Wilson's disease

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Progressive hepatolenticular degeneration, or Wilson's disease, is a genetic disorder of copper metabolism. Knowledge of the clinical presentations and treatment of the disease are important both to the generalist and to specialists in gastroenterology and hepatology, neurology, psychiatry, and paediatrics. Wilson's disease invariably results in severe disability and death if untreated. The diagnosis is easily overlooked but if discovered early, effective treatments are available that will prevent or reverse many manifestations of this disorder. Studies have identified the role of copper in disease pathogenesis and clinical, biochemical, and genetic markers that can be useful in diagnosis. There are several chelating agents and zinc salts for medical therapy. Liver transplantation corrects the underlying pathophysiology and can be lifesaving. The discovery of the Wilson's disease gene has opened up a new molecular diagnostic approach, and could form the basis of future gene therapy.

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Wilson's disease is a rare autosomal recessive genetic disorder of copper metabolism, which is characterised by hepatic and neurological disease. The disease affects between one in 30 000 and one in 100 000 individuals, and was first described as a syndrome by Kinnier Wilson in 1912.[1] The past two decades have seen major advances in our understanding of the pathogenesis, cellular biology, and molecular genetics of the disease. Most symptoms first appear in the second and third decades of life. In affected individuals, there is accumulation of excess copper in the liver caused by reduced excretion of copper in bile. The great danger is that Wilson's disease is progressive, can remain undiagnosed, and is thought to be fatal if not treated.

Hepatic pathology

In the early stages of the disease, diffuse cytoplasmic copper accumulation can be seen only by special immunohistochemical stains for detecting copper, which are not routinely available. This early accumulation of copper is associated with macrosteatosis, microsteatosis, and glycogenated nuclei which are features that can be seen in various other disorders—eg, nonalcoholic steatohepatitis.[2] The ultrastructural abnormalities range from enlargement and separation of the mitochondrial inner and outer membranes, with widening of the intercristal spaces, to increases in the density and granularity of the matrix, or the occurrence of large vacuoles. In the absence of cholestasis, these changes are regarded as pathognomonic of Wilson's disease. Ultrastructural analysis might be useful for helping to distinguish between heterozygous carriers and patients.

The initial stages of Wilson's disease progress to an intermediate stage, which is characterised by periportal inflammation, mononuclear cellular infiltrates, erosion of the limiting plate, lobular necrosis, and bridging fibrosis, and these features are indistinguishable from those of autoimmune hepatitis.[3,4] Mallory bodies can be seen in up to 50% of biopsy specimens.[5] Cirrhosis almost invariably follows with either a micronodular or a mixed macronodular–micronodular histological pattern. In patients with fulminant hepatic failure, parenchymal apoptosis, necrosis, and collapse might predominate, often with a background of cirrhosis.[6] There are rare reports of older individuals, who present with the disease but do not seem to have liver cirrhosis, although they have neurological disease.

Histochemical confirmation of excess copper can be helpful in diagnosis, but if absent does not exclude Wilson's disease. The lack of immunoreactivity to copper-binding protein can occur because of the diffuse presence of copper in the cytoplasm and because of the assay's low sensitivity. Rhodamine and rubeanic acid stains can show dense granular lysosomal copper deposition in hepatocytes at the stage of cirrhotic nodular regeneration, with noticeable variability from nodule to nodule.[7]

Molecular pathogenesis

The gene responsible for Wilson's disease (on chromosome 13) was identified almost simultaneously by three separate laboratories.[8–11] The gene (*ATP7B*) is highly expressed in the liver, kidney, and placenta. *ATP7B* encodes a transmembrane protein ATPase (ATP7B), which functions as a copper-dependent P-type ATPase. The ATP7B transporter has dual synthetic and excretory roles, functioning in the transport of copper into the trans-Golgi compartment, for incorporation into the plasma protein caeruloplasmin, and into the bile, for excretion of excess stores. Defective ATP7B function results in hepatic copper accumulation, which leads to the hepatic and neurological features of Wilson's disease.

Search strategy and selection criteria

We searched the MEDLINE database from January, 1966, to June, 2006, for specific topics in relation to the search terms "Wilson disease" or "Wilson's disease" in combination with the terms "genetic", "liver disease", "neurology", and "psychiatric". We largely selected publications in the past 5 years, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy, and selected those we judged relevant. Several review articles or book chapters were included because they provide comprehensive overviews that are beyond the scope of this Seminar. We prioritised articles published in high-quality journals, natural history studies, and randomised controlled trials. Personal knowledge and clinical experience was used to complete the review, where gaps in knowledge still remain.

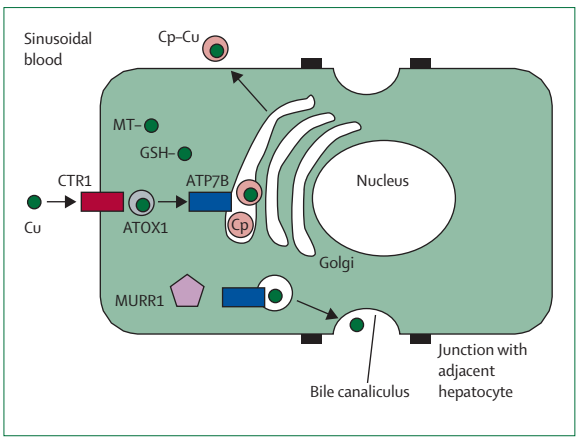

Figure 1: **pathways of copper metabolism in the hepatocyte**
Cu=copper. CTR1=copper transporter 1. MT=metallothioneins. GSH=glutathione. Cp=caeruloplasmin.

Healthy function of ATP7B includes trafficking of the protein between different cellular compartments in response to copper. Studies of polarised HepG2 hepatoma cells cultured in low-copper medium (<1 μM) showed ATP7B localised to the trans-Golgi network. The addition of copper resulted in the redistribution of ATP7B to vesicles and then to vacuoles.[12] This effect is reminiscent of patterns of ATP7B immunohistochemistry seen in human liver, showing punctate immunoreactivity within hepatocytes and staining of the apical membrane, adjacent to the bile canaliculi.[13] Copper-induced trafficking of ATP7B can therefore show the transitions in its cellular role. In low and normal copper states, ATP7B is present in the trans-Golgi network, and is important in the biosynthesis of holocaeruloplasmin. When excess copper is present, the protein moves towards the canalicular aspect of the hepatocyte, where it takes on an excretory role in promoting biliary copper excretion.

For **the HUGO Wilson's disease database** see http://www.uofa-medical-genetics.org/wilson/index.php

Mutation of the protein ATP7B can interrupt its normal cellular processing. The most common ATP7B mutation found in patients of European origin is the histidine to glutamate substitution at aminoacid 1069 (H1069Q). In the hepatocytes of patients with Wilson's disease homozygous for H1069Q, ATP7B was mislocalised to the endoplasmic reticulum consistent with a failure of the mutant protein to undergo normal trafficking to its usual resident position in the trans-Golgi network.[14] By use of baculovirus to express wild-type and mutant ATP7B in insect SF9 cells, the H1069Q mutation was shown not to result in major misfolding, but the normal catalytic phosphorylation of ATP7B by adenosine triphosphate (ATP) was substantially decreased. Histidine 1069 is located in a conserved motif within the ATP-binding domain of ATP7B. Therefore, histidine 1069 might be needed for the correct orientation of ATP in the ATP7B catalytic site before ATP hydrolysis.[15]

In the human hepatocyte, ATP7B represents one step in the network of copper metabolism pathways that are increasingly being characterised at molecular level (figure 1). Dietary copper is absorbed in the stomach and duodenum and transported via the portal vein to the liver, which is the main organ responsible for copper homoeostasis. Copper is taken up into the hepatocyte via copper transporter 1 (CTR1) on the sinusoidal aspect of the hepatocyte. In mice, CTR1 is essential for healthy development and is thought to be the main mechanism for uptake of copper into mammalian cells. Knockout of the *CTR1* gene results in embryonic lethality.[16,17] In the cytoplasm, glutathione and metallothionein proteins are important scavengers, protecting the cell from copper's toxic effects. A specific copper chaperone, ATOX1, delivers copper to the Wilson's disease protein, ATP7B, by copper-dependent protein-protein interaction.[18] ATP7B brings about transport of copper into the trans-Golgi and holo-caeruloplasmin and, under conditions of copper loading, into vesicles for export of copper into bile.

The biliary excretion process includes another protein, COMMD1 (originally called MURR1), which interacts directly with ATP7B.[19] Mutation of COMMD1 causes the copper toxicosis of Bedlington terriers—an autosomal recessive disorder that involves hepatic copper overload and deficient biliary copper excretion.[19] However, sequencing and haplotype analysis reported no evidence to implicate the COMMD1 protein in copper-storage disorders of undefined aetiology in human beings.[20] The copper metabolism pathway has been found to regulate the metabolism of chemotherapeutic drugs containing platinum—eg, cisplatin. CTR1 has been identified as the uptake mechanism for entry of these agents into tumour cells, and the copper-transporters ATP7A and ATP7B regulate their efflux. Thus, understanding the copper transport pathway could provide insight into the development of resistance to these anticancer agents.[21]

Clinical applications of Wilson's disease genetics

The Human Genome Organisation (HUGO) database for Wilson's disease lists roughly 300 different mutations described in the disease, which are distributed across the *ATP7B* gene. As a result, the distribution of *ATP7B* genotypes is complex and most patients are compound heterozygotes, having two different mutations of the *ATP7B* gene. In general, a few mutations predominate, depending on the population tested. Therefore, in molecular diagnosis, selected exons are chosen for initial screening according to the population group. Vrabelova and colleagues[22] reported that screening of five prevalent mutations in patients from the Czech Republic and Slovakia detected 70% of Wilson's disease mutant alleles.

The H1069Q mutation represents 37–63% of mutations in studies of white populations. Other mutations are prevalent in non-European populations and in isolated ethnic groups. In Chinese patients, H1069Q seems to be absent, but R778L has been reported to represent 34–38% of mutations.[23,24] In a study of Indian patients with the disease, neither H1069Q nor R778L was detected.[25] In

Saudi Arabian patients, a founder effect was detected, with a predominant 4193delC mutation present in many members of one tribe.[26] Prevalent disease mutations have also been detected in isolated populations, including Iceland and Gran Canaria (one of the Canary Islands).

The homozygous H1069Q genotype has been detected frequently in several studies of white patients with Wilson's disease, and has therefore been widely used as a model to investigate possible genotype-phenotype relations. Although an association has not been universally reported, homozygous H1069Q has been associated with late onset and neurological disease in several studies, including a meta-analysis of 577 patients.[22,27]

Wilson's disease is an autosomal recessive disorder, which means that there is a 25% chance that a sibling of the index case has Wilson's disease. Once homozygous or compound heterozygous mutations in *ATP7B* have been established in the index patient, then mutation detection is valuable in family screening. The same genotype in asymptomatic family members confirms diagnosis of the disease, thus allowing early treatment before the onset of complications. In family members in whom clinical and biochemical features are uncertain, the demonstration of either heterozygous (carrier) or wild-type gene sequence prevents unnecessary treatment.[28]

If the index patient has a secure diagnosis of Wilson's disease on the basis of clinical and biochemical evidence but testing for ATP7B mutations is not available, family screening can be done by haplotype analysis of polymorphic markers flanking the disease gene.[29] In this instance, the rare possibility of recombination events (typically between 0·5 and 5% of cases) needs to be considered. The rate of recombination is dependent on which flanking markers are studied.

Genetic testing for *ATP7B* mutations can be valuable to confirm a diagnosis of Wilson's disease, especially when presentation is unusual. This situation has been drawn attention to by the molecular confirmation of early-onset hepatic disease in a 3-year-old child.[30] Mutation analysis has also confirmed late-onset disease, including the case of two siblings in their 70s—the oldest reported patients so far at time of diagnosis.[31–33]

ATP7B mutation analysis makes an important contribution to clinical practice. Unfortunately, systematic genetic testing for Wilson's disease is still difficult and fairly expensive because of many different mutations, the occurrence of regulatory mutations in non-coding sequence, the large size of the gene that spans around 80 kb, and the restrictions of present methods. However, technical advances allowing high-throughput screening could be applied to the disease.[34] This new apparatus can sequence six million base pairs of DNA per hour with an accuracy greater than 99%. Such advances might permit specialised laboratories to sequence the entire genomic Wilson's disease gene from patients, including not only the translated exons, but also the important non-coding sequences that are not normally investigated, to detect all mutations.

Clinical manifestations and range of disease

The clinical range of Wilson's disease is wide, and knowledge of the various disease presentations is important (panel 1). In broad terms, patients can present acutely with liver failure, haemolysis, or both, or more chronically with liver disease, neurological disease, or both.

Patients who first present with neurological or psychiatric signs tend to be older than those with hepatic features alone. Most patients with CNS involvement are believed to have liver disease at the time of presentation but they are often not symptomatic from their liver disease. However, hepatic histology is not generally available for these patients because the diagnosis is usually established on the basis of Kayser-Fleischer (K-F) rings (an ophthalmic manifestation of the disease) and decreased caeruloplasmin concentrations.

Hepatic disease

Wilson's disease can present as fulminant hepatic failure—ie, worsening coagulopathy and encephalopathy with an associated Coombs negative haemolytic anaemia,

***Panel 1:* Clinical manifestations of Wilson's disease**

Hepatic
- Persistently elevated serum aminotransferases
- Chronic hepatitis
- Cirrhosis (decompensated or compensated)
- Fulminant hepatic failure (+/- haemolytic anaemia)

Neurological
- Tremor
- Choreiform movements
- Parkinsonism or akinetic rigid syndrome—ie, partial parkinsonism
- Gait disturbances
- Dysarthria
- Pseudobulbar palsy
- Rigid dystonia
- Seizures
- Migraine headaches
- Insomnia

Ophthalmic
- K-F rings
- Sunflower cataracts

Psychiatric
- Depression
- Neuroses
- Personality changes
- Psychosis

Other systems (rare)
- Renal abnormalities: aminoaciduria and nephrolithiasis

renal failure, and substantially increased serum and urinary concentrations of copper. Around 5% of patients present in this manner[35] with most patients being in the second decade of life, when K-F rings might not yet be apparent. Almost all patients are already cirrhotic, though some might show evidence of massive necrosis with only bridging fibrosis, which clearly would progress to cirrhosis with time. Concentrations of serum alkaline phosphatase are frequently depressed, and this feature has led to the finding that a ratio of alkaline phosphatase concentration (IU/L) to bilirubin concentration (mg/dL) of less than two might be diagnostic of Wilsonian fulminant hepatitis.[36,37] Transient low-grade haemolysis can take place even when liver disease is not clinically evident.[38]

The clinical picture can be similar to other forms of chronic hepatitis, which emphasises the need to screen such patients for Wilson's disease. In patients presenting with liver disease, neurological features (if they occur) usually do so 2–5 years later.[39,40]

Cirrhosis

The patient might present with insidious cirrhosis. Clinical features of this disease include spider naevi, splenomegaly, portal hypertension, and ascites. In some patients cirrhosis is well compensated. All young patients with unexplained chronic liver disease, with or without cirrhosis, should be screened for Wilson's disease.

Hepatocellular carcinoma is rarely associated with Wilson's disease, but may occur in the setting of cirrhosis and chronic inflammation.[41,42] 11 cases have been reported thus far, and it is thought that men with longstanding treated Wilson's disease have the greater risk of developing hepatocellular carcinoma.

Eye changes

Ophthalmic findings include K-F rings (figure 2) and sunflower cataracts.[43,44] Both findings are reversible with medical therapy or after liver transplantation. The reappearance of either of these eye changes in a medically treated patient suggests non-compliance with therapy.[45,46]

K-F rings are most apparent at the periphery of the cornea. They are caused by the granular deposition of copper on the inner surface of the cornea in Descemet's membrane. The upper pole is affected first. The rings have a golden brown appearance. Although sometimes visible to the naked eye, slit lamp examination is necessary to confirm the presence or absence of K-F rings. Rings indistinguishable from K-F rings have also been seen in other forms of chronic liver diseases, especially longlasting cholestasis and cryptogenic cirrhosis.[47,48]

Sunflower cataracts are brilliantly multicoloured and are visible only by slit-lamp examination. They do not impair vision. Other less common findings include night blindness, exotropic strabismus, optic neuritis, and optic disc pallor.[49]

Neurological and neuropsychiatric disease

Neurological and neuropsychiatric signs are the presenting features in 40–50% of patients with Wilson's disease.[50] The neurological abnormalities can be classified as: (a) an akinetic-rigid syndrome similar to Parkinson's disease, (b) pseudosclerosis dominated by tremor, (c) ataxia, and (d) a dystonic syndrome.[51] Subtle signs can appear before the characteristic neurological features, including changes in behaviour, deterioration of school work, or an inability to carry out activities that need good hand-eye coordination. Handwriting might deteriorate and micrographia—as in Parkinson's disease—could develop.[52] Other common neurological findings include tremor, lack of motor coordination, drooling, dysarthria, dystonia, and spasticity.[53] Migraine, headaches, and insomnia have also been reported, although seizures could be more common.[54–56] Along with behavioural changes, other psychiatric manifestations include depression, anxiety, and frank psychosis.[57–60]

Advances in neuroimaging have helped to improve our understanding of the pathophysiology of Wilson's disease. Structural brain MRI in patients with the disease has shown widespread lesions in the putamen, globus pallidus, caudate, thalamus, midbrain, pons, and cerebellum as well as cortical atrophy and white matter changes. In general, these lesions show high-signal intensity on T2 weighted images and low-intensity on T1 scan.[61,62] Although MRI changes are present in many Wilson's disease patients, even patients without neurological symptoms, these changes tend to be more severe and widespread in patients with neurological Wilson's disease.[63] Proton-density MRI sequences seem to be especially sensitive in showing the extent of the neuropathology.[64] Histologically, there is an increase in astrocytes within the grey matter, associated with swollen glia, liquefaction, and appearances of spongiform degeneration. Neuronal loss is often accompanied by gliosis and active glial fibrillary protein. The characteristic astrocytes are Alzheimer type 1 and 2 cells. Opalski cells are distinctive for Wilson's disease,[65] and these are fairly large (up to 35 μm in diameter), with fine granular

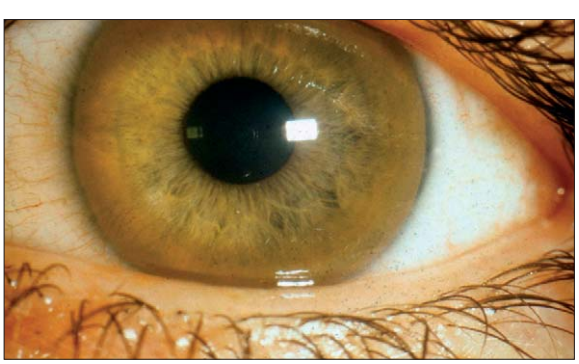

Figure 2: **Kayser-Fleischer (K-F) ring**
There is a brown discolouration at the outer margin of the cornea because of the deposition of copper in Descemet's membrane. Here it is clearly seen against the light green iris. Slit lamp examination is required for secure detection.

cytoplasm and slightly abnormal nuclei (single or multiple). These cells are thought to originate from degenerating astrocytes.[66]

Cognitive dysfunction in patients with Wilson's disease can accompany neurological deficits, often in the absence of detectable cortical association or hepatic encephalopathy, which lends support to the importance of pathological changes in basal ganglia as the primary cause of cognitive deficits in the disease.[67]

Neurological assessment should be undertaken on all patients with Wilson's disease. Patients with obvious symptoms or signs should be seen by a neurologist or movement disorder specialist before treatment. A specific rating scale (based on that for Huntington's disease) has been used in clinical trials to assess patients[68]—however, this scale has never been tested outside of this research setting.

Other changes

Pathological changes of bone and periarticular abnormalities have been recorded to account for osteomalacia, osteoporosis, spontaneous fractures, adult rickets, osteoarthritis, osteochondritis dissecans, chrondocalcinosis, subchondral cyst formation, and azure lunulae of the fingernails.[69] The knee joints and spine are the most common sites for skeletal and articular abnormalities.[70] Myocardial copper accumulation can cause cardiomyopathy and arrhythmias, although these are clinically rare.[71,72] Other rare extrahepatic manifestations include hypoparathyroidism,[73,74] infertility, repeated miscarriages,[75–77] and renal abnormalities,[78,79] including aminoaciduria and nephrocalcinosis.

Establishing a diagnosis

There is no one test for the diagnosis of Wilson's disease (panel 2). The diagnostic challenge is that the symptoms are often non-specific and the disease affects many different organ systems, which results in confusion with other disorders. The diagnosis is easy to establish in individuals with neurological symptoms, K-F rings, and a low caeruloplasmin concentration. The absence of K-F rings does not necessarily exclude the possibility of this disease but in patients with predominantly neurological disease, K-F rings are absent in only 2% or less of cases. In patients with liver disease as the presenting feature, the diagnosis can be more difficult. Molecular analysis of ATP7B mutations, (if available), can potentially be diagnostic. However, this method is expensive, and will not necessarily detect all disease producing mutations.

To help with diagnosis, Ferenci and co-workers[80] proposed a scoring system. Clinical, biochemical, and histological features were allocated a score and the total accumulated score indicated the possibility of the patient having Wilson's disease. Although helpful if a diagnosis of the disease is being considered, this proposed scoring system has not been assessed prospectively.

***Panel 2:* Diagnostic recommendations in Wilson's disease**

- Wilson's disease should be considered in any individual with liver abnormalities of uncertain cause and those with new onset movement disorders.
- Assessment should include history, physical examination, liver function tests, full blood count, serum copper and caeruloplasmin, and 24-h urinary copper excretion. Liver biopsy quantitative copper concentrations remain the best biochemical evidence for Wilson's disease.
- Kayser-Fleischer rings should be sought using slit-lamp examination by a skilled examiner.
- Family screening of first-degree relatives must be undertaken. When possible, genetic diagnosis should be used, especially in patients with indeterminate clinical and biochemical features.

Roberts and Schilsky[81] provide formal guidelines for current diagnostic approaches and treatment. These guidelines were prepared for the American Association for the Study of Liver Diseases and provide specific recommendations on the basis of previous published work and the researchers' experience in caring for paediatric and adult patients with Wilson's disease.

Serum aminotransferase activity is generally abnormal in this disease except at a very early stage. For many individuals, the degree of raised aminotransferese activity might be mild and does not necessarily reflect the severity of the liver disease.

Caeruloplasmin

A caeruloplasmin concentration of less than 0·2 g/L (normal laboratory range 0·2 to 0·5 g/L), has been regarded to be consistent with Wilson's disease and diagnostic in association with K-F rings. Up to 95% of homozygotes and 20% of asymptomatic heterozygotes have serum caeruloplasmin values less than 0·2 g/L. 5% of homozygotes, and in some studies up to 50% of affected individuals with severe decompensated liver disease, have normal caeruloplasmin concentrations.[82] One explanation for this finding is that caeruloplasmin is an acute-phase reactant and concentrations can be raised into the normal range by inflammation. Conversely, low concentrations of caeruloplasmin can be seen in hypoproteinaemic states. Low concentrations also occur in Menke's disease and acaeruloplasminaemia—both of which are very rare disorders.[83,84]

Hepatic copper

The normal copper content of liver is less than 55 µg/g dry weight. Accurate analysis needs an adequate sample of liver (at least 1 cm of a 1·6 mm diameter core). A hepatic copper concentration greater than 250 µg/g dry weight is usual in homozygous Wilson's disease and with some caveats remains the best biochemical test for the disease. Measurement of copper content in liver is the most important diagnostic test in patients in whom other data are suggestive but not diagnostic of disease.

Diagnosis is sometimes only considered retrospectively, after liver biopsy has been done. Under these circumstances, liver biopsy specimens can be retrieved from paraffin blocks for quantitative copper measurement. However, the sensitivity and specificity of this test have never been clearly established.

Thus, liver copper content is an important indicator of disease, although a value below 250 µg/g dry weight does not exclude the possibility of disease. Specimens with extensive fibrosis and few parenchymal cells can provide copper concentrations that are falsely low. Furthermore, greatly increased hepatic copper concentrations can be seen in long-term cholestasis. Therefore, the results of the hepatic copper concentration estimation should be taken in the context of the histological, clinical, and biochemical data.[85]

Urinary excretion of copper

Urinary copper is derived from the so-called free (non-caeruloplasmin-bound) copper circulating in plasma. In Wilson's disease, the 24-h urinary copper excretion is increased, and the concentration taken as suggestive of disease is greater than 100 µg per 24 h (>1·6 µmol/24 h).[86–88] The reference limits for normal 24-h excretion of copper vary between laboratories, with many taking 40 µg per 24 h (0·6 µmol/24 h) as the upper limit of normal. This limit seems to be a better threshold for diagnosis because testing sensitivity is increased. Results can be difficult to assess unless strict precautions are taken. Wide-necked bottles with copper-free disposable polyethylene liners have been recommended.

Interpretation of 24-h urinary copper excretion can be difficult because of an overlap with results in other types of liver disease, especially severe liver injury. Heterozygotes could also have intermediate levels for 24-h copper excretion. Urinary copper excretion with penicillamine administration can also be a useful diagnostic adjunctive test. This test has only been standardised in the paediatric population, in whom 500 mg of d-penicillamine was given orally at the beginning and again 12 h later during 24-h urine collection. Copper excretion greater than 25 µmol per 24 h (1600 µg copper/24 h) was regarded as diagnostic for paediatric Wilson's disease.[89] In adults and heterozygote carriers, the predictive value and usefulness of using penicillamine in testing is unknown.

Family screening

First-degree relatives must be screened for Wilson's disease. The probability of finding a homozygote in siblings is 25% and in the children is roughly 0·5%. Liver function tests, serum copper and caeruloplasmin concentration, and urinary copper analysis are done for relatives. If necessary, investigations should be extended to test for K-F rings. 24-h urinary copper might be difficult to interpret in Wilson's disease heterozygotes.

The diagnosis could remain contentious when individuals without K-F rings have a low caeruloplasmin concentration. These individuals might need a liver biopsy for hepatic copper quantification to eliminate the diagnosis. Molecular genetic analysis is becoming more widely available and is useful for families in whom both mutations have been detected in the index patient, allowing molecular analysis for the same mutations in siblings.

Haplotype analysis of markers around the *ATP7B* gene on chromosome 13 has been used in families to establish whether siblings of affected individuals have inherited the same pair of chromosomes. This approach would be useful when it has not been possible to detect both mutations in the index case by mutation analysis.

Treatment

The drug treatment of Wilson's disease is based on the use of copper chelators to promote copper excretion from the body, or zinc to reduce copper absorption, or both. Liver transplantation is successful for patients with liver failure that is unresponsive to medical treatment.

Wilson's disease was progressive and fatal until 1951, when the first chelating agent dimercaprol given intramuscularly was used. In 1956, John Walshe[90] reported the clinical benefit of the orally active chelator penicillamine, which revolutionised treatment of the disease. However, some patients did not tolerate penicillamine, and in 1969, trientine was introduced as an alternative chelator.[91] These two agents have remained the mainstay of chelation treatment for patients with Wilson's disease. Ammonium tetrathiomolybdate, used by veterinarians for treating copper poisoning in animals, is another chelator, which is under assessment in the USA for treatment of patients with neurological Wilson's disease.[92] This chelator remains an investigational drug, not yet available in the UK nor outside clinical trials in the USA. Zinc was first used in treatment in the early 1960s and has been studied in the USA particularly, gaining recognition for asymptomatic and presymptomatic patients and as maintenance therapy after an initial period of treatment with a chelator.[93]

Historically, penicillamine has been the treatment of choice, on the basis of clinical data and many years of experience. However, side-effects and neurological deterioration in some patients after starting treatment have led to the suggestion that trientine is an effective and safer alternative initial therapy. A randomised double blind study[94] comparing trientine with ammonium tetrathiomolybdate in patients with neurological presentation found that tetrathiomolybdate might be better than trientine. No randomised trials exist in patients with liver disease. After initial chelation therapy, usually until clinical improvement had been noted, the choice for maintenance therapy is between reduction in the dose of chelator or zinc monotherapy.

The best therapeutic approach remains controversial and there is no universally accepted regimen (panel 3). We have to emphasise two aspects of care to optimise

clinical outcome—first, proper monitoring of patients and second, support to ensure compliance with whichever regimen is used. Compliance is a problem for patients, because they find it difficult to take life-long treatment when they feel healthy.

Penicillamine

Penicillamine is cysteine, doubly substituted with methyl groups. A free sulphydryl group acts as the copper-chelator. Total bioavailability after oral administration is 40–70%. More than 80% of penicillamine excretion is in urine, with chelated copper. Penicillamine can also induce metallothionein, a cysteine-rich protein that is an endogenous chelator of metals. Thus, penicillamine enhances urinary copper excretion but can also lead to the sequestration of free intracellular copper.

The initial dose of penicillamine is 1000–1500 mg per day in two to four divided doses. The treatment is best taken 1 h before or 2 h after food. Absorption might only be 50% if it is taken with a meal. The use of lower initial doses, 250–500 mg per day, increasing over a few weeks, can increase tolerance to the drug. Regular monitoring of full blood count and urinary protein (using dipsticks) is recommended because of possible adverse effects, which occur in 10–20% of patients and can be severe enough to lead to the treatment being stopped.[95] Early side-effects in the first 1–3 weeks include sensitivity reactions with fever, rash, lymphadenopathy, neutropenia, thrombocytopenia, and proteinuria.[95] If these adverse effects are noticed, then penicillamine should be stopped and an alternative treatment used. Later side-effects include nephrotoxicity (a lupus-like syndrome) and bone marrow suppression (eg, thrombocytopenia and aplasia). Skin complications have arisen with long-term use of penicillamine, including progeriatric changes (with long-term doses greater than 1000 mg per day), elastosis perforans serpiginosa, and aphthous stomatitis.[96] Furthermore, penicillamine can affect pyridoxine metabolism, and this vitamin (vitamin B6) should therefore be given (50 mg weekly) to children, pregnant women, and patients with malnutrition or an intercurrent illness.[97]

The clinical benefit of penicillamine in Wilson's disease is well documented.[96,98] In patients with severe liver disease—eg, patients with high bilirubin or low albumin concentrations, prolonged prothrombin time, ascites, or high Child-Turcott score—hepatic function usually improves. In those who deteriorate, either the penicillamine dose can be increased for a trial period or the patient listed for urgent liver transplantation. In patients with neurological disease, gradual clinical and cerebral MRI improvement is well documented.[62]

However, the side-effects and the initial neurological deterioration, reported in 20–50% of patients with a neurological presentation[99,100] and which in some cases cannot be reversed, have led to other agents being considered for first-line treatment.

***Panel 3:* Medical treatment recommendations in Wilson's disease**

- Treatment recommendations for Wilson's disease are in transition. There is increasing confidence in the use of trientine rather than penicillamine for initial chelation therapy of neurological and hepatic disease.
- Zinc alone has been advocated for presymptomatic and asymptomatic Wilson's disease, although some clinicians still prefer a chelator. For maintenance therapy, reduced dose of chelator or replacement with zinc alone are options.
- There is a need for lifelong follow-up by specialist units to monitor clinical progress (symptoms, signs, and laboratory tests), to be alert to the side effects of drugs, and to encourage compliance.
- To encourage clinicians in structured monitoring, standardised regimens and assessment, with the publication of outcomes data. Databases (eg, http://www.eurowilson.org) and future international collaborations will play an important part in providing these data.
- Patients' associations have an important role in supporting patients and families affected by Wilson's disease.

Trientine

As evidence grows for the effectiveness of trientine, with fewer side-effects arising than with penicillamine, trientine is now regarded as an accepted alternative to penicillamine for initial treatment of Wilson's disease. Trientine has a polyamine structure, which chelates copper by the formation of stable complexes with the four constituent nitrogens in a planar ring. Although commonly regarded as a weaker chelator of copper than penicillamine, there is still some debate—it could be that these two chelators mobilise different pools of body copper. The initial dose is 1200–1800 mg per day in two to three divided doses. Maintenance therapy is 900–1200 mg per day. As in the case of penicillamine, the timing of oral administration in relation to food is important.

Trientine is becoming recognised as an effective initial treatment[100,101] since there are few reported side-effects—pancytopenia occurs rarely and hypersensitivity reactions and renal effects have not been reported. Sideroblastic anaemia and hepatic siderosis can occur if copper deficiency develops because of excessive treatment. The frequency of neurological deterioration is thought to be less with trientine than with penicillamine, but could still arise.[94] Data for patients with severe liver disease have been reported. Askari and colleagues[102] studied nine adults with severe liver disease identified over a 10 year period, who received initial treatment with trientine (1000 mg/day) and zinc (150 mg/day). Only one patient had hepatic encephalopathy. One patient developed mild neurological symptoms and was given ammonium tetrathiomolybdate and zinc after 2 weeks of the original treatment. In the eight patients receiving trientine and zinc, the combination was given for at least 4 months and then maintenance zinc treatment was used. Over the first 12 months of treatment, prothrombin time and raised bilirubin and albumin concentrations returned to normal, and ascites disappeared. Benefit was maintained over 12 months to 14 years of follow-up. These are encouraging data. The

For **the EuroWilson database** see http://www.eurowilson.org

European database, EuroWilson, set-up for newly diagnosed patients with Wilson's disease, might add information regarding the effectiveness of penicillamine and trientine for patients with severe hepatic presentation.

Ammonium tetrathiomolybdate

Ammonium tetrathiomolybdate forms a complex with copper and protein. Taken with meals, the drug forms complexes with copper in the food and that secreted into the intestine, thus preventing absorption. Taken between meals, the drug is absorbed and complexes copper in the blood with albumin. This complex is metabolised by the liver and excreted in bile.[103] A randomised trial[94] compared the efficacy of ammonium tetrathiomolybdate in patients with neurological Wilson's disease with that of trientine (both groups also received zinc). In the ammonium tetrathiomolybdate group, one of 27 patients had neurological deterioration, compared with five of 27 patients in the trientine group. Anaemia or leucopenia occurred in three patients in the ammonium tetrathiomolybdate group, and four patients had increased aminotransferase concentration, although these side-effects resolved on dose reduction.

Zinc

Zinc induces intestinal metallothionein, which preferentially binds to copper within the duodenal enterocyte. Copper absorption into the circulation is reduced, and copper is lost when the enterocyte is shed during normal cell turnover. Without normal absorption but with continuing copper losses there is a negative copper balance. Furthermore zinc can induce copper-binding metallothionein in hepatocytes, thereby reducing the damaging effects of free copper.

The dose of zinc for adults is 150 mg per day of elemental zinc given in three doses. Food interferes with absorption but patients vary in their ability to comply with the recommended separation from food. However, if this recommendation is difficult for the patient the dose can be adjusted. Dyspepsia can be a troublesome side-effect, and changing the formulation (to acetate, sulphate, or gluconate) and timing of administration can help.

Zinc has been used successfully in asymptomatic or presymptomatic affected family members of individuals with Wilson's disease.[93] Czlonkowska and co-workers[104] reported that zinc is equally as effective as penicillamine in a group of patients predominantly with neurological disease, with a follow-up of 12 years. In patients with severe hepatic disease, maintenance therapy with zinc was effective after an initial period of treatment with trientine and zinc (given at separate times).[102]

Present choice of medical treatment

Virtually all the data on the treatment options are from clinical series of patients rather than randomised studies, which makes definitive recommendations difficult.[81,99,100] Moreover, clinical deterioration has been reported or alluded to in reviews for all treatment methods, showing that none is totally effective or reliable. Variables confounding outcome include the clinical phase and pattern of disease when treatment starts and patients' compliance with treatment.

We can conclude that treatment for Wilson's disease is generally very effective. Although many physicians still use penicillamine as the first choice chelator for symptomatic patients, data suggest that trientine is as effective and has fewer side-effects, especially in those with neurological onset. Combined treatment with zinc, separated appropriately from trientine, has been used without any disadvantage. For asymptomatic patients many will use this approach initially, although others judge that zinc therapy is sufficient.[93] For maintenance therapy of patients who are initially symptomatic and have responded to chelator treatment, the dose of the chelator can be reduced or replaced with zinc. Clinical follow-up and monitoring of copper concentrations and excretion are essential, coupled with patients' support to encourage compliance with which ever therapy is used.

The most difficult challenge is how to manage patients who deteriorate, despite what seems to be best possible therapy. Liver transplantation is available for hepatic disease, but for patients with neurological deterioration no such option is available. Studies that attempt to identify clinical features associated with neurological deterioration have identified possible brain MRI changes that need further assessment.[105] Although some clinicians start with a lower dose of chelator and then increase the dose subsequently, currently there is no evidence that this approach reduces the risk of neurological deterioration. If neurological deterioration takes place, withdrawal of the chelator and then reintroduction of the chelator at low-dose with escalation has been reported with some benefit,[105] but data supporting this approach are only anecdotal. The decision of whether to continue with the chosen treatment or change to or add another agent, or administer dimercaprol intramuscularly (despite associated discomfort and potential complications) is difficult even for clinicians with experience of treating Wilson's disease. If ammonium tetrathiomolybdate were to become widely available, patients would have another potentially useful treatment option, and Brewer and colleagues[94] suggest that this drug could have a lower risk of neurological deterioration.

Other therapeutic agents

Toxic concentrations of copper in the liver produce oxidant damage to mitochondria with lipid peroxidation, which can be reduced experimentally by vitamin E administration. Vitamin E concentrations may be low in patients with Wilson's disease.[106] However, there are no data to substantiate the administration of vitamin E in patients with disease, which is also the case for N-acetylcysteine.

Functional imaging can prove useful in treating patients, especially in exploring possibilities for new therapies. Magnetic resonance spectroscopy has shown reduced amounts of striatal N-acetylaspartate, a marker of neuronal health, in Wilson's disease patients with neurological symptoms compared with those without disease.[64] If this reduction is proven to be caused by reversible persistent neuronal dysfunction, it could provide a target for neuronal rescue therapy. Single-photon-emission computed tomography (SPECT) imaging has shown presynaptic and postsynaptic deficits in the dopaminergic system of patients with this disease.[64] SPECT and positron-emission tomography might therefore have a role in identifying a subpopulation of patients with predominantly presynaptic dopaminergic deficits, who could be potential candidates for dopamine replacement therapy.[107,108] There is no evidence for the potential role of functional neurosurgery in the management of neurological symptoms, including dystonia, in Wilson's disease.

Diet

Some foods—eg, chocolate, liver, nuts, mushrooms, and shellfish—contain high concentrations of copper and in general are best avoided.

Monitoring of medical treatment

Patients on initial therapy should have follow-up appropriate to the severity of their neurological or hepatic features. Neurological assessment and monitoring of liver function tests should be done, and signs of hepatic decompensation assessed.

During chelation therapy, 24-h urinary copper excretion is measured and an output of 3–8 μmol per day (200–500 μg) denotes adequate treatment. Some experts recommend collection of urine after stopping chelator for 48 h (no chelator is taken on the third day), to assess the urinary copper excretion while off treatment. Which of these approaches is best is not known. During zinc therapy, 24-h copper excretion is also measured with a target of less than 2·0 μmol per day (less than 125 μg) to suggest satisfactory treatment. Urinary output of zinc is also measured to show whether sufficient zinc is being taken and absorbed, and to show patients' compliance.

During all treatments, whether with a chelator or zinc, or both, an estimation of non-caeruloplasmin-bound (ie, so-called free) copper is made from the measurements of total copper and caeruloplasmin. However, caeruloplasmin is almost universally measured with an immunological rather than enzymatic assay, which challenges the accuracy of this assessment. The target is a non-caeruloplasmin-bound copper concentration of between 50 and 150 μg/L.

Liver transplantation for Wilson's disease

The oldest Wilson's disease patient to undergo a liver transplantation is now 30 years past his initial transplant.[109] Since that original report, there have been more than 370 reported liver transplantations for the disease.[109,110] Although transplantation is an effective cure, there are risks associated with the procedure and the immunosuppressive therapy that follows.[46] Ideally, family screening and development of efficient population screening for Wilson's disease will eventually reduce the number of patients with this disorder requiring liver transplantation.

Liver transplantation is clearly indicated for patients with acute fulminant hepatic failure from Wilson's disease. Fulminant hepatic failure describes the development of coagulopathy and encephalopathy as a result of acute hepatic deterioration within 8 weeks from the onset of illness. There are some reports of cure by medical therapy of rare patients who have acute liver disease and even haemolytic anaemia caused by Wilson's disease.[111] However, failure to act promptly to stabilise and transplant those with true fulminant failure is important to avoid progressive encephalopathy and cerebral oedema and multiorgan failure. Liver transplantation of these individuals can be achieved by a cadaveric donor or living donor transplant, even if the donor is a heterozygous carrier.

Liver transplantation is also indicated for patients with Wilson's disease in whom medical therapy is ineffective, as defined by a failure to stabilise and prevent progressive hepatic insufficiency.[92] These patients include those whose disease was discovered after manifestation of cirrhosis and severe hepatic insufficiency, and those who might have been successfully treated but discontinued their treatment and then deteriorated or developed some secondary liver injury, leading to worsening disease. This group includes a subset of patients, who have neurological and hepatic disease, but in whom hepatic symptoms are predominant. The difficulty is in how to define an adequate treatment trial for these patients and what constitutes a true failure of medical therapy. In view of the delay between the initiation of treatment and measurable objective laboratory response, often a gap of up to 6–8 weeks, the previous recommended interval for a medical trial was for 3 months' time. Although this 3-month suggested trial is not absolute since some patients will deteriorate before this time has elapsed, careful monitoring is essential to detect these individuals for whom transplantation might be more urgently needed. If the patient stabilises in this time, there is hope for the long-term use of medical therapy and avoidance of liver transplantation.

In whom would liver transplantation for Wilson's disease be medically futile? Individuals who are not suitable candidates for transplantation under any circumstances include those with liver failure, severe cerebral oedema, long-term reduction of cerebral perfusion pressure, active infections, malignancies, or those with severe psychiatric disease with suicidal ideation. Similarly, patients with severe long-standing neurological impairment from Wilson's disease are unlikely to recover after transplantation. The failure of transplantation to

improve neurological disease and the increased frequency of post-transplant complications caused by calcineurin inhibitors used to prevent rejection are not routinely reported, but have occurred on both sides of the Atlantic (unpublished findings). In view of the acute shortage of donor organs and our growing waiting lists for liver transplant recipients, the use of an organ for liver disease that can be stabilised medically is not easily justified. This is especially true when the risk of potential worsening of neurological disease is uncertain.

A new factor in this dilemma has arisen, with the arrival of living-donor liver transplantation.[112] This procedure removes the concern about misallocation of a donor organ from the public donor pool, but has different risks associated with the procedure for the donor and recipient. However, many treatment programmes that undertake this procedure insist that the same indications for deceased liver transplant be applied to those for living donor transplant to avoid undue risk to the donor and recipient.

Patients with less severe neurological impairment caused by Wilson's disease present a unique dilemma with respect to treatment by liver transplantation.[113,114] There are reports of improved neurological and psychiatric disease in patients with this disorder who had liver transplant for their liver disease, and in some patients for whom transplantation was undertaken for their neurological impairment. The decision of whether to choose transplantation rather than medical treatment is complicated because of the long period (up to 4 years) over which neurologically affected patients can improve while on medical therapy. The difficulty is that the time needed to find out whether medical treatment for neurological disease has failed, is probably longer than the window of opportunity for transplantation to prevent progression. This argument remains unresolved, and although we continue to transplant for liver disease as the primary indication, continued improvements in safety and management of transplant patients, and the growing use of living-donor liver transplants, will probably add impetus to this continued debate. The development of better prognostication for neurological progression or improvement of Wilson's disease by MRI findings or other clinical or biochemical variables will help improve our ability to make treatment choices.

The future

Why is there a need for a cure for a disease that has available medical therapy? Patients faced with a lifelong need for medication and physicians faced with the results of non-adherence to therapy are the two main arguments.

Genetic therapy and hepatocyte transplantation represent future curative treatments for Wilson's disease, along with currently available liver transplantation.[115] However, both cell and liver transplants need immunosuppression to maintain grafted cells. Future use of stem-cells, ex-vivo modification of cells by gene therapy, or better means of inducing immune tolerance might obviate the difficulty of immunosuppresion and provide a cure for this disease by cell transplantation.[116] With respect to gene therapy, we have learned from cell transplant studies in a rodent model for Wilson's disease that not only can disease progression be prevented, but also that only 30–50% of the liver mass need be functionally healthy with respect to copper metabolism, to provide protection for the remaining liver cells.[117] This finding suggests that gene therapy need not achieve 100% efficiency with respect to transduction of all of the hepatocytes. In preclinical studies the transduced *ATP7B* gene can result in expression and function in liver cells.[118] Although there are still many hurdles to overcome in Wilson's disease, present gene therapy trials and continuing research will hopefully achieve both the safety and effectiveness of gene transfer, and overcome hurdles to permit efficient transduction of even large genes, such as *ATP7B*.

Conflict of interest statement

We declare that we have no conflict of interest.

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