestinal bleeding or hepatic encephalopathy. Spontaneous bacterial peritonitis can be diagnosed by paracentesis and should be treated with antibiotics. Patients with liver cirrhosis are susceptible to bacterial infections of any cause and sepsis is one of the leading causes of death in these patients. In advanced stages of cirrhosis, hepatic encephalopathy and renal function impairment (hepatorenal syndrome) may occur and show high mortality.

Acute presentation with rapid deterioration may also occur in patients with WD who were previously treated but stopped their medications. Failure to comply with lifelong therapy can lead to significant progression of liver disease and liver failure, the latter requiring liver transplantation for survival (ref: Roberts 2008; EASL 2012). Studies suggest that up to 45% of patients treated with current therapies have poor or problematic long-term adherence (ref: Maselbas et al. Neurol Neurochir Pol 2010;44:260–263; Dziezyc et al. Eur J Neurol 2014;21:332–37) and adherence should be carefully monitored in WD patients with all forms of presentation (ref: Roberts 2008; EASL 2012).

Neurological manifestations.—After hepatic manifestations, neurological symptoms are the most frequent clinical symptoms of WD. Initial neurological presentation occurs in 18–68% of patients (depending on the referral centre), with mean age at symptom onset of 20–30 years[102]. However, the youngest reported WD patient with neurological presentation was 6 years old, and the oldest, 72 years[4]. The main clinical spectrum of neurological symptoms includes different movement disorders with a wide spectrum of involuntary movements, which often overlap[1,4]. However, summarising the most common neurological features of WD, we can distinguish clinical forms where there is a predominance of tremor, dystonia, parkinsonism, all of which are often associated with dysarthria, gait and posture disturbances, drooling and dysphagia[103,104] These disturbances may severely affect the activities of daily living[77,103,104].

A characteristic and frequent neurological symptom of WD is tremor, which is experienced by up to 55% neurological WD patients at diagnosis. It can be resting, postural or kinetic and may start initially as unilateral or bilateral tremor with mainly distal upper extremities involvement. However, over time, legs, head or even the whole body may be affected, usually in a bilateral manner. Specific involuntary movements mimicking tremor, called asterixis or flapping tremor, is a negative myoclonus affecting the hands, which can be observed in WD patients with liver failure as a symptom of hepatic encephalopathy[103,104].

Dystonia is reported as the first WD symptom in 11–65% of patients. It can be focal (involves one body part, e.g. one hand), segmental (involves one segment, e.g. upper extremity), multisegmental (involves multiple segments, e.g. face and leg) or even generalised (Figure 6). The most characteristic WD dystonic presentation is abnormal facial expression or risus sardonicus, which presents as a fixed smile due to dystonia of the risorius muscle. During untreated disease, symptoms usually progress to generalised dystonia, contractures and terminal dystonic state.

Parkinsonism occurs in 19–62% of WD patients and usually presents as symmetric bradykinesia, rigidity, hypomimia, gait and posture disturbances as well as dysarthria, dysphagia and drooling[77,103,104]. Ataxia as a symptom of cerebellar dysfunction (ataxic gait and posture, impaired coordination, intentional tremor, dysarthria) occurs in 30% of WD patients, usually not as a solitary symptom but in combination with other movement disorders[77,104]. Chorea, characterised by rapid, irregular involuntary movements of face, head, trunk or extremities occurs rarely in patients with WD (6–16%). In addition, gait and posture disturbances are reported in 44–75% of WD patients with neurological presentation and 57% have impaired hand writing, often as an early sign of the disease (ref: Czlonkowska A et al. BMC Neurol. 2018;18:34)

Dysarthria appears to be the most frequent neurological symptom, being reported in up to 97% of WD neurological patients. In some cases, dysarthria may be so severe and persistent that verbal communication is impossible for the rest of their lives. There is no specific WD dysarthria, but manifestations can be divided based on the predominance of clinical symptoms: 1) cerebellar; 2) extrapyramidal (dystonic, parkinsonian) and 3) mixed (unclassified due to symptoms overlapping)[77,103–105].

Dysphagia, defined as difficulties in any phase of swallowing (oral preparation, oral transit, swallowing, drooling), is reported in up to 50% of neurological WD patients[77,103,104,106–108]. Dysphagia varies from mild to severe and may lead to severe general health complications, including bronchoaspiration, pneumonia, malnutrition and weight loss. Dysphagia should be routinely checked in all patients with WD, especially in neurological WD patients[106]. Drooling is a classic neurological symptom of WD, which is observed in almost 68% of neurological WD patients[104], occurring as a result of dysphagia or orofacial dystonia.

In addition to the classical movement disorders of WD, it is notable that other neurological syndromes like epilepsy may occur[104,109–112]. Current epidemiological studies suggest that epilepsy occurs in 6.2–8.3% of WD patients, which is more than 10-fold greater frequency

than in healthy populations[80,109]. Seizures are usually generalised, less frequently partial and can occur at every stage of disease. It has been suggested that additional risk factors of seizures include lesions of white matter tracts in the cortex and may be due to WD overtreatment and treatment-induced copper deficiency; however, this hypothesis requires further confirmation[113,114]. Other neurological symptoms have been described in patients with WD, including olfactory dysfunction (suggested marker of neurodegeneration), neuropathy (due to liver failure, or treatment-induced copper deficiency), restless leg syndrome (RLS), rapid eye movement (REM) sleep behaviour disorder and other sleep abnormalities, tics, myoclonus, headache, pyramidal signs, oculomotor impairment, and taste dysfunctions; however, there is lack of studies describing their frequency as well as significance[1,4,94,104,114–118]. Due to wide heterogeneity as well as combined neurological symptoms occurring in the course of WD, the clinical scales like the Unified Wilson's Disease Rating Scale (UWDRS) or Global Assessment Scale for WD assessing neurological deficits and functional impairment were established[4,105,114,119].

Currently, brain MRI is the most important neuroradiological examination for diagnosis and may be helpful for treatment monitoring (discussed below) [4,119]. Usual abnormalities in WD brain MRI include symmetric hyperintense changes visualised in T2-weighted images located in basal ganglia (mainly putamen and caudate nuclei), thalami, midbrain, and pons[94,114,120–123] (Figure 7). In more advanced cases, these abnormalities can be visualised in T1-weighted images as hypointense changes reflecting severe tissue damage (Figure 7a & b). The most spectacular WD changes are described as the 'face of the giant panda' in the midbrain (up to 20% cases of neurological WD) (Figure 7c), while 'miniature panda' in the pons occurs less frequently[114] and injuries of neuronal tracts can also be seen (Figure 7d). There is diffuse brain atrophy that is accentuated particularly in the subcortical region and upper brain stem. Atrophy can be present even in patients diagnosed with mild neurological symptoms but is more evident in severe cases (Figure 7e). Particularly in WD cases with cirrhosis and portosystemic shunting, increased signal in T1-weighted images may be observed in the globus pallidus as well as substantia nigra, probably due to manganese accumulation[124] (Figure 7f&g). Characteristic brain MRI changes occur in almost 100% of drug-naive neurological WD patients, 40–75% of hepatic cases and 20–30% of presymptomatic patients. There are no clear correlations between lesion localisation and symptoms; however, changes located in the thalamus and pons are suggested to be unfavourable[125]. Very rarely, diffuse white matter changes are observed with preservation of the cortex, probably due to myelin destruction (Figure 7h). During correct WD treatment, at least partial recovery of neuroimaging pathology is usually observed, especially in early diagnosed patients[114]. As the presence of MRI brain changes is observed in hepatic or presymptomatic cases, MRI seems to be justified in all patients before initiation of therapy. Transcranial ultrasonography hyperechogenicity is observed in lenticular nucleus and corresponds with changes in MRI, but this method requires specialist skills[126].

New neuroimaging techniques such as MR quantitative susceptibility mapping, diffusion tractography, and positron emission tomography may, in future, broaden our knowledge regarding in vivo brain pathology[76,120,127,128].

Ophthalmological manifestations.—Ophthalmological manifestations of WD include the Kayser-Fleischer ring (Figure 8) and sunflower cataract[114, 129], which are caused by pathological copper accumulation in the eyes. The Kayser-Fleischer ring is caused by copper accumulation in the Descement membrane and appears as golden, brown or green colouration at the periphery of the cornea. Kayser-Fleischer rings occur in almost 100% of neurological WD patients, 40–50% of hepatic and 20–30% of presymptomatic WD patients and are an important diagnostic feature[4], although a slit-lamp examination by an experienced observer is required for correct identification. False positive changes, similar to Kayser-Fleischer rings, are observed in disorders such as primary biliary cirrhosis, cholestasis and neoplastic disorders with high serum copper level for example multiple myeloma; as well as during oestrogen intake[94]. Recently, it was shown that anterior segment optical coherent tomography (OCT), a non-contact ophthalmological device that provides images and quantitative analysis of ocular tissue (including cornea), can be an alternative method for detecting copper depositions in cornea (Figure 8)[130]. Sunflower cataract appears as a central disc with radiating petal-like fronds located under the lens capsule and occurs rarely (2–20%)[81]. The most recent ophthalmologic studies based on OCT and electroretinography suggest that the retina and optic nerve may also be affected in WD[81,114].

Psychiatric manifestations.—Psychiatric symptoms occur frequently in the clinical presentation of WD[4], mostly secondary to the somatic and brain pathology of the disease. Co-morbidity of psychiatric illness (for example major depressive disorder, bipolar disorder) and WD may also be considered, especially in cases of strong family history of a given psychiatric illness.

The most comprehensive review of psychiatric aspects of WD was performed in 2014[131]. The authors concluded that psychiatric symptoms can occur before, concurrent with or after the diagnosis and treatment of WD. In their review, 20% of patients had seen a psychiatrist prior to their WD diagnosis, and 30–40% had psychiatric manifestations at the time of diagnosis. Epidemiological data suggest that up to 30% of WD patients initially manifest with psychiatric symptoms. The first psychiatric manifestation of WD may even occur in childhood as a decline in school performance, inappropriate behaviour or impulsiveness[4]. Some clinical observations also suggest that acute psychiatric symptoms may manifest themselves after initiation of anti-copper agents or sometimes in the first months of treatment, paradoxically even when neurological status of the patient is improving[132,133].

Mood disturbances are the most common psychiatric manifestation of WD. Between 20–60% of WD patients develop depression, with a high rate of suicidal attempts ranging between 4–16% of WD patients[14,131,134,135]. High frequency of depressive syndromes in WD may be facilitated by a reaction of the patient to the chronic disease as well as to physical incapacity due to neurological deficits. Bipolar disorder has been reported in WD more frequently than in the healthy age- and gender- matched population, amounting to 14–18%[136]. However, studies on the prevalence and incidence of bipolar disorder among WD patients rarely consider a differentiation between bipolar mania/hypomania and symptoms due to brain damage. Emotional lability, irritability and aggression, shallow cheerfulness, euphoria, social disinhibition, hypersexuality, lack of criticism and deficits in planning and anticipating social consequences can be due to the lesions of frontal lobe or its pathways[136].

A few epidemiological studies show that psychosis occurs not more frequently in WD than in general population, however, more often in patients with neurological symptoms[131]. It seems that no specific clinical manifestations of psychosis occur, and patients with WD with psychiatric symptoms have usually an initial diagnosis of schizophrenia, schizoaffective and delusional disorder. Psychotic symptoms occurring as the first manifestation of WD may present both diagnostic and therapeutic challenges[137]. As approximately 3% of first-episode psychosis may be due to 'organic' causes, some diagnostic guidelines suggest screening for WD in first episode psychotic patients.

Behavioural and personality disorders also belong to frequent psychiatric disturbances of WD, the most common manifestations being irritability, aggression, and antisocial behaviour[138]. A few other psychiatric conditions such as catatonia, anorexia nervosa, bulimia, obsessive-compulsive disorder and attention-deficit hyperactivity disorder have also been reported in WD[139,140]. In most described cases, psychiatric manifestations lead to a delay in WD diagnosis.

Diagnosis

As described above, clinical presentation varies widely in patients with WD and in most cases, a combination of clinical features and laboratory parameters (Table 1) are required to establish the diagnosis.

Serum ceruloplasmin is typically decreased in patients with neurologic WD but may be in the low normal range in about half of patients with active liver disease[141]. The predictive value of ceruloplasmin for diagnosis of WD in patients with liver disease is poor[142,143]. When WD is suspected, levels of serum ceruloplasmin can be measured enzymatically or by nephelometric immunoassays. Immunological assays may overestimate ceruloplasmin concentrations since they do not discriminate between apoceruloplasmin and holoceruloplasmin[144].

Total serum copper is usually decreased in proportion to reduced ceruloplasmin in the circulation. Normal or elevated serum copper levels in the face of decreased levels of ceruloplasmin indicate an increase in the concentration of copper which is not bound to ceruloplasmin in the blood (non-ceruloplasmin-bound copper), which is suggestive of WD. Non-ceruloplasmin-bound copper concentration can be estimated by subtracting ceruloplasmin-bound copper from the total serum copper concentration[145]. However, the calculation of non-ceruloplasmin-bound copper concentration depends on the correct determination of functional ceruloplasmin, which is more precisely addressed when using enzymatic assays. Exchangeable copper is an experimental technique to determine bioavailable copper in the plasma compartment[146] but does not reliably measure non-ceruloplasmin-bound copper concentration.

24-hour urinary copper excretion is helpful to diagnose and to monitor treatment in WD. The conventional level taken as diagnostic of WD is >100 μg/24 h (>1.6 μmol/24 h) in symptomatic patients (ref: Roberts 2008). However, basal 24-hour urinary copper excretion may be <100 μg at presentation in 16–23% of patients diagnosed with WD, especially in children and asymptomatic siblings (ref: EASL 2012; Roberts 2008). Interpreting 24-h

urinary copper excretion can be difficult due to overlap with findings with other types of liver disease, in particular during acute hepatic injury or failure of any origin and the reference limits for normal 24-h excretion of copper vary among clinical laboratories. Many laboratories take 40 μg/24 h (0.6 μmol/24 h) as the upper limit of normal, which appears to be a better threshold for diagnosis (ref: Roberts 2008).

A >5-fold increase of urinary copper excretion after an oral d-penicillamine challenge has been used for diagnostic purposes in children[147]. Although this test is used in some centres, it's value is not confirmed[6,148] and may not be required if the lower threshold for urinary copper excretion of 40 μg/24 h is applied. The radioactive copper test which shows incorporation of copper into ceruloplasmin can be used when standard copper metabolism tests and genetic tests are inconclusive (for example, to distinguish heterozygotes from homozygotes)[149]; however, this test is only available in highly specialised centres.

A liver biopsy with measurement of hepatic parenchymal copper concentration is required if the clinical signs and noninvasive tests do not allow a final diagnosis or if there is suspicion of other or additional liver pathology[4]. Hepatic copper content >250 μg (4 μmol)/g dry weight is considered as the best biochemical evidence for WD. In a large prospective study, 209 μg (3.3 μmol)/g dry weight provided the highest diagnostic accuracy for WD[150] (sensitivity: 99.4%; specificity: 96.1%). However, the concentration can be underestimated due to sampling errors[151,152]. Of note, hepatic copper content may also be increased in cholestatic disorders[151].

Mutational analysis is a further important diagnostic tool. However, the results of direct molecular-genetic diagnosis may take time to obtain; analysis is difficult because there are more than 700 possible mutations[153,154](ref: https://portal.biobase-international.com/hgmd/pro/all.php) and many patients are compound heterozygotes. However, sequencing is becoming continually faster and cheaper and will become a commonly used diagnostic test in the future.

Since none of the available laboratory tests are perfect and specific for WD and typical clinical symptoms may be absent, a diagnostic score based on all available tests was proposed by the Working Party at the 8th International Meeting on Wilson's disease, Leipzig 2001[155] (Table 2), was found to have good diagnostic accuracy and was adopted in the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines for Wilson disease[4].

Prevention

WD is one of the few metabolic disorders that can be successfully pharmacologically treated if diagnosis is early established — by family screening of first-degree relatives and the differential diagnosis of other liver and movement disorders — and the disease is correctly treated. It should be emphasised that the one of the most significant factors that determines poor treatment outcome is delayed diagnosis[4,5,155,156]. The greatest risk of the disease is among siblings of the index case where obligatory fast diagnosis is needed despite lack of any symptoms and age[157]. Based on a large cohort of 760 Polish patients, offspring are at about 4% risk of WD and there is increased risk of the disease among cousins; WD has even

been diagnosed in asymptomatic parents (ref: Dzieżyc et al. Mov Dis 2014;29:1828–32). When presymptomatic are treated, they have similar rates of survival as the general population, providing good adherence to treatment[3]. Genetic screening is not always available or conclusive and copper metabolism norms are not well established in small children, so diagnosis must be supported by careful clinical observation. In small infants, it is not clear when anti-copper therapy should be started to avoid the consequences of copper deficiency. Knowledge about WD at all stages among patients and their families is very important and there is a valuable place for patient support organisations[158].

Management

General remarks

If the diagnosis is established, treatment for WD should always be lifelong[4,5,155]. Current management options include pharmacological therapies and liver transplantation[114,159].

Pharmacological treatment.—The pharmacological treatment of WD is based on drugs that create negative copper body balance and include the chelators (d-penicillamine and trientine) that increase urinary copper excretion and zinc salts that decrease copper absorption from the digestive tract[13–15,160,161]. With correct and early pharmacological treatment (before advanced liver or brain injury), liver function and transaminases improve in >90% of patients, usually in 2–6 months, while neurological improvement is observed in 50–60% of patients over a longer time course of 1–3 years[4]. For long-term success of treatment, adherence plays the most important role both in symptomatic and presymptomatic cases[3,162].

At least twice a year, monitoring should be performed to determine adequacy of treatment with regards to clinical improvement and biochemical changes (e.g., urinary copper excretion and non-ceruloplasmin-bound copper levels), to assess adherence as well as to detect any treatment-induced adverse events (ref: Roberts 2008). For patients on chelation therapy, elevated values for urine copper may suggest nonadherence to treatment and hepatic deterioration may follow. Low values for urine copper excretion for patients on chelation treatment can also indicate overtreatment, and this finding is accompanied by very low values for estimates of non–ceruloplasmin bound copper. Neutropenia, anaemia and hyperferritinaemia may also indicate copper deficiency. Data about WD drug interactions, treatment monitoring, effectiveness and adherence assessment as well as adverse events are presented in Table 3. It should be emphasised that there are very limited data from prospective head-to-head clinical trials comparing the safety and efficacy of different treatment pharmacological options in WD[4,5,114]. There are conflicting data from retrospective studies according to the superiority or equality of different drugs, what is mainly based on centre- or country-specific experience with different drugs[4,5,13–15,114,135,160,161,163,164]

A particularly important and well-discussed adverse event that can occur with each type of treatment (including liver transplantation) is paradoxical neurological deterioration, with rapid appearance of new neurological signs or worsening of existing neurological signs[125]. Paradoxical neurological deterioration has prompted the discussion whether d-penicillamine

should be used in neurological WD patients and led to the search for safer treatments[92,165]. The mechanism of paradoxical neurological deterioration is still not proven but may be caused by rapid mobilisation of copper from different tissues, upon chelator initiation, with extreme secondary increase of non-ceruloplasmin-bound copper into the blood, which then causes increased oxidative stress and cell damage[4,114,166]. Based on this theory, chelators are now introduced slowly with dose titration and the occurrence of paradoxical neurological deterioration with different treatments seems similar (about 10%)[125,161,163]; other suggested risk factors for paradoxical neurological deterioration include additional drugs (e.g. neuroleptics and antidepressants) and advanced neurological and brain MRI changes[13,125]. Additionally, it is sometimes impossible to distinguish treatment-induced worsening from the natural course of the disease, especially in late-diagnosed cases (ref: 13 Czlonkowska 2005, ref 125 Litwin 2015).

Current guidelines of EASL[4] and the American Association for the Study of Liver Diseases (AASLD)[5] recommend the use of chelators as the initial treatment of symptomatic WD patients, with the suggestion that trientine is better tolerated. Zinc is generally reserved for maintenance treatment, although it is also used as first-line therapy, most commonly for asymptomatic or presymptomatic patients (ref: EASL 2012; Roberts 2008). First-line zinc monotherapy appears to be effective and well tolerated in neurological WD patients; however, caution is needed in patients with hepatic WD due to the risk of hepatic deterioration that has been occasionally reported (ref: EASL 2012).The use of zinc monotherapy in patients with mild liver disease diagnosed in childhood is supported by retrospective data (ref: Ranucci et al. Orphanet J Rare Dis. 2014;9:41).

Liver transplantation.—A surgical and more complex option for the treatment of WD treatment is liver transplantation[159,167,168]. As WD is primarily a liver disease characterised by copper defects in hepatocytes, liver transplantation can be considered a phenotypical correction of the gene defect and can restore copper homeostasis. According to EASL and AASLD recommendations[4,5] liver transplantation in WD patients is indicated in cases with acute liver failure or decompensated liver cirrhosis with lack of pharmacological treatment effect. A transplant indication in the setting of acute liver failure should be based on a specific WD clinical scale, commonly, the revised King's prognostic Wilson Index (Table 4)[168]. Whether uncontrolled neurological disease constitutes a treatment indication, as a 'last chance' treatment option, is highly controversial[159,167,168]. Paradoxical neurological deterioration may be observed in liver transplantation patients; however, the mechanism is unclear. Acute liver failure is often connected with a rapid increase of copper in blood, causing acute haemolysis and copper penetration to the brain. Anaesthetics may also induce neurological deterioration and other complications may cause deterioration.

Symptomatic therapies.—Dietary copper restriction may be helpful in controlling copper excess in some patients. Patients with WD should generally avoid foods with very high concentrations of copper (shellfish, nuts, chocolate, mushrooms, and organ meats) at least in the first year of treatment[5,45] As well as specific anti-copper treatments, symptomatic therapies are important, particularly to address complications of liver damage (or failure)[169] as well as neurological symptoms[170]. In the case of documented liver disease

(steatosis, liver fibrosis or cirrhosis), symptomatic management may include avoidance of further liver damage due to potentially hepatotoxic substances intake (for example, alcohol, herbal essences, drugs) and screening and/or re-evaluations for oesophageal or gastric varices[169] and hepatocellular carcinoma. In the case of decompensated liver cirrhosis, symptomatic management also includes treating the complications of portal hypertension treatment, namely, gastroesophageal varices prophylaxis and treatment, ascites, hepatic encephalopathy and spontaneous bacterial peritonitis and hepatorenal syndrome. Symptomatic liver therapy should always be introduced in accordance with good hepatology practice without any delay while waiting for a response to anti-copper treatment effects[169].

Symptomatic treatment should also be considered in WD patients with persistent or deteriorating neurological symptoms during WD treatment[170]. Symptomatic therapy of neurological impairments depends mainly on predominant signs, such as dystonia, parkinsonism, tremor and is based on experiences from treatment in other neurodegenerative diseases. No symptomatic treatments have been tested in clinical studies. It is difficult to distinguish a positive effect of symptomatic treatments from an improvement due to anti-copper therapy or a lack of effect from the natural course of the disease changes[13]. In severe neurological cases, there is often either a weak or no effect with symptomatic treatments. Tube feeding may be important in cases of severe dysphagia and percutaneous gastrostomy should be discussed to avoid bronchopneumonia and malnutrition. Drooling may be successfully treated with botulinum toxin injections in salivary glands[170].

To summarise management: 1) WD may be successfully treated with pharmacological agents if diagnosed early, and life-long adherence is maintained; 2) the drug choice should be discussed with patients based on possible adverse events, availability and costs; 3) treated WD patients should undergo continued monitoring to assess adherence with treatment, including copper status, liver function, neurological and psychiatric status, and any effects of the disease on other organs; 4) symptomatic treatment of liver, neurological or psychiatric manifestations should be applied if needed and reassessed over time.

Pregnancy

Amenorrhoea and frequent abortions may precede the onset of other symptoms of WD and, in symptomatic cases, are more frequent than in the general population[171,172] (Add ref: Klee 1979). Pregnancy in WD patients with compensated liver disease is safe and most patients have successful pregnancies. Management of anti-copper therapy during pregnancies should focus on prevention of spontaneous abortions, maternal disease control, and minimisation of putative drug-induced teratogenicity. Pre-conception counselling might address both the risks of the medication for the pregnancy, but also the risks of uncontrolled disease, as women may choose to not take their medication during the pregnancy[171,173,174].

In the case of successful conception, a biochemical and clinical baseline assessment is mandatory, including the appraisal of portal hypertension in cirrhotic patients to plan the delivery type due to the increased risk of peripartal variceal haemorrhage[175]. As prevention of symptomatic deterioration of the mother is the primary concern, there is no rationale for discontinuation of any anti-copper therapy during pregnancy, which may lead to severe liver

or neurological exacerbation[171]; however, dose reduction may prevent over-decoppering, with adjustment to account for increased foetal copper demand[176].

For patients on a chelator (d-penicillamine or trientine), a reduced daily dose might be appropriate during the first and second trimester. At the beginning of the third trimester, on an individual per case basis, which takes into account the course of liver function test during pregnancy, a further daily dose reduction can be considered[173]. After delivery, up-dosing of the chelating agents to the level before pregnancy is reasonable. There is no evidence that switching the medical therapy to zinc prior to conception decreases the risk of miscarriage or occurrence of birth defects[173]. From clinical experience, in patients treated with zinc a daily dose of elementary zinc should be maintained during pregnancy in almost all cases[173]. As all available anti-copper drugs pass into milk and may cause infantile copper deficiency, breastfeeding is not generally recommended[5,176].

Management of psychiatric symptoms

Management of psychiatric disturbances should consider the limitations of pharmacotherapy in liver impairment and potential effect of drugs on worsening neurological signs[177]. Selective serotonin reuptake inhibitors (SSRI) may be used first-line treatment for depression. Also, serotonin-norepinephrine reuptake inhibitors (SNRI), serotonin antagonist and reuptake inhibitors (SARI) as well as electroconvulsive therapy (ECT) have been used with good effect[178,179]. Antidepressants with high risk of liver injury, such as iproniazid, phenelzine, imipramine, amitriptyline, duloxetine, bupropion, or agomelatine should be avoided[4,180]. For treatment of mania/hypomania, mood stabilisers are used, such as lithium and antiepileptic drugs (e.g. carbamazepine and lamotrigine, but avoid valproate due to hepatotoxicity)[136,181].

Antipsychotic drugs are applied in severe mania and for the treatment of psychotic symptoms. In WD, they pose a risk of neurological deterioration and hepatic injury. Therefore, antipsychotics with low risk for extrapyramidal symptoms, such as clozapine or quetiapine[182], should be used. Clozapine, due to increased risk of leukopenia and dose-related seizures, should be reserved for the most severe and treatment-resistant cases, with regular blood analysis[4]. Olanzapine is an effective anti-manic drug with low extrapyramidal risk[136], while olanzapine and quetiapine have also a moderate risk in patients with liver injury[180]. Aripirazole has a good safety profile and sulpiride and amisulpride are interesting options because they are not metabolised in the liver and have low risk of extrapyramidal symptoms, especially at lower doses. Antipsychotic drugs, even those considered as having low effect on the extrapyramidal system, should be used only in severe cases, at the lowest effective dose and for the shortest possible duration[104,119,177].

Psychiatric treatment of obsessive-compulsive disorder should include SSRIs with behavioural therapy, and, for catatonia, lorazepam followed by ECT should be considered[178]. For behavioural symptoms and personality disorders, SSRIs, antiepileptics and benzodiazepines can be used[177].

Quality of life

Quality of life (QoL) is one of the most important objective patient-reported outcomes of the treatment of chronic disorders; however, QoL has not been clearly investigated in WD. Only 4 studies (1 with only liver transplanted WD patients) have been performed, which aimed to verify the Health Survey Questionnaire (SF-36) and the Brief Questionnaire (WHO-BREF for QoL) in WD patients[183,184,185,186]. The main conclusion from these studies is poor QoL in patients with a long delay without WD treatment[183], highlighting the significance of early WD diagnosis. Due to the wide spectrum of symptoms, non-homogenous presentation, limited numbers of studies (none with drug naïve patients; most without healthy control groups), it is difficult to recommend any QoL scale (or their subscales) to use routinely in WD. Additionally, it should be noted that large cohorts of WD patients presenting with psychiatric problems, including cognitive, behavioural or criticism disturbances, would decrease the reliability of assessment (authors unpublished data). Further studies are needed, based on larger groups of patients, as well as including the objective correction of self-reported scales, to include QoL scales in the routine assessment of WD patients.

Outlook

Improved diagnostic techniques and more systematic approaches to disease diagnosis once WD has been considered have created an easier path to confirmation or exclusion of a diagnosis of WD. Currently used zinc salts and chelators are mostly effective but have some limitations[19,187]. We need better information about dosing, monitoring and studies of the comparative effectiveness of available medical therapies and standardisation of definitions for treatment success and failure. A recent clinical trial with tetrathiomolybdate that leads to the formation of an inert complex of tetrathiomolybdate with copper and albumin in the circulation and increases biliary copper excretion seems very promising, especially with respect to low potential for neurological worsening with initiation of therapy[188]. Due to the lifelong nature of WD treatment, new therapies must be accessible and affordable if they are to be clinically useful, particularly when healthcare resources are stretched and as regulatory authorities consider WD a rare disease.

New advances in molecular genetics may soon enable translation of successful studies of gene therapy using viral vectors with *ATP7B* constructs in animal models of WD to patients with WD, potentially to provide a ‘cure’ for this disorder[189]. Future applications of somatic gene modification may permit correction of some *ATP7B* mutations by restoring functional copper transport in liver cells. Hepatocyte transplantation may offer another modality for treatment, but as of now requires immunosuppression to prevent rejection of the transplanted cells and would not necessarily correct complications of portal hypertension as accomplished by orthotopic LT. Future manipulation of stem cells may allow their transformation to functional liver cells and eliminate the need for subsequent immunosuppression to maintain these cells. Other treatments may focus on the ability to restore some function to some mutant ATP7B proteins by changing their conformation or intracellular localisation[190]. These novel therapies may offer the opportunity to lower or even discontinue current standard of care treatments for WD.

Despite current advances, there are still unmet needs for patients with WD and other unexplained issues regarding the wide phenotypic expression. The development of neonatal screening techniques that can detect most WD patients prior to symptom development is an important priority[191]. To increase understanding of phenotypic variation, the complex interrelationships between environmental and host factors needs to be further explored[192]. Some studies have showed that genetic and epigenetic factors influence whether neurological disease may progress differently in some individuals or whether their livers develop steatosis or handle copper differently, all independent of *ATP7B* mutations. Other studies in animal models have shown changes in gene expression by alteration in DNA modification induced by nutritional changes[193]. A deeper understanding of these factors along with more careful phenotypic characterisation of patients will help us to explore whether we can individually predict the natural history of the disease and response to therapy.

Overall, the outlook for patients with WD is very bright, for never in its prior history have there been as many opportunities for early disease diagnosis, successful medical therapies and rescue therapies, such as liver transplantation. New opportunities for diagnosis and treatment of WD will augment those in use now, and even better outcomes may be achievable.

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Box 1.

Other genetic disorders of copper metabolism.

The X-linked recessive disorder, Menkes disease, is characterised by impaired copper absorption due to mutations in *ATP7A*[1]. Menkes disease typically presents in boys aged 2–3 months, with the neurodevelopmental delay and degeneration, seizures and failure to thrive. Without early treatment with parenteral copper replacement, death usually occurs several years after onset. Occipital horn syndrome is a milder allelic variant of Menkes disease, with a less severe neurological phenotype (slight generalised muscle weakness, dysautonomia, including syncope, orthostatic hypotension, and chronic diarrhoea) that is often not diagnosed until mid-childhood or later. Another *ATP7A* allelic variant, distal motor neuropathy without overt copper metabolic abnormalities, presents with progressive distal motor neuropathy (curled fingers, foot deformities, and diminished deep tendon reflexes), with minimum or no sensory symptoms.

Huppke-Brendel syndrome results from mutations in *SLC33A1*, which encodes the acetylCoA transporter, AT-1. Low serum copper and ceruloplasmin concentrations are observed along with congenital cataracts, hearing loss, and severe developmental delay. Similar to Menkes disease, pronounced cerebellar hypoplasia and hypomyelination are also seen.

The newly recognised copper metabolism disorder, MEDNIK syndrome, is an autosomal recessive trait due to mutations in the *AP1S1* gene, which encodes the σ 1A small subunit of the adaptor protein complex-1 (AP1) involved in intracellular trafficking of transmembrane proteins. Abnormalities in copper ATPase trafficking result in hypocupraemia, hypoceruloplasminaemia and hepatic copper accumulation, similar to that seen in WD. MEDNIK is an acronym for the syndromic constellation of symptoms observed: including mental retardation, enteropathy, deafness, neuropathy, ichthyosis, and keratodermia.

Ceruloplasmin is involved in iron metabolism and the genetic defect, aceruloplasminemia, shows neurological disorders, diabetes mellitus and microcytic anaemia associated with excessive systemic iron accumulation, which is typically accompanied by low serum copper concentrations (ref: Bandmann 2015). Defects in copper metabolism are also seen in some patients with congenital disorders of glycosylation and may be accompanied by severe liver disease (ref: Girard M et al. Mol Genet Metab. 2018 May 9. pii: S1096–7192(18)30158–6).

Box 2.

ATP7B structure (ref: Chang IJ, Hahn SH. Handb Clin Neurol. 2017; 142: 19–34)

ATP7B belongs to class 1B of the highly conserved P-type ATPase superfamily responsible for the transport of copper and other heavy metals across cellular membranes. The protein contains 1465 amino acids organised into the phosphatase domain (PD), the phosphorylation domain, nucleotide-binding (N) domain and M-domain, which is comprised of eight transmembrane (Tm) ion channels that span the phospholipid bilayer of the membrane (M). The N-terminal metal-binding domain (MBD) is composed of six copper-(Cu) binding sites, which play a central role in accepting copper from copper chaperone ATOX1 through protein–protein interactions.

Different *ATP7B* mutations affect function in different ways. One of the most common WD mutations, p.H1069Q, occurs in the SEHPL motif in the N-domain, resulting in protein misfolding, abnormal phosphorylation in the P-domain, decreased ATP binding affinity, thermal instability and abnormal localisation to the trans-Golgi network. Mutations in p.E1064A, also found in the SEHPL motif, completely disable ATP-binding affinity but do not result in protein misfolding, transport abnormalities or thermal instability. The p.R778L mutation affects transmembrane transport of copper while p.G943S and p.M769V mutations result in defective copper metabolism but preserved ceruloplasmin levels. Other prevalent mutations, such as protein-truncating nonsense mutations and frameshift mutations, are predicted to cause decay of mRNA or a severely truncated protein, resulting in absent or diminished levels of protein.

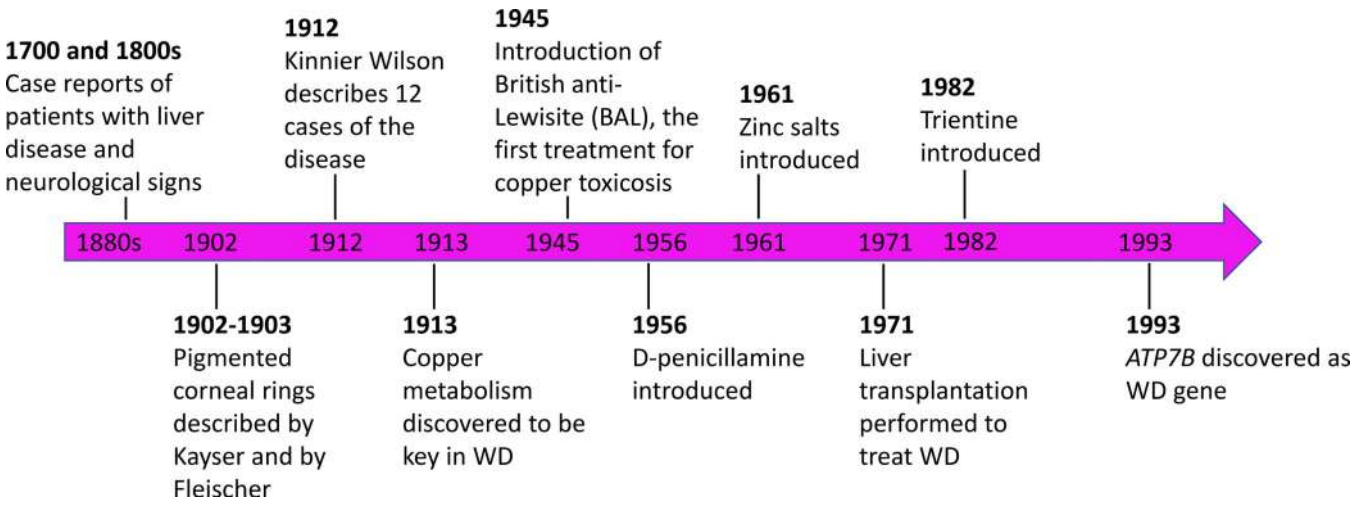

Figure 1. A timeline of key discoveries in WD

Samuel Alexander Kinnier Wilson described the Wilson disease (WD) in 12 patients in 1912. However, the first cases of WD with dominant tremor symptoms were described in 1883 by Westphal, while the corneal rings pathognomonic of WD were described by Kayser and by Fleischer in 1902–1903. Then followed the discovery of disturbances in copper metabolism as the aetiology of WD and the autosomal recessive inheritance. In 1945, the first treatment for copper toxicosis in WD, British anti-Lewisite (BAL), was introduced. In 1956, d-penicillamine became the first oral drug for WD, which was followed by the availability of zinc salts in 1961 and trientine in 1982. Liver transplantation, as an ultimate treatment for WD, was performed by Starzl and coworkers in 1971. In 1993, the WD gene, *ATP7B*, was located to chromosome 13q and found to code a P-type ATPase involved in copper transport[194].

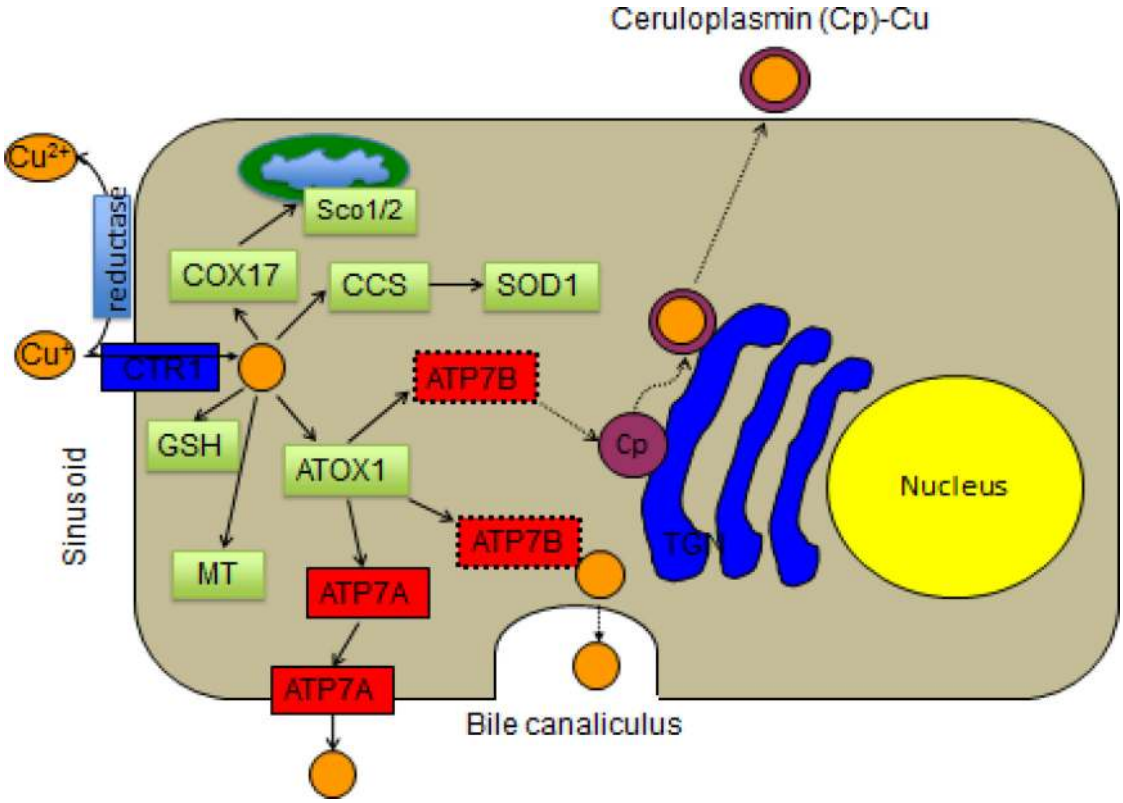

Figure 2. Copper homeostasis in the hepatocytes

Cellular copper (Cu) uptake in hepatocytes and other cells is primarily mediated by copper transporter 1, CTR1. A yet unknown cuprireductase and/or extracellular ascorbate provide the reduced copper species for uptake by CTR1. Specialised chaperons shuttle copper to its specific cellular targets: the copper chaperon for superoxide dismutase (CCS) to superoxide dismutase 1 (SOD1), Cox17 to SCO1/2 for subsequent incorporation into cytochrome c oxidase, and antioxidant protein 1 (ATOX1) shuttles copper to the copper transporting ATPases, ATP7A and ATP7B, in the trans-Golgi network (TGN). In the TGN, ATP7B activates ceruloplasmin (Cp) by packaging six copper molecules into apoceruloplasmin, which is then secreted into the plasma. In the cytoplasm, ATP7B sequesters excess copper into vesicles and excretes it across the apical membrane into bile.In the liver, ATP7B provides copper for incorporation into ceruloplasmin (Cp) and is also required for biliary copper excretion. Pathways altered in WD are marked by dashed lines. With reduced/absent levels of ATP7B in WD, there is reduced biliary copper excretion and reduced incorporation of copper into ceruloplasmin. (ref for text content: 195 Scheiber 2017)

Image provided by Petr Dusek and Valentina Medici.

GSH, glutathione; MT, metallothioneins

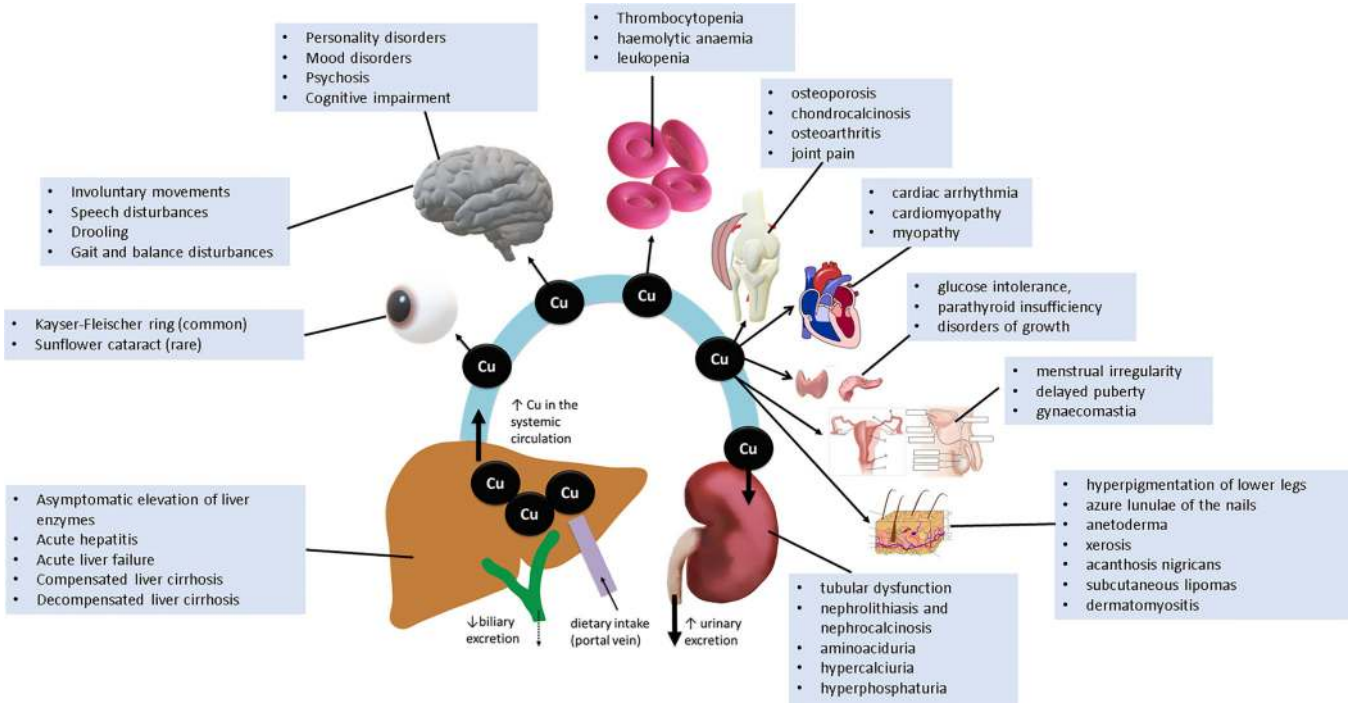

Figure 3. Copper toxicity in the pathogenesis of Wilson disease

Dietary copper (Cu) is transported via portal vein and sequestered in liver which is the central organ for systemic copper balance. Impairment of biliary copper excretion in Wilson disease caused by ATP7B dysfunction leads to gradual copper accumulation in liver. When the liver capacity to store copper is exhausted, excessive quantities of non-ceruloplasmin-bound copper enter systemic circulation and is deposited in various organs exerting extrahepatic copper toxicity. Copper accumulates in the cornea, brain, red blood cells, skeletal and cardiac muscle cells, synovial membranes of large joints, and renal parenchyma. Non-ceruloplasmin-bound plasma copper is filtrated by renal tubular epithelium and is excreted via urine.

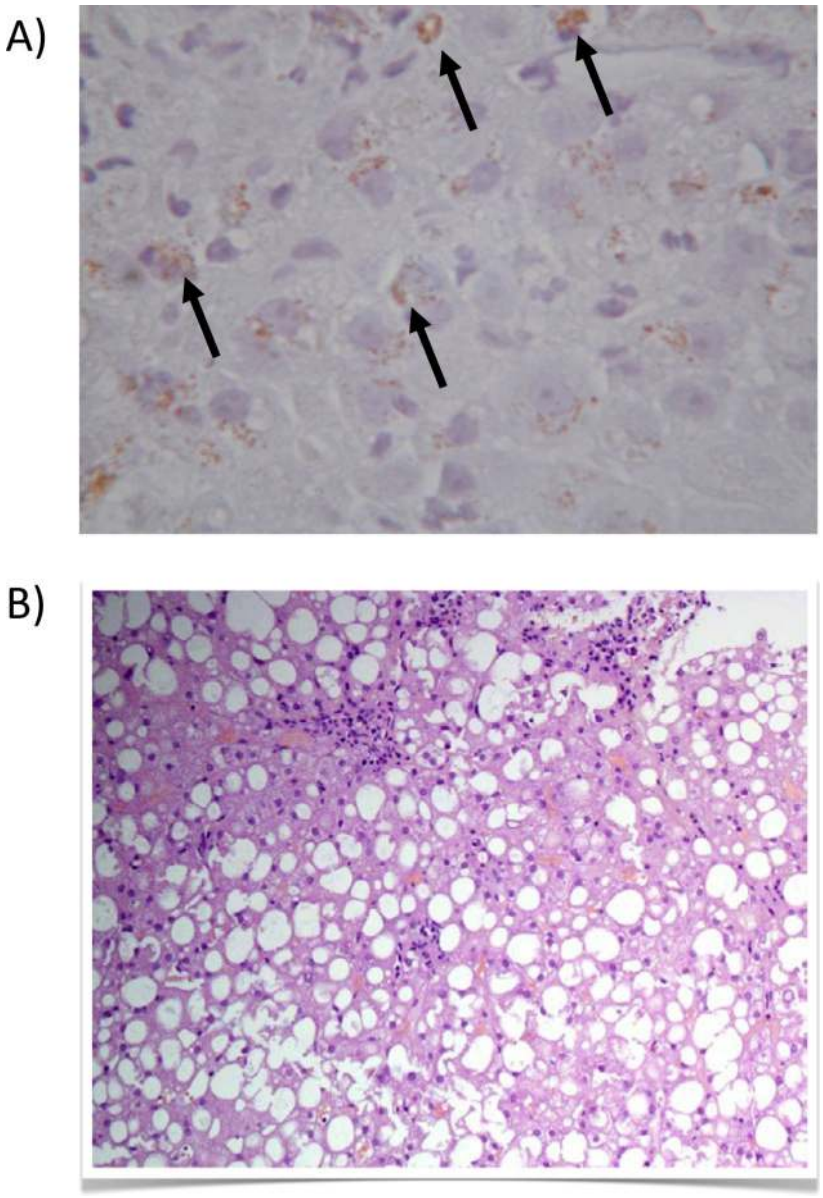

Figure 4. Liver pathology of WD

The histochemical demonstration of hepatic copper can be observed as rhodanine-positive granules (part a, black arrows). Early characteristic alterations of the liver pathology in WD include steatosis (part b), which is sometimes indistinguishable from non-alcoholic fatty liver disease. Image b) has been adapted from REF[24].

[Image a) was provided by Professor Ferenci as an original image]

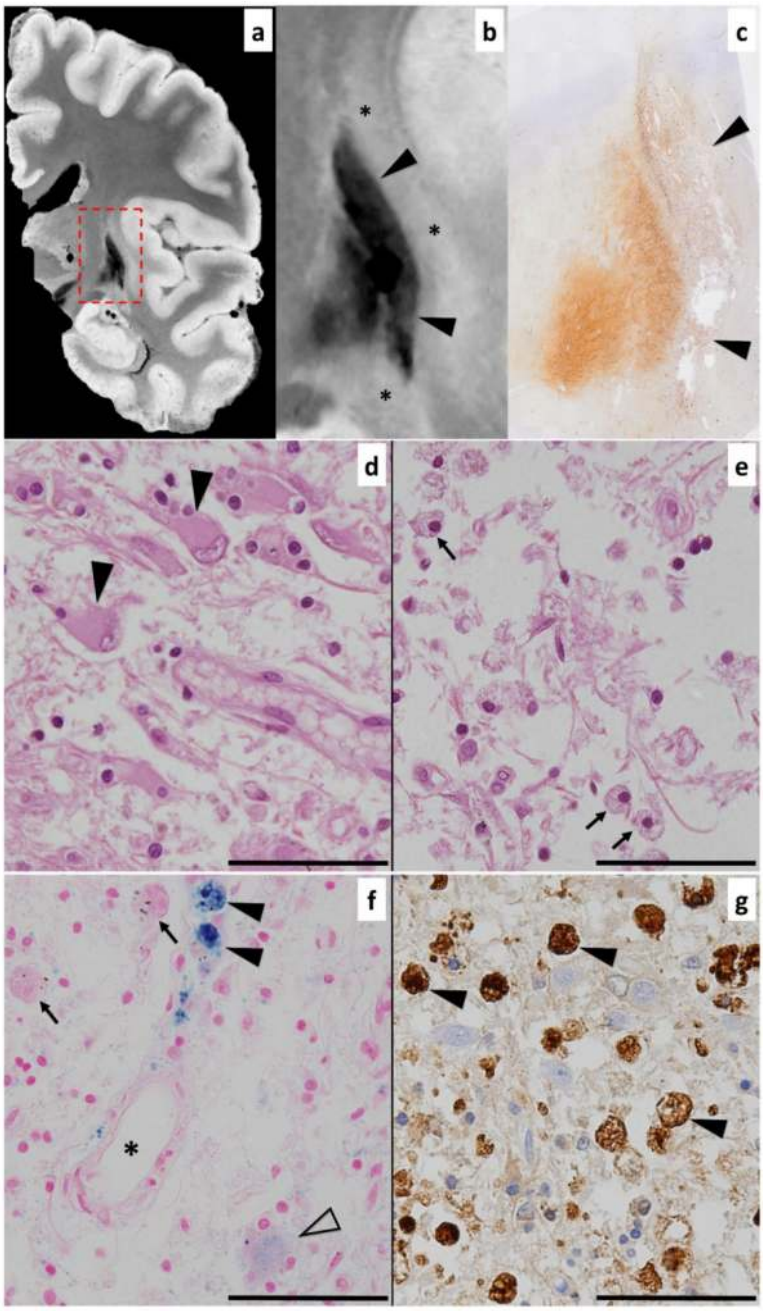

Figure 5. Post mortem MRI and histopathology in neurological Wilson disease

a) T_2*-weighted post mortem MRI acquired at 7T scanner; b) magnification of the basal ganglia region marked by dashed red rectangle in a), note shrunken and markedly hypointense putamen and globus pallidus (marked by black arrowheads) surrounded by mildly hyperintense area (marked by asterisks); c) low power magnification of Turnbull iron staining displaying corresponding area with MRI on image b), iron staining corresponds with low MRI T_2* signal; d & e) Hematoxylin-eosin staining showing reactive astrocytes with large pale nuclei (black arrowheads) and severely damaged tissue with macrophages (black arrows), note that d) corresponds to T_2* hyperintense area directly adjacent to

putamen while e) corresponds to rarefied area in central putamen; f) Berlin-blue staining showing iron-negative (black arrows) and iron-positive (black arrowheads) macrophages, iron is faintly present also in astrocytes (empty arrowhead) and as iron dust with dominant perivascular distribution (vessel marked by asterisk); g) Ferritin staining shows numerous strongly positive macrophages (black arrowheads) which drive the MRI T_2* signal drop. Scale bars in images D-G represent 50 μm.

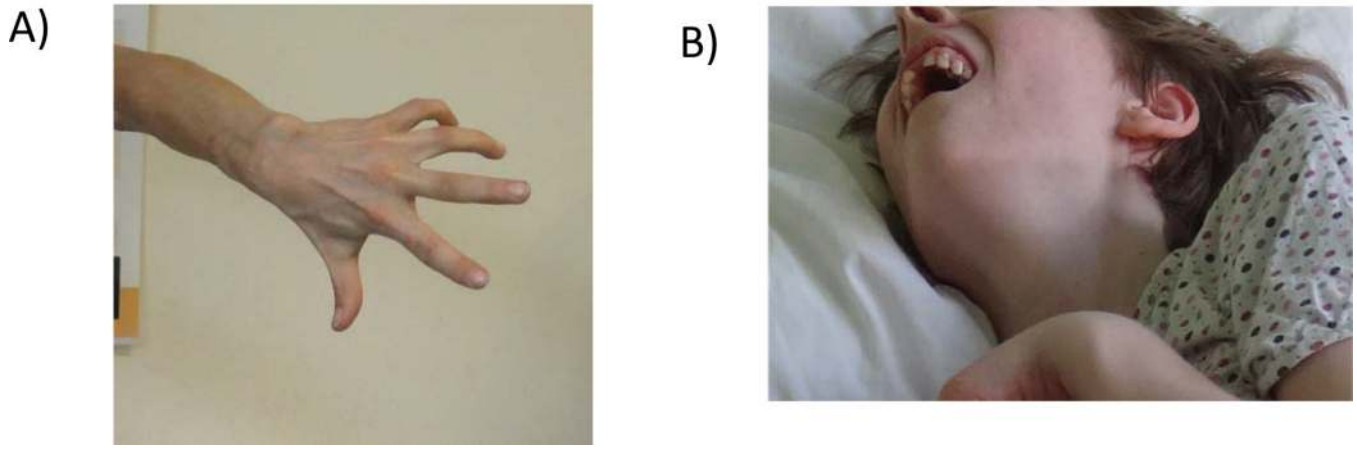

Figure 6. Dystonia, a characteristic symptom in WD

Dystonia is present in at least a third of all patients with a neurological presentation of WD and can be generalised, segmental, multifocal, or focal (part a; focal hand dystonia)[1]. The most characteristic WD dystonic presentation is abnormal facial expression or risus sardonicus, which presents as a fixed smile due to dystonia of the risorius muscle (part b, severe dystonia).

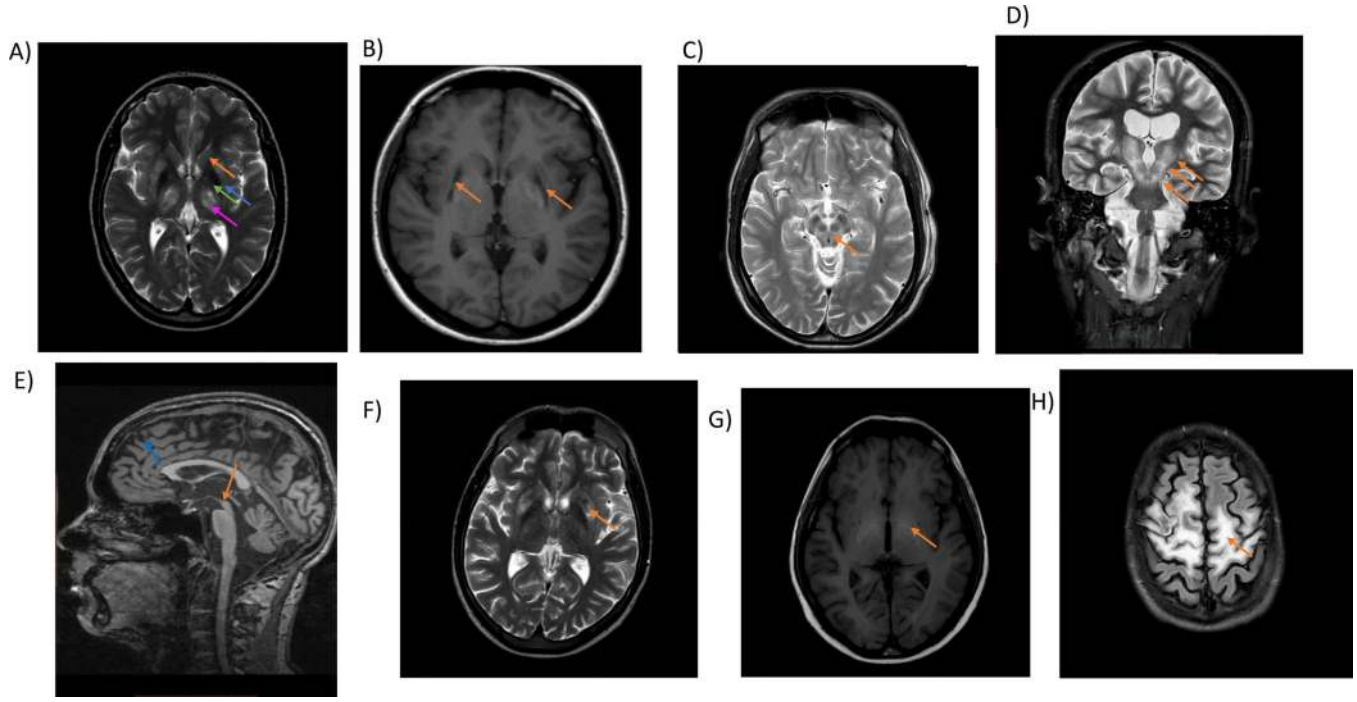

Figure 7. Brain MRI changes in WD.

Usual abnormalities in brain MRI in patients with WD include symmetric hyperintense changes visualised in T_2-weighted images of the basal ganglia, particularly the putamen (blue arrow), caudate nuclei (orange arrow), thalami (pink arrow) and globi pallidi (green arrow)^{89,108,114–117}(part a). In more advanced cases, severe tissue damage can be visualised in T_1-weighted images as hypointensity in the putamen (part a, blue arrow; part b, orange arrows). The most spectacular WD changes are described as the 'face of the giant panda' in the midbrain (part c). Another common MRI abnormality is increased T_2 signal along the dentato-rubro-thalamic pathway (part d), which is a major efferent pathway from the cerebellum involved in movement disorder symptoms, including ataxia, tremor and dystonia.

Particularly in severe cases of WD, diffuse brain atrophy (part e) can be seen in the midbrain (orange arrow) and cortex (blue arrow). In brain MRI scans from a 21-year old male with severe liver failure and discrete neurological signs, hyperintense changes in putamina can be visualised in T_2-weighted images, which is characteristic of early stages of brain involvement (part f). Hyperintense changes in the globi palidi in T_1-weighted images in the same patient are presumably due to manganese accumulation, which is characteristic of hepatic encephalopathy and hypointense changes due to neurodegeneration in the putamina (part g). Very rarely, diffuse white matter changes in both brain hemispheres are observed with preservation of the cortex, probably due to myelin destruction (part h). MRI, magnetic resonance imaging; WD, Wilson disease.

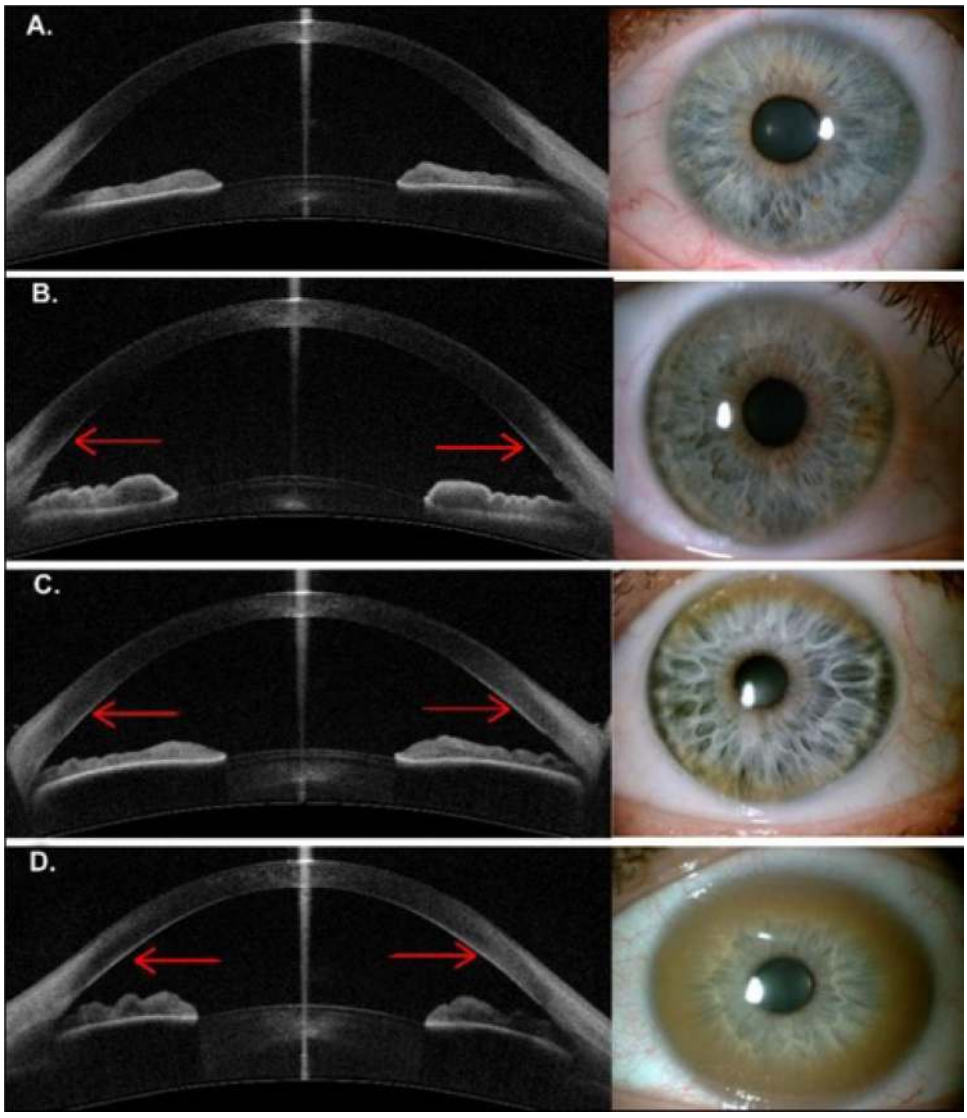

Figure 8. Kayser-Fleischer rings visualised by anterior segment optical coherent tomography.
In some patients and in healthy individuals, corneal copper deposits are not seen (part a). However, on other WD patients, copper deposits can be visualised as hyperreflective points by anterior segment optical coherence tomography that are either discrete (part b), on the superior and inferior part of the cornea (part c) or form a complete Kayser-Fleischer ring (part d).
Kindly provided by Dr Karina Broniek and Professor Jacek Szaflik from the Department of Ophthalmology, Medical University of Warsaw, Poland

Table 1.

Routine tests for the diagnosis of Wilson disease [Ref EASL 2012]

Test	Typical finding	False 'negative'	False 'positive'
Serum ceruloplasmin	Decreased	Normal levels in patients with marked hepatic inflammation Overestimation by immunological assay Pregnancy, oestrogen therapy	Low levels in patients with: - malabsorption, malnutrition - aceruloplasminemia - heterozygotes
24-hour urinary copper	Adults: >100 μg (1.6 μmol)/24 h Child: >40 μg (0.64 μmol)/24 h	Normal: - incorrect collection - children without liver disease	Increased: - hepatocellular necrosis - cholestasis - contamination
Non-ceruloplasmin-bound copper	>100 μg/l (1.6 μmol/l)	May appear normal if ceruloplasmin is overestimated by immunological assay A negative value may be obtained if ceruloplasmin is measured immunologically	
Hepatic copper	250 μg/g (4 μmol)/g dry weight	Due to regional variation: - in patients with active liver disease - in patients with regenerative nodules	Cholestatic syndromes Idiopathic copper toxicosis disorders
Kayser-Fleischer rings by slit-lamp examination	Present	Absent: - in up to 50% of patients with hepatic Wilson's disease - in most asymptomatic siblings	Primary biliary cholangitis (primary biliary cirrhosis)

Table 2.

Diagnostic scoring system developed at the 8th International Meeting on Wilson disease, Leipzig 2001[155]

Typical clinical symptoms and signs		Other tests	
Kayser-Fleischer rings		**Liver copper** (in the absence of cholestasis)	
Present	2	>250 μg (>4 μmol)/g dry weight	2
Absent	0	50–249 μg (0.8–4 μmol)/g	1
Neurologic symptoms**		Normal: <50 μg (<0.8 μmol)	−1
Severe	2	Rhodanine-pos. granules*	1
Mild	1	**Urinary copper** (in the absence of acute hepatitis)	
Absent	0	Normal	0
Serum ceruloplasmin		1–2 × ULN	1
Normal (>0.2 g/l)	0	>2 × ULN	2
0.1–0.2 g/l	1	Normal but >5 × ULN after d-penicillamine	2
<0.1 g/l	2	**Mutation analysis**	
Coombs-negative haemolytic anaemia		On both chromosomes detected	4
Present	1	On 1 chromosome detected	1
Absent	0	No mutations detected	0
TOTAL SCORE		**Evaluation:**	
4 or more		Diagnosis established	
3		Diagnosis possible, more tests needed	
2 or less		Diagnosis very unlikely	

ULN, upper limit of normal.

*If no quantitative liver copper available

**Or typical abnormalities at brain magnetic resonance imaging.

Table 3.

Drugs used in the treatment of WD.

Drug	Mode of action	Interactions	Frequency of adverse events leading to discontinuation of treatment	Assessment of effectiveness of treatment and adherence
D-penicillamine (DPA)	Promotes urinary excretion of copper	- Do not combine with myelosuppressive agents, cytostatic, antimalarials, gold therapy, oxyphenbutazone, phenylbutazone - DPA interacts with heavy metals (iron salts if needed should be given after 2 h break)	20–30% during treatment[168,170] Can be divided into: - 'early' AEs (first 3 weeks) hypersensitivity: fever, cutaneous manifestations including generalised pruritus, rashes, urticarial eruptions and exfoliative dermatitis, accompanied by lymphadenopathy, arthralgia, leukopenia thrombocytopenia, and proteinuria - 'late' AEs (3 weeks to few years) include: paradoxical neurological worsening, renal insufficiency in the course of Goodpasture syndrome with fatal glomerulonephritis and intra-alveolar haemorrhage, myasthenia-like syndrome; lupus-like syndrome, or fatal bone marrow aplasia to mild symptoms, such as gastric symptoms, hair loss or hypogeusia	- Copper urinary excretion 200–500 μg/24 h (at the beginning of the treatment >1000 μg/24 h) - Serum NCC 5–15 μg/dl - Normalisation of copper urinary excretion 2 days after stopping the treatment with DPA
Trientine (TN)	Promotes urinary excretion of copper	- Mineral supplements should be avoided (iron chelation)	7.1%; AE frequency is 4-fold less than with DPA[170] - Gastritis - Sideroblastic anaemia - Lupus like reactions - Loss of taste	- Copper urinary excretion 200–500 μg/24 h (at the beginning of the treatment >1000 μg/24 h) - Serum NCC * 5–15 μg/dl - Normalisation of copper urinary excretion 2 days after stopping the treatment with trientine
Zinc salts (ZS)	Blocks intestinal absorption of copper	- ZS diminish absorption of tetracyclines and chinolones - Diuretics increase urinary zinc excretion - Iron salts, milk, milk products, wholegrain bread, products containing phytates, high-fibre products and chelating agents diminish absorption of ZS	3–7%[168] - Gastritis - Biochemical pancreatitis - Immunosuppression - Bone marrow depression	- Copper urinary excretion <75 μg/24 h - Serum NCC 5–15 μg/dl; (>12 months of treatment)

NCC, non-ceruloplasmin-bound copper. NCC is not routinely used.

* NCC <5 μg/dL indicates over treatment and modification of therapy needed.

Decrease of NCC during treatment is currently used as an efficacy measure[194]

Table 4.

Wilson disease prognostic index*

	0 points	1 point	2 points	3 points	4 points
Serum bilirubin (μmol/l)	0–100	101–150	151–200	201–300	>301
Aspartate aminotransferase (U/l)	0–100	101–150	151–300	301–400	>401
International normalized ratio	0–1.29	1.3–1.6	1.7–1.9	2.0–2.4	>2.5
White blood cell count (10^9/l)	0–6.7	6.8–8.3	8.4–10.3	10.4–15.3	>15.4
Albumin (g/l)	>45	34–44	25–33	21–24	<20

*New Wilson Index modified from the Nazer scoring system[196] by Dhawan et al[168]. A score of ≥11 points is associated with high probability of death without liver transplantation and is an indication for liver transplantation.