Wilson's disease and other neurological copper disorders

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The copper metabolism disorder Wilson's disease was first defined in 1912. Wilson's disease can present with hepatic and neurological deficits, including dystonia and parkinsonism. Early-onset presentations in infancy and late-onset manifestations in adults older than 70 years of age are now well recognised. Direct genetic testing for *ATP7B* mutations are increasingly available to confirm the clinical diagnosis of Wilson's disease, and results from biochemical and genetic prevalence studies suggest that Wilson's disease might be much more common than previously estimated. Early diagnosis of Wilson's disease is crucial to ensure that patients can be started on adequate treatment, but uncertainty remains about the best possible choice of medication. Furthermore, Wilson's disease needs to be differentiated from other conditions that also present clinically with hepatolenticular degeneration or share biochemical abnormalities with Wilson's disease, such as reduced serum ceruloplasmin concentrations. Disordered copper metabolism is also associated with other neurological conditions, including a subtype of axonal neuropathy due to *ATP7A* mutations and the late-onset neurodegenerative disorders Alzheimer's disease and Parkinson's disease.

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Introduction

2012 saw the centenary of Samuel Alexander Kinnier Wilson's seminal publication *Progressive Lenticular Degeneration: A Familial Nervous Disease Associated with Cirrhosis of the Liver*.[1,2] Wilson noticed several key features of this condition—namely, its hereditary nature, the co-occurrence of liver cirrhosis with neurological deficits, and the predominantly extrapyramidal nature of these signs and symptoms. The discovery of increased copper concentrations in the brain and liver of patients with Wilson's disease led to the introduction of British antilewisite and later penicillamine as the first chelating agents for Wilson's disease.[3] Great progress has been made since, most notably the discovery of *ATP7B* as the causative Wilson's disease gene and introduction of other chelating and non-chelating treatments.

This Review will focus on recent advances in our understanding of Wilson's disease with particular reference to clinically relevant aspects, such as our improved understanding of the clinical presentation, diagnostic guidelines, and an up-to-date discussion of treatment of this disorder. Additionally, we will describe clinically relevant aspects of other copper disorders (several of which have only recently been discovered), how to distinguish them from other heavy metal diseases, and summarise the evidence for disturbed copper metabolism in Alzheimer's and Parkinson's diseases.

Genetics

Wilson's disease is a monogenic, autosomal recessive condition. The causative gene, *ATP7B*, encodes a copper-transporting P-type ATPase.[4–6] More than 500 *ATP7B* mutations have now been identified. Most of these are missense mutations, small deletions or insertions in the coding region, or splice junction mutations. Less common genetic mutations, including whole exon deletions, promoter region mutations, multiple mutations, and monogenic disomy, have also been reported[7,8] but are comparatively rare. *ATP7B* mutation hot spots exist but vary substantially between different populations.[9] The point mutation 3207C→A leading to His1069Gln substitution is the most common *ATP7B* mutation in patients from central, eastern, and northern Europe, and 50–80% of patients with Wilson's disease from these countries carry at least one 3207C→A allele.[9] Table 1 shows common *ATP7B* mutations in different populations.

Basic aspects of normal copper metabolism and the molecular mechanisms of the different copper transport diseases are summarised in figure 1. Mutations resulting in completely absent or non-functional ATP7B protein activity are associated with early-onset, typically hepatic, severe Wilson's disease; these mutations are comparatively rare.[11,12] Systematic attempts to establish firm genotype–phenotype correlations for other, more common *ATP7B* mutations have largely failed.[13,14] An association between particular point mutations, such as 3207C→A, and the late-onset neurological presentation of Wilson's disease has been suggested but not confirmed in independent cohorts.[14–16] The absence of genotype–phenotype correlations, the clinical variability, and the variable penetrance suggest the existence of modifier genes that determine an individual's level of copper tolerance or copper storage capacity. Genetic modifiers, such as the presence of the *APOE ε4* allele or polymorphisms in the methylenetetrahydrofolate reductase gene (*MTHFR*), might contribute to the age of Wilson's disease onset, but these findings[17,18] await confirmation in larger, independent populations.

At the cellular level, the functional consequences of pathogenic ATP7B aminoacid substitutions vary greatly,

For the **database of ATP7B mutations** see http://www.wilsondisease.med.ualberta.ca/database.asp

	DNA nucleotide change	Protein aminoacid change	Exon	Frequency	Other common mutations
East Asia	2333G→T	Arg778Leu	8	30–50%	2871delC
Europe	3207C→A	His1069Gln	14	35–45%	2299insG 1934T→G
India	813C→A	Cys271Stop	2	~20%	3305T→C 2975C→T
Middle East	4196A→G	Gln1399Arg	21	~30%	

Table 1: **Common *ATP7B* mutations in different populations. Modified from Ferenci[9] and Weiss[10]**

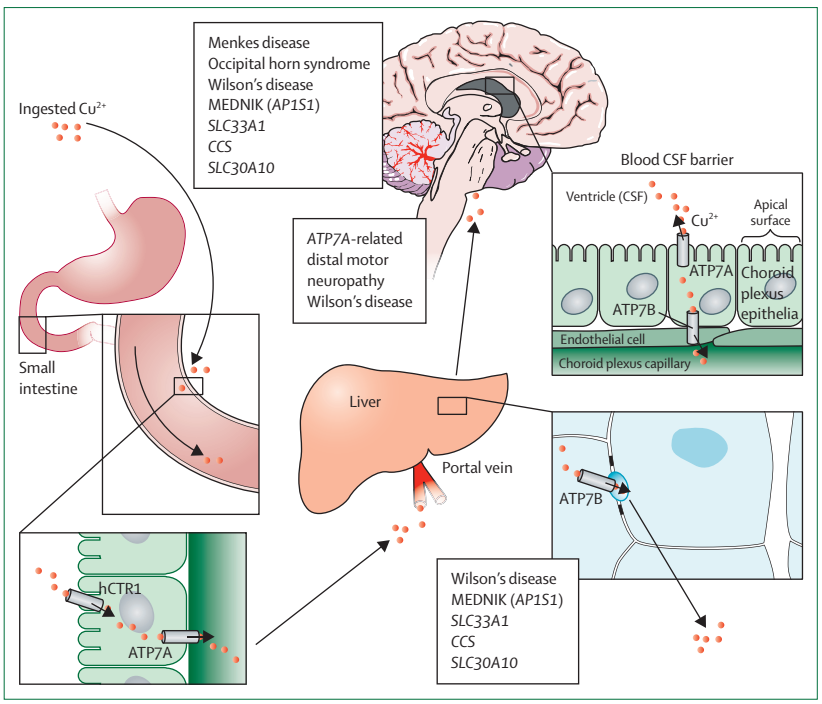

Figure 1: **Healthy copper metabolism and the molecular mechanisms of copper disease**
Copper absorption occurs in the small intestine via enterocyte uptake by human copper transporter 1 (hCTR1) and passage into the blood mediated by ATP7A at the basolateral aspect of duodenal epithelia. Copper is conveyed to the liver via the portal circulation and excess copper is removed by excretion into the bile at the apical aspect of hepatocytes, a process impaired by mutations in *ATP7B*. Copper diseases of the liver also involve mutations in the *AP1S1* gene implicated in MEDNIK syndrome, the acetyl CoA transporter SLC33A1, and the cytosolic copper chaperone CCS. Mutations in the manganese transporter SLC30A10 produce hepatic cirrhosis due to manganese accumulation that can mimic Wilson's disease. *ATP7A* and *ATP7B* are believed to mediate copper entry and exodus, respectively, at the blood–CSF barrier of the choroid plexus epithelia. Brain copper deficiency (Menkes disease) or excess (Wilson's disease), respectively, result from mutations in these essential copper transporters. The CNS is also affected by alterations in *AP1S1*, *SLC33A1*, *CCS*, and *SLC30A10*. Isolated motor neuron degeneration occurs in association with unique *ATP7A* missense mutations affecting axonal trafficking, and sensory peripheral neuropathy can be a component of Wilson's disease. MEDNIK=mental retardation, enteropathy, deafness, neuropathy, ichthyosis, and keratodermia.

even when the substitutions are in the same functional domain.[19] Increased intracellular copper concentrations lead to oxidative stress, free radical formation, and mitochondrial dysfunction arising independently of oxidative stress. These combined effects can result in cell death in hepatic tissue, brain tissue, and other organs.[20,21]

Prevalence

The widely cited prevalence figure of 1 in 30 000 for Wilson's disease with a heterozygous *ATP7B* mutation carrier frequency of 1 in 90 was estimated in 1984 and thus predates the identification of *ATP7B* as the causative gene. This prevalence estimate was at least partly based on assumptions, and has been questioned.[22] Mass screening studies undertaken in east Asia[23,24] suggested a substantially higher prevalence of Wilson's disease (1 in 1500 to 1 in 3000) based on concentrations in serum of the copper carrying protein ceruloplasmin in large cohorts. We completed the first genetic prevalence study of Wilson's disease in the UK.[7] The entire *ATP7B* coding region and adjacent splice sites were sequenced in 1000 apparently healthy neonatal controls. Our data suggested an unexpectedly high rate (about 1 in 40) of *ATP7B* heterozygote mutation carriers, predicting a 1 in 7000 prevalence in the UK population.[7] The prevalence of Wilson's disease might be substantially higher in isolated populations.[25] The large discrepancy between our calculated genetic prevalence data for Wilson's disease (suggesting about 9000 current cases of Wilson's disease in the UK) and the substantially lower number of clinically diagnosed patients with Wilson's disease is likely to be at least partly due to the reduced penetrance of *ATP7B* mutations. However, our study also raises concerns that the disease might still be unrecognised in a substantial proportion of affected individuals. Late diagnosis is the most common cause of death in Wilson's disease.[26]

Clinical manifestations

Neurological symptoms in Wilson's disease typically begin in the second or third decade of life.[27] Only 46 of 1223 (3·8%) patients with Wilson's disease in one large cohort study became symptomatic after 40 years of age, and about two-thirds of these patients had neurological symptoms.[28] However, late-onset Wilson's disease manifesting in people older than 70 years of age is well documented.[29] Thus, the diagnosis of Wilson's disease should never be excluded because a patient is too old. Conversely, Wilson's disease with onset in early infancy has also been reported, the youngest age of onset being 9 months.[30] Although all children diagnosed in early infancy with genetically confirmed Wilson's disease present with hepatic symptoms, whether Wilson's disease should also be considered in infants who present with neurological impairment at such a young age is unclear. Paediatric neurologists should nevertheless be aware of unusual presentations of Wilson's disease in children, such as spasmodic muscle cramps and myopathy.[31]

The frequency of distinct neurological features described in patients with Wilson's disease, such as dystonia or parkinsonism, varies widely in different case series. The presence of classic wing-beating tremor or flapping tremor in combination with dysarthria strongly suggests the diagnosis of Wilson's disease. However, any of the other more common forms of tremor, such as rest, action, or intention tremor, can occur as well. The most common form of tremor in Wilson's disease is an irregular, and somewhat jerky, dystonic tremor. Dystonia is present in at least a third of all patients with a neurological presentation of Wilson's disease and can be generalised, segmental, multifocal, or focal.[32] Isolated cervical dystonia is nevertheless unlikely to be due to Wilson's disease.[33] Dysarthria is frequently combined with slow tongue movements and orofacial dyskinesias, including the risus sardonicus, a term which describes involuntary grimacing with the mouth open and the upper lip contracted. Slowness of movement and other neurological features

that are typically observed in Parkinson's disease, such as hypomimia, shuffling gait, impaired fine finger movements, and foot tapping, are also typical features. The presence of three distinct neurological presentations of Wilson's disease has been suggested: a dystonic syndrome, an ataxic syndrome, or a parkinsonian syndrome. However, most patients with Wilson's disease will present with a combination of these features.[27,34] Furthermore, some neurological features, such as dystonic action tremor or the inability to walk heel-to-toe because of lower limb dystonia, might be misinterpreted as cerebellar impairment with gait and limb ataxia. Pyramidal features, such as pathologically brisk deep tendon reflexes, can be present but paralysis is rare. The presence of sensory impairment makes the diagnosis of Wilson's disease highly unlikely. Seizures might also be the presenting symptom of Wilson's disease; these can occur at any stage of the illness, but might be more common after treatment has been initiated.[35,36] Abnormal vertical smooth pursuit has been reported in 85% of patients with Wilson's disease on formal testing with electro-oculography, but vision was normal.[37]

Psychiatric symptoms can occur in both untreated and treated patients with Wilson's disease.[38,39] According to one literature review, 20% of people with Wilson's disease will have seen a psychiatrist before a formal diagnosis of Wilson's disease was reached. The average time between the onset of psychiatric symptoms and the diagnosis of Wilson's disease was 864 days for patients with Wilson's disease in whom psychiatric symptoms preceded neurological or hepatic damage.[39] The most common psychiatric features are abnormal behaviour (typically increased irritability or disinhibition), personality changes, anxiety, and depression. Psychosis is much less common. A history of jaundice, a positive family history of neuropsychiatric disease, and increased sensitivity to neuroleptics can be diagnostic clues for Wilson's disease in people with a history of psychiatric disease.[40] Cognitive impairment might be global in patients with advanced, untreated Wilson's disease but is typically limited to impaired executive function involving frontostriatal circuits in treated patients, with the preservation of verbal intelligence and episodic memory.[41] Attention deficits can be identified in symptomatic and asymptomatic patients with Wilson's disease.[42]

It is beyond the scope of this Review to describe the full range of non-neurological presentations of Wilson's disease, particularly the hepatic presentation, but neurologists need to be aware of the fact that Wilson's disease can present either with acute liver failure or chronic liver disease that might clinically be indistinguishable from other hepatic conditions. Neurologists also need to consider a diagnosis of Wilson's disease if asked to see a patient with unexplained hepatic encephalopathy. Conversely, the absence of clinical or biochemical evidence of liver disease does not exclude Wilson's disease.

Diagnostic investigations

Ophthalmological and laboratory investigations

A scoring system for Wilson's disease diagnosis was developed by attendees at an international meeting in Leipzig, Germany, in 2001 (table 2). This approach collates biochemical, clinical, and genetic data from individual patients to provide a quantitative score and is now included in the European Association for the Study of the Liver (EASL) clinical practice guidelines for Wilson's disease.[43] Clinical validation will be needed before the effectiveness of this scoring system in clinical practice is known. Typically, the presence of Kayser-Fleischer rings (figure 2) and serum ceruloplasmin concentrations of less than 100 mg/L are sufficient to establish the diagnosis. Intermediate ceruloplasmin concentrations below the normal range but above 5 mg/L might suggest that the patient is a heterozygote *ATP7B* mutation carrier. However, Kayser-Fleischer rings are absent in about half of all hepatic patients,[45] and these rings can easily be missed on bedside testing, especially in patients with dark eyes. Referral to an ophthalmologist for slit-lamp examination should therefore be advised in most suspected cases. Occasionally, Kayser-Fleischer rings can be present in other hepatic conditions, such as primary biliary cirrhosis. Ceruloplasmin concentrations can also be low in other conditions, such as hepatic insufficiency due to advanced liver disease. Of note, the oral contraceptive pill can increase low ceruloplasmin concentrations to the normal range. False normal ceruloplasmin values can also result from inflammatory conditions because ceruloplasmin is an acute phase reactant. Laboratory findings that further support the diagnosis of Wilson's disease include low serum copper concentrations, increased hepatic transaminase concentrations, aminoaciduria, and haemolytic anaemia. However, transaminase concentrations might be normal in patients with Wilson's disease who present with neurological symptoms.[45] Analysis of 24 h copper excretion in urine is an easy and important diagnostic test for Wilson's disease. Acid-washed (copper-free) collection containers should be used. Urinary copper excretion greater than 100 μg per 24 h, in the absence of cholestatic liver disease, is typical for Wilson's disease. Values greater than 40 μg per 24 h (0·64 μmol per 24 h) are suggestive of Wilson's disease in asymptomatic children.[46] In most centres, the penicillamine challenge has only been validated in paediatric patients. 500 mg of penicillamine are given orally after collecting baseline 24 h urine (urine collected over 24 h). The penicillamine dose is repeated after 12 h, at the midpoint of a second 24 h urine collection. A several-fold increase in copper excretion in the second collection is highly suggestive of Wilson's disease.[47] A liver biopsy for measurement of hepatic copper might rarely be indicated in patients with the neurological presentation of Wilson's disease, in whom other investigations are ambiguous. Hepatic copper values greater than 250 μg per gram of dry weight (normal 20–50 μg per gram) are

	Diagnosis score
Kayser-Fleischer rings	
Present	2
Absent	0
Neurological symptoms*	
Severe	2
Mild	1
Absent	0
Serum ceruloplasmin	
Normal (>200 mg/L)	0
100–200 mg/L	1
<100 mg/L	2
Haemolytic anaemia	
Present	1
Absent	0
Liver copper (in absence of cholestasis)	
>5×ULN (>250 µg/g)	2
50–250 µg/g	1
Normal (<50 µg/g)	−1
Rhodanine positive granules†	1
Urinary copper (in absence of acute hepatitis)	
Normal	0
1–2×ULN	1
>2×ULN	2
Normal, but >5×ULN after penicillamine	2
Mutation analysis	
Homozygous	4
Heterozygous	1
No mutations detected	0

ULN=upper limit of normal. *Typical abnormalities on brain MRI. †If no quantitative liver copper available. 4 or more=diagnosis established. 3=diagnosis possible, more tests needed. 2 or less=diagnosis very unlikely.

***Table 2*: Scoring system for Wilson's disease diagnosis developed by attendees at the international Wilson's disease meeting in Leipzig, Germany, in 2001**

characteristic of Wilson's disease. Intermediate values (50–200 µg per gram of dry weight liver tissue) suggest heterozygote *ATP7B* mutation carrier status.[48] Incorporation of ^{64}Cu into serum ceruloplasmin is a further highly specific diagnostic test. Radioactive copper ^{64}Cu in the serum is measured after an oral load, with most patients with Wilson's disease only incorporating very little ^{64}Cu into ceruloplasmin. The usefulness of the ^{64}Cu incorporation test is restricted because the isotope is not widely available.

Crucially, family screening, including assessment of both clinically unaffected siblings and their parents for Wilson's disease, is mandatory to detect presymptomatic Wilson's disease. As a minimum, this screening should include clinical examination for Kayser-Fleischer rings, and measurement of copper and ceruloplasmin concentrations. Ideally, genetic testing should also be done on all first-degree relatives of patients.

Imaging

MRI abnormalities were present in all 56 patients with Wilson's disease included in a large case series of 100 patients with early-onset extrapyramidal disorders. However, the so-called face of the giant panda sign was only detected in 14·3%. Other abnormalities, such as tectal plate hyperintensity (75%), central pontine myelinolysis-like abnormalities (62·5%), and concurrent signal changes in the basal ganglia, thalamus, and brainstem (55·3%) were much more common (figure 3).[49] Rarely, Wilson's disease can result in diffuse white matter abnormalities and thus needs to be regarded as a possible cause of diffuse leukencephalopathy.[50] Other imaging techniques, such as 7 T MRI, T2-weighted imaging, MR spectroscopy, transcranial brain parenchyma sonography (TCS), or SPECT are currently only being used in research.[51–54] Importantly, MRI brain abnormalities can completely regress after successful treatment.[55]

Genetic testing

Previously, the usefulness of direct genetic testing for *ATP7B* mutations was limited.[56] However, direct genetic testing is likely to play an increasingly important part in the confirmation of the clinical diagnosis of Wilson's disease owing to improved techniques and a steady reduction in the costs of genetic investigations. We have recently shown that two pathogenic *ATP7B* mutations can be detected in 98% of patients with clinically confirmed Wilson's disease if the entire *ATP7B* coding region and adjacent splice sites are sequenced.[7] Promoter mutations or gene dosage problems, such as whole exon deletions, are rare and therefore do not need to be included in a routine genetic workup of *ATP7B*. In many populations, a step-wise approach, initially focusing on sequence analysis of *ATP7B* mutation hotspots, is likely to save costs and time. Such sequence analysis of *ATP7B* can be particularly helpful in patients with unusual presentations.[31] We and others have reported the presence of Wilson's disease in two or more generations of the same family, an example of pseudo-dominant inheritance, presumably due to the relatively high prevalence rate of *ATP7B* mutations in some populations.[7,57] Thus, the diagnosis of Wilson's disease cannot be excluded simply because the family history misleadingly suggests an autosomal dominantly inherited gene defect.

Treatment

Medical therapy in Wilson's disease must be lifelong because abnormal copper accumulation cannot be controlled by a low copper diet. Ideally, treatment should have two phases: an initial, acute de-coppering therapy and a subsequent maintenance therapy. This takes into account that, after the initial more aggressive treatment phase, a reduced dose or less toxic medical approach might be sufficient to maintain normal copper homoeostasis. Adjustment of the maintenance treatment dose

also helps to prevent overtreatment that can result in copper deficiency.[58]

Generally, treatment options include the copper chelators (penicillamine, trientine, and tetrathiomolybdate), zinc salts, or both. Chelating agents bind copper directly in blood and tissues and facilitate its excretion, whereas zinc interferes with the intestinal uptake of copper. The zinc interference mechanism includes induction of metallothionein synthesis in intestinal epithelial cells; increased metallothionein synthesis leads to preferential binding of dietary copper to metallothionein in these intestinal cells, which are subsequently shed. Thus, zinc impedes further copper accumulation but has lower de-coppering potential and thus less potential to mobilise copper from tissues that are already overloaded.

Because few prospective controlled studies have been done, we will discuss the different treatment approaches mostly investigated in retrospective studies. These studies emphasise the difficulty of choosing the appropriate de-coppering compound, especially in a patient with Wilson's disease who has neurological symptoms, and recommend careful monitoring of the risks associated with treatment.

A retrospective European multicentre study analysed the treatment outcome in 405 patients receiving penicillamine or trientine for at least 6 months.[59] Although chelation therapy—irrespective of the particular drug—led to hepatic improvement in more than 90% of patients, the response rates for symptomatic neurological patients were much less favourable. After 4 years of therapy, an improvement of neurological symptoms was only seen in 88 (62%) of 143 patients. The failure to respond to chelation therapy in 38% of these patients with neurological presentation might suggest irreversible brain damage.

Paradoxical worsening of the clinical neurological presentation is reported in up to 20% of patients after initiation of chelation therapy with either penicillamine or trientine. The mechanism of this neurological deterioration is not fully understood, but seems to be related to dose, given that a high starting dose could increase the risk of rapid chelator-induced paradoxical worsening. To mechanistically understand these dose effects, a concept of different copper pools has emerged that differentiates between high-affinity bound copper (bound to ceruloplasmin) and free copper (not bound to ceruloplasmin). A plausible mechanism of the paradoxical neurological deterioration could be over-mobilisation of copper by chelator therapy, leading to an increased free copper pool with toxic effects. This hypothesis is supported by a landmark study by Brewer and colleagues[60] that showed an association between an unfavourable neurological course and an increased free copper pool. Results from animal studies also showed an increase of low-affinity bound copper in the CNS associated with enhanced oxidative stress after initiation of penicillamine therapy in the toxic milk mouse model.[61] Thus, a flexible dose increase adjusted to control the free copper pool might help to avoid neurological deterioration. Also worrisome is the observation of long-term neurological worsening in ten of 143 patients on chelation therapy, which raises questions about the efficacy of penicillamine and trientine to control Wilson's disease-related brain involvement.[59]

In our view, the available data do not allow a strong recommendation of one chelating compound above

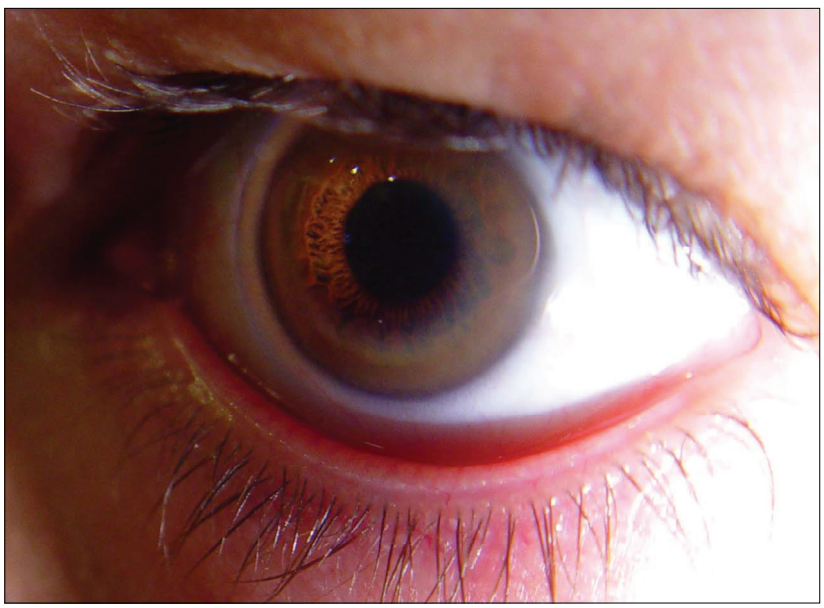

Figure 2: **Kayser-Fleischer ring with copper deposition in Descemet's membrane, leading to brown discolouration at the outer margin of the cornea**
With permission from Elsevier.[44]

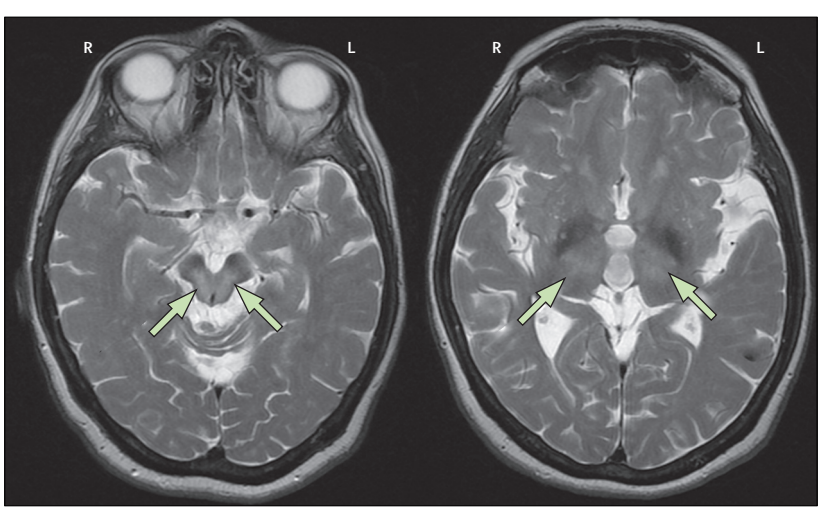

Figure 3: **Representative T2-weighted axial brain MRI scan showing symmetrical T2-weighted hyperintense lesions (solid green arrows) in the tectal midbrain on the left and in the ventromedial thalami and posterior limbs of the internal capsule on the right**
MRI image provided by Dr Mirko Pham, Department of Neuroradiology, Heidelberg University Hospital, Heidelberg, Germany.

others. We suggest that the advantages and disadvantages of penicillamine and trientine are discussed with the patient and a decision is then made on the basis of their individual needs and the respective side-effects profile.

A different chelating compound, tetrathiomolybdate, might be a promising alternative because it is fast acting and can restore copper balance within several weeks compared with the several months needed when treating with copper chelators or zinc.[60] However, clinical experience with this drug is insufficient, and the ammonium formulation of tetrathiomolybdate has proven too unstable for routine clinical use. Of note, a bis-choline formulation of tetrathiomolybdate has become available on a named patient basis in the EU and USA and is more stable than the ammonium formulation. Further studies, especially long-term follow-up studies, including direct comparisons with the traditional copper chelating compounds, are needed to establish the efficacy of tetrathiomolybdate in both formulations for routine clinical practice.

Because of the different modes of action, treatment with zinc salts is less often associated with paradoxical deterioration after initiation of therapy, but this potential advantage might be outweighed by overall lower treatment efficacy. With zinc, a comparatively long time (4–6 months) is needed to generate a negative copper balance when used in the initial stages of treatment, which might account for reports of non-response or worsening under zinc therapy.[45] Nonetheless, zinc used as the primary treatment in asymptomatic or neurologically affected patients with Wilson's disease has shown long-term outcomes similar to penicillamine in some centres.[62,63] A major concern in patients with Wilson's disease on zinc monotherapy is control of the hepatic disease. We noted[45] insufficient hepatic treatment efficacy under zinc monotherapy, with increased liver enzymes and urinary copper excretion in 14 (16%) of 88 patients. Others have reported[63] development of liver disease during zinc monotherapy in 20% of patients with initial neurological presentation.

The debate between chelating compounds and zinc will only be settled if a randomised controlled trial is done to compare both treatments directly. In our view, patients with newly diagnosed neurological Wilson's disease should be started on chelation therapy to ensure adequate de-coppering within an acceptable timeframe. Patients (and physicians) need to be warned that the switch to maintenance therapy can easily take 1–3 years. Close monitoring of clinical and laboratory parameters might be more important than the actual choice between penicillamine, trientine, or zinc for subsequent maintenance therapy. We typically aim for a copper concentration of less than 0·6 mg per 24 h urine (collected off medication) in patients with Wilson's disease on pencillamine as maintenance therapy.

The safety profile of all oral de-coppering compounds seems acceptable, although data about long-term use of penicillamine in a case series indicated that 99 (31·6%) of 313 patients discontinued penicillamine treatment because of marked side-effects. These included nephrotoxicity, haematological abnormalities, and elastosis perforans serpiginosa (usually on the neck and axillae).[59] Adverse events associated with trientine and tetrathiomolybdate use include bone marrow toxicity, whereas zinc therapy is often associated with gastrointestinal discomfort. Independent of the chosen medical regimen, non-adherence or discontinuation of medical therapy is associated with the risk of intractable hepatic decompensation.[64,65]

Accordingly, easy to follow drug regimens—especially once daily schemes, as for tetrathiomolybdate—or those under investigation for trientine (NCT01472874), could result in improved compliance. Those clinicians who favour a combination of zinc and a copper chelator need to be aware of a possible interaction between the drugs.

Lifelong presymptomatic treatment for Wilson's disease is mandatory in all clinically unaffected relatives with unequivocal biochemical or genetic evidence of preclinical Wilson's disease (table 2). It can consist of either chelating compounds or zinc.[43]

Monitoring of Wilson's disease patients

Timing and investigations

Even with asymptomatic or stable disease, monitoring intervals of 6 months seem reasonable to ensure a clinical and biochemical response and to identify adverse events in a timely fashion.[43] Laboratory testing should include serum parameters of copper metabolism, urinary copper, and liver function tests. Very low urinary copper concentrations and pancytopenia might suggest overtreatment. Similar to other chronic liver diseases, ultrasound screening for hepatocellular carcinoma is justified. However, newly developed hepatic or neurological symptoms, or re-occurrence of clinical findings, such as Kayser-Fleischer rings, are red flags that suggest a non-controlled copper state.

Liver transplantation

Liver transplantation is a rare consideration in Wilson's disease because the condition is usually responsive to medical therapy. Liver transplantation, which corrects the hepatic genotype and restores copper excretion capacity, should be considered in patients with Wilson's disease presenting with acute liver failure (fulminant Wilson's disease) or decompensated (chronic) cirrhosis due to Wilson's disease. Liver transplantation as a treatment option for patients presenting with severe neurological symptoms is controversial.[66–68]

Symptomatic treatment

In line with the lack of improvement of many neurological patients despite adequate de-coppering therapy, about a third of all neurological patients with Wilson's disease can be on symptomatic treatment,

typically botolinum toxin injections for dystonia or primidone for tremor.[69] Thalamotomy or deep brain stimulation might also be beneficial in carefully selected patients with otherwise treatment-resistant tremor or patients who are non-compliant with chelation therapy.[70]

Other copper disorders and mimics

Menkes disease and variants

Mutations in *ATP7A* are typically detected in Menkes disease, an X-linked recessive disorder of impaired copper absorption.[71] Menkes disease typically presents in boys at age 2–3 months, with a subsequent loss of previously obtained developmental milestones and the onset of hypotonia, seizures, and failure to thrive. White matter abnormalities on MRI show impaired myelination. Diffuse brain atrophy, ventriculomegaly, and tortuosity of the cerebral vasculature are also present. Without early treatment, death usually occurs several years after onset. Current management is limited to parenteral copper replacement, which can be highly successful depending on the *ATP7A* mutation type, and the timing of treatment.[72] Adeno-associated virus-mediated gene therapy is emerging as a prospective treatment, which is especially relevant for patients with severe *ATP7A* defects.[73]

The occipital horn syndrome is a milder allelic variant of Menkes disease, so-named in reference to the pathognomonic wedge-shaped calcifications that form within the trapezius and sternocleidomastoid muscles at their attachments to the occipital bone in patients. This protuberance can be palpated in some patients and is shown on lateral and Towne's view skull radiographs, MRI, or sagittal CT images. Occipital horn syndrome has the hair and connective tissue abnormalities associated with classic Menkes disease. Because the neurological phenotype in this variant is mild (slight generalised muscle weakness, dysautonomia, including syncope, orthostatic hypotension, and chronic diarrhoea), affected individuals are often not diagnosed until mid-childhood or later.[71]

A third *ATP7A* phenotype, distal motor neuropathy without overt copper metabolic abnormalities, is associated with mutations in the *ATP7A* copper transporter.[74] This newly recognised *ATP7A* allelic variant includes progressive distal motor neuropathy with minimum or no sensory symptoms. Signs include distal muscle weakness with curled fingers, foot deformities, and diminished deep tendon reflexes. In 12 affected individuals from the two families in which this phenotype was first discovered, the age of onset ranged from 5 to 50 years, with a median onset at 14 years. This wide range of age of onset is similar to that seen in patients with Wilson's disease, implying that environmental or other genetic influences, or both, might be relevant. With the most severe phenotype, the *ATP7A*-related motor neuron phenotype is reminiscent of type 2 Charcot-Marie-Tooth disease (CMT2).[75]

Neurophysiological studies show reduced compound motor amplitudes with normal conduction velocities. Patients with the two ATP7A mutations (Thr994Ile, Pro1386Ser) associated with this phenotype do not manifest the severe infantile CNS deficits reported in Menkes disease, the signs of autonomic dysfunction associated with occipital horn syndrome, the hair and connective tissue abnormalities identified in both conditions, nor any of the typical biochemical features of these well characterised phenotypes, emphasising the distinction between distal motor neuropathy without overt copper metabolic abnormalities and Menkes disease and occipital horn syndrome. The type 2 Charcot-Marie-Tooth disease-like phenotype is due to the altered intracellular localisation of ATP7A and the interaction of ATP7A with P97/VCP, a ubiquitin-selective chaperone.[76] Of note, autosomal dominantly inherited P97/VCP mutations have been identified as a cause of familial amyotrophic lateral sclerosis.[77]

MEDNIK syndrome Two further newly recognised copper metabolism disorders (MEDNIK and Huppke-Brendl syndrome) are inherited as autosomal recessive traits. MEDNIK is an acronym for the syndromic constellation of mental retardation, enteropathy, deafness, neuropathy, ichthyosis, and keratodermia. The causative gene for MEDNIK is *AP1S1,* which encodes the σ 1A small subunit of the adaptor protein complex-1 (AP1). AP1 normally mediates intracellular trafficking of transmembrane proteins.[78] Mutations in *AP1S1* affect systemic copper metabolism by perturbing copper ATPase trafficking, resulting in hypocupremia, hypoceruloplasminemia, and hepatic copper accumulation, similar to that seen in Wilson's disease. Liver disease in patients with MEDNIK might improve strikingly in response to therapy with zinc.[79]

Huppke-Brendl syndrome

Low serum copper and ceruloplasmin concentrations are also characteristic in infants and children homozygous or compound heterozygous for mutations in *SLC33A1,* which encodes the acetylCoA transporter AT-1.[80] AT-1 is needed for acetylation of many gangliosides and glycoproteins, and perhaps the two copper ATPases. AT-1 deficiency causes reduced ceruloplasmin secretion in vitro, suggesting that post-translational acetylation of ceruloplasmin is necessary for secretion. The biochemical phenotype is only one aspect of this condition, now called Huppke-Brendl syndrome, a lethal autosomal-recessive disorder with congenital cataracts, hearing loss, and severe developmental delay. Cerebral MRI in patients with Huppke-Brendl syndrome shows pronounced cerebellar hypoplasia and hypomyelination that is similar to that seen in Menkes disease.[75]

Manganese storage disorder

A newly identified manganese storage disorder, due to autosomal recessively inherited mutations in the manganese transporter gene *SLC30A10,* has been described according to some authors as the new Wilson's disease.[81] Patients with this disorder typically develop

generalised dystonia in childhood and adolescence (2–14 years) or asymmetrical parkinsonism and early postural instability in adulthood (47 and 57 years for the two cases reported with this presentation so far).[82,83] Of note, similar to the hepatolenticular degeneration seen in the natural history of Wilson's disease, patients with this condition also develop hepatic cirrhosis. Typical laboratory findings include polycythaemia, depleted iron stores with low ferritin, high total iron binding capacity, increased serum manganese concentrations, and basal ganglia hyperintensities on T1 brain MRI imaging.[81] The treatment of choice for these patients is repeated intravenous $CaNa_2$-EDTA infusions, leading to a large increase in urinary manganese excretion. Both parkinsonian and dystonic features can greatly improve after such infusions, but a successful treatment response might depend on early diagnosis and initiation of treatment.[81,84]

Aceruloplasminaemia

Aceruloplasminaemia belongs to a group of neurodegenerative disorders in which iron accumulates in the brain. Aceruloplasminaemia can be confused with Wilson's disease because of the very low or absent serum ceruloplasmin (protein) concentrations in patients who present with symptoms and brain imaging findings suggestive of Wilson's disease.[85] Aceruloplasminaemia is thought to be a disorder of iron (not copper) metabolism caused by autosomal recessive mutations in the ceruloplasmin gene. Although ceruloplasmin binds copper, it also acts as an iron oxidase. Loss of ceruloplasmin ferroxidase activity leads to iron accumulation in the pancreas, liver, and brain, whereas iron accumulation does not occur in Wilson's disease.[86] Clinically, aceruloplasminemia is characterised by the triad of retinal degeneration, diabetes, and neurological symptoms, which are present in about two-thirds of all cases.[87] Neurologically, patients typically manifest in the fifth or sixth decade of life with ataxia (in about 70% of patients); involuntary movements, including dystonia, chorea, and tremor (about 60%); cognitive dysfunction (60%); or parkinsonism (20%).[87] Of note, heterozygote ceruloplasmin mutation carriers might also manifest these signs and symptoms.[88]

Search strategy and selection criteria

We identified articles for this Review through PubMed searches using the search terms "Wilson disease", "Wilson's disease", "Menkes disease", "Aceruloplasminemia", "copper and Parkinson's disease", "copper and Alzheimer's disease", "MEDNIK", "ATP7B", "ATP7A", "AP1S1", "SLC33A1", and "SLC30A10" from Jan 1, 2009 to June 30, 2014. Further articles were selected through searches of the reference lists of the articles identified with the above search terms and of the authors' own files. All references used were published in English and were selected according to originality and relevance to the content of this Review.

Diabetes (typically type 2) can precede the neurological symptoms by several decades.[89] In addition to low or absent ceruloplasmin, patients with aceruloplasminaemia typically have high serum ferritin concentrations, low serum iron concentrations, microcytic anaemia, and low serum copper concentrations, with normal urinary copper concentrations. Brain imaging shows hypointensities in the basal ganglia and dentate nucleus, and thalamic signal changes. Attempts to treat aceruloplasminaemia with the iron chelator desferrioxamine have led to inconsistent and largely disappointing results.[87]

Copper in other neurodegenerative disorders

Copper and ceruloplasmin have been implicated in the pathogenesis of both Alzheimer's disease and Parkinson's disease.[90] Copper enhances dimerisation of amyloid precursor protein and promotes amyloid β (Aβ) production.[91] Meta-analyses reported an increase of free (non-ceruloplasmin bound) copper concentrations in the serum and CSF of patients with Alzheimer's disease.[92,93] Furthermore, high free copper concentrations in serum might be associated with a decline in cognitive function in Alzheimer's disease.[94] Copper is essential for LDL receptor-related protein-1 (LRP-1)-mediated Aβ clearance across the blood–brain barrier in mice. In a mouse model of Alzheimer's disease, copper added to the drinking water affected Aβ production and neuroinflammation, even at low copper concentrations.[95] An association between *ATP7B* haplotypes and Alzheimer's disease has been reported in one candidate gene study,[96] by contrast with a recent meta-analysis of genome-wide association (GWAS) studies in Alzheimer's disease, in which the *ATP7B* locus was not identified as an Alzheimer's disease risk locus.[97]

The synucleins are a family of redox-active copper binding proteins.[98] α-synuclein has two copper binding sites, and increased formation of α-synuclein aggregates is the direct result of the formation of an α-synuclein–copper complex.[99] However, copper concentrations are decreased in both the serum and the substantia nigra of patients with Parkinson's disease, making the direct implication of copper in the pathogenesis of Parkinson's disease unlikely.[100,101] By contrast, iron concentrations are increased in Parkinson's disease brain tissue.[90] The copper-containing protein ceruloplasmin has ferroxidase activity, and decreased ceruloplasmin concentrations lead to increased iron deposition in the substantia nigra in Parkinson's disease; therefore, lower ceruloplasmin concentrations might be associated with earlier onset of this condition.[102,103] Concentrations of oxidised ceruloplasmin with reduced ferroxidase activity are increased in the CSF, and there is about an 80% loss of ceruloplasmin ferroxidase activity in the substantia nigra of patients with Parkinson's disease.[104,105] Of note, peripheral infusion of ceruloplasmin attenuated neurodegeneration and nigral iron increase in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease.[104]

Thus, ceruloplasmin upregulation might be a potential disease-modifying strategy for Parkinson's disease.

Conclusions and future directions

The usefulness of direct genetic testing for the diagnosis of Wilson's disease has led to the possibility of a substantially higher prevalence of Wilson's disease than previously estimated, and has also drawn attention to the advantages and disadvantages of the currently available treatments for Wilson's disease. The phenotype for other copper disorders, such as those due to *ATP7A* mutations, has been widened and new early-onset disorders of copper metabolism have been identified. However, little progress has been made to address at least some key issues, including the risk of neurological deterioration after initiation of therapy and the lack of improvement in about a third of all patients with neurological Wilson's disease. Preclinical assessment of experimental therapies for the CNS manifestations of Wilson's disease is complicated by the absence of a neurological phenotype in all *ATP7B* mutant and knockout rodent models of Wilson's disease.[106] A new animal model of Wilson's disease with clear symptomatic brain involvement would therefore be very valuable. Nonetheless, it is plausible to hypothesise that the combination of standard Wilson's disease drugs with other compounds, such as antioxidants, mitochondrial rescue treatments, or compounds to lower sphingomyelinase activity—reported to be increased in Wilson's disease—might improve neurological outcomes.[107] Clinical trials designed to assess these hypotheses are needed. Such trials will be facilitated by the development of a validated neurological rating scale for Wilson's disease.[108]

Gene therapy approaches are novel and potentially effective management strategies for Wilson's disease.[109,110] Liver-directed adeno-associated viral gene therapy could be further assessed in animal models, but questions regarding probable costs and an acceptable benefit versus risk profile remain. Gene-based or cell-based therapies offer the potential to simplify Wilson's disease management by reducing reliance on daily use of therapeutic compounds. Finally, assessment of new and existing treatment strategies by prospective randomised clinical trials would be facilitated by the identification of biomarkers for Wilson's disease, which would enable more objective monitoring of treatment response and disease progression.[111]

Contributors

OB developed the concept of this report and had overall responsibility for the decision to submit for publication. All authors contributed to the writing of the manuscript and subsequent editing.

Declaration of interests

We declare no competing interests.

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