

# Wilson's disease: update on pathogenesis, biomarkers and treatments

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## ABSTRACT

Wilson's disease is an autosomal–recessive disorder of copper metabolism caused by mutations in *ATP7B* and associated with neurological, psychiatric, ophthalmological and hepatic manifestations. Decoppering treatments are used to prevent disease progression and reduce symptoms, but neurological outcomes remain mixed. In this article, we review the current understanding of pathogenesis, biomarkers and treatments for Wilson's disease from the neurological perspective, with a focus on recent advances. The genetic and molecular mechanisms associated with *ATP7B* dysfunction have been well characterised, but despite extensive efforts to identify genotype–phenotype correlations, the reason why only some patients develop neurological or psychiatric features remains unclear. We discuss pathological processes through which copper accumulation leads to neurodegeneration, such as mitochondrial dysfunction, the role of brain iron metabolism and the broader concept of selective neuronal vulnerability in Wilson's disease. Delayed diagnoses continue to be a major problem for patients with neurological presentations. We highlight limitations in our current approach to making a diagnosis and novel diagnostic biomarkers, including the potential for newborn screening programmes. We describe recent progress in developing imaging and wet (fluid) biomarkers for neurological involvement, including findings from quantitative MRI and other neuroimaging studies, and the development of a semiquantitative scoring system for assessing radiological severity. Finally, we cover the use of established and novel chelating agents, paradoxical neurological worsening, and progress developing targeted molecular and gene therapy for Wilson's disease, before discussing future directions for translational research.

## INTRODUCTION

Wilson's disease is an autosomal–recessive disorder of copper metabolism caused by *ATP7B* mutations. Originally described as *hepatolenticular degeneration*, it classically presents with the combination of liver disease and a movement disorder during adolescence or early adulthood, although with a highly variable phenotype. Up to 60% of patients have neurological or psychiatric symptoms at onset and are referred to as having *neurological* presentations, irrespective of the severity of any underlying liver disease.<sup>1</sup> Patients presenting primarily with liver disease ranging from asymptomatic deranged liver function tests, hepatomegaly or splenomegaly

to acute liver failure are referred to as having *hepatic* presentations. Chelating agents are used to 'decopper' patients, but neurological outcomes are unpredictable; symptoms usually improve, but a minority have persistent or progressive neurological disability.<sup>2,3</sup>

Our understanding of genetic and molecular mechanisms, neuroimaging abnormalities and other biomarkers for neurological involvement in Wilson's disease has advanced in recent years. Novel chelating agents, adeno-associated virus-based gene therapy and variant-specific treatments are also in development and may help improve neurological outcomes. Clinical presentations and hepatic aspects of Wilson's disease have been comprehensively covered elsewhere. In this narrative review, we discuss the pathogenesis, biomarkers and treatment of Wilson's disease from the neurological perspective, with a focus on recent developments and future directions.

## PATHOGENESIS

### Structure and function of *ATP7B*

*ATP7B* is a copper-transporting P-type ATPase that consists of six metal-binding domains and eight transmembrane domains, which form a pore for ATP-dependent transport of copper across membranes.<sup>4</sup> This protein has two specific functions in hepatocytes. First, it supplies copper to the trans-Golgi network (TGN) for incorporation into caeruloplasmin, the main copper-transport protein that is secreted into the bloodstream. Second, it facilitates the biliary excretion of excess copper by translocating to late endosomal or lysosomal compartments and sequestering copper into vesicles for export across the apical (canalicular) membrane.

*ATP7B* is expressed in other human tissues. Davies *et al* found immunohistochemical staining for *ATP7B* in all human brain regions they tested including the striatum, substantia nigra, anterior cingulate and visual cortices and cerebellum.<sup>5</sup> Animal studies suggest that *ATP7B* indirectly regulates the activity of copper-dependent enzymes, such as dopamine beta-hydroxylase and copper/zinc superoxide dismutase-1 (SOD1), in specific neuronal subpopulations.<sup>6</sup> It also interacts with *ATP7A*, another copper-transporting ATPase that is widely expressed in the brain.<sup>7</sup> Interestingly, alternative splicing of *ATP7B* mRNA in the brain was described more than two decades ago. This suggests different isoforms of *ATP7B* may be expressed in

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neurons and glia but has not, to the best of our knowledge, been explored further.<sup>8</sup>

### Genetics

Over 900 pathogenic mutations in *ATP7B* have been reported, although ever some variants with high allele frequencies probably exhibit low penetrance.<sup>9</sup> The majority of mutations are missense, nonsense or frameshift mutations. Additional mechanisms including exon skipping, large deletions and intronic variants have recently been described but are probably rare. The most common mutation among patients from Northern and Eastern Europe is H1069Q, but its frequency varies significantly between countries. In a genetic study of 248 patients from Poland, 45% were homozygous and 41% were heterozygous for this mutation.<sup>10</sup> In a similar study of 181 patients from the UK, H1069Q mutations were identified in 19% of patients.<sup>11</sup> Other variants are much more prevalent in non-European populations. For example, R778L is the most common mutation among patients from China.

The genetic basis for phenotypical variation remains elusive despite extensive efforts to identify genotype–phenotype correlations. There was no association between *ATP7B* genotype and the presence of neurological involvement in a cohort of 1172 European patients.<sup>12</sup> Patients with I1148T or R919G variants were more likely to have neurological involvement in a cohort of 1222 Chinese patients, although both variants were rare.<sup>13</sup> Several genetic modifiers have been proposed. Schiefermeier *et al* and Litwin *et al* found an association between the ε3/ε3 ApoE genotype and delayed onset of symptoms in H1069Q homozygotes, particularly women, but not with clinical presentation.<sup>14</sup> Roy *et al* subsequently suggested that *APOE* and *PRNP* genotypes may be associated with cognitive or behavioural symptoms.<sup>15</sup> A whole-exome sequencing study examining genes in the *ATP7B* interactome suggested rare variants in *ESD* and *INO80* might also affect phenotype.<sup>10</sup>

### Molecular mechanisms

The functional consequences of the most common *ATP7B* variants have now been characterised. The H1069Q and R778L variants cause retention of *ATP7B* in the endoplasmic reticulum (ER) leading to impaired copper export. Using hepatocyte-like cells derived from patients with homozygous H1069Q mutations, Parisi *et al* recently demonstrated that around two-thirds of the mutant protein is retained in the ER where it undergoes more rapid degradation. However, the ability to recruit *ATP7B* to the TGN in response to increasing copper is preserved with important implications for using targeted molecular therapies to rescue *ATP7B* function.<sup>16</sup>

Huster *et al* explored the effects of 28 *ATP7B* variants in Sf9 and HEK293 cells.<sup>17</sup> There were marked differences in copper transport, phosphorylation activity, protein expression and subcellular localisation between variants. In a similar study, Roy *et al* characterised the functional properties of further variants demonstrating differential effects on intracellular copper concentrations.<sup>18</sup> Intriguingly, they also found that coexpressing the G1061E variant, which is usually retained in the ER, with the A595T variant restored localisation of *ATP7B* to the TGN. *ATP7B* has previously been shown to form stable dimers in vitro, and so genetic and functional studies may need to consider interactions between heterozygous variants in the future.

### Neurodegeneration

An inability to excrete copper in bile leads it to accumulate in the liver with subsequent accumulation of copper in other organs, including the brain. Postmortem studies confirm that patients with Wilson's disease have dramatically increased copper content in the basal ganglia and cortex.<sup>7,19</sup> However, advanced liver disease is not necessarily a prerequisite to developing neurological disease. Przybyłkowski *et al* characterised the liver disease in newly diagnosed patients with neurological patients and found that, while every patient had at least one feature of liver disease on ultrasound examination, around half of patients had clinical or radiological features of cirrhosis.<sup>20</sup> In a cohort of 131 patients who underwent liver biopsy at diagnosis, Ferenci *et al* found that 8 of 34 patients with neurological presentations had normal histology or steatosis (without hepatitis fibrosis or cirrhosis) Interestingly, four patients with neurological presentations (and two pathogenic *ATP7B* mutations) had hepatic copper content less than 250 µg/g (dry weight), including one patient with hepatic copper content of 50 µg/g.<sup>21</sup> Low hepatic copper content may result from sampling error, where an area of relatively normal tissue is taken during liver biopsy; however, the possibility of neurological involvement developing without increased liver copper content cannot be excluded.

Patients may also have increased brain copper content without developing neurological or psychiatric symptoms. In a cohort of 11 patients who underwent brain copper quantification post-mortem, all had dramatically increased copper content in the putamen and frontal cortex, irrespective of presentation.<sup>22</sup> It therefore appears that a model where copper accumulates in the liver, overflows into the systemic circulation and is then deposited in the brain may be an oversimplification, and that a subset of patients seems to be more vulnerable to developing neurodegeneration in response to copper deposition in the brain, although the mechanism for this is unclear.

Histopathological changes in the brain are heterogeneous and include neuronal loss, spongiosis, cavitation, demyelination and reactive astrogliosis.<sup>7</sup> These findings can be seen in the basal ganglia, thalamus, brainstem and cerebral white matter but are usually most prominent in the putamen. Animal models of Wilson's disease do not produce a convincing neurological phenotype but do accumulate copper in the brain and have provided some insights on the mechanisms by which this could lead to neuropathology.<sup>23</sup> Mitochondrial dysfunction appears to play a critical role and brain iron metabolism is also affected. These processes and the broader concept of selective neuronal vulnerability in Wilson's disease are discussed further.

### Mitochondrial dysfunction

Copper is an essential cofactor for energy production in mitochondria but has deleterious effects on the organelle in excess. The prevailing view up until recently has been that copper-induced mitochondrial dysfunction is mediated by the generation of reactive oxygen and nitrogen species in Fenton-like reactions. However, several findings point towards alternative mechanisms for mitochondrial dysfunction:

- ▶ Marked mitochondrial copper accumulation, with concentrations up to 200 times normal, occurs in the hepatocytes from *ATP7B* knockout rats prior to signs of oxidative damage.<sup>24</sup>
- ▶ Loss of mitochondrial membrane potential and reduced ATP production develop in response to exogenous copper in healthy rat brain prior to the increased generation of reactive

oxygen species, potentially as a result of direct interactions with protein thiol groups.<sup>25</sup>

- ▶ Copper interrupts mitochondrial DNA replication, leading to mitochondrial DNA depletion in human blood samples independently of oxidative stress-mediated damage.<sup>26</sup>

These observations suggest that copper toxicity causes mitochondrial dysfunction through both direct and indirect mechanisms. However, studies using induced pluripotent stem cell models of human neurons and glia are needed to further elucidate the precise mechanisms linking mitochondrial dysfunction to neuronal and glial cell death.

### Iron metabolism

Several converging lines of evidence indicate brain iron metabolism is disrupted in Wilson’s disease. Susceptibility-weighted imaging (SWI) shows increased signal in the basal ganglia of patients with neurological involvement.<sup>27</sup> Cerebral iron uptake, measured using <sup>52</sup>Fe-citrate positron emission tomography, is also increased. In a histopathological study of nine patients, Dusek *et al* confirmed that SWI abnormalities in the caudate, putamen and pons represent abnormal iron deposition, mainly caused by an increase in iron-containing macrophages, and identified an association between iron accumulation and pathological severity in the putamen.<sup>28</sup> The basis for abnormal iron deposition in Wilson’s disease is unclear; however, copper-dependent enzymes play an important role in iron metabolism. For example, caeruloplasmin, which is also expressed in the brain, exhibits ferroxidase activity, and loss of function in caeruloplasminaemia leads to brain iron accumulation.<sup>28</sup>

### Selective neuronal vulnerability

There are several potential reasons why neurons, specifically those within the basal ganglia, might be particularly vulnerable to copper toxicity in Wilson’s disease:

- ▶ Medium spiny neurons of the striatum and dopaminergic neurons of the substantia nigra have unusually high energy requirements, making them more susceptible to perturbations in mitochondrial function.<sup>29</sup>
- ▶ Endogenous copper-scavenging proteins, metallothionein 1/2 and Cu/Zn SOD1, are differentially expressed between neuronal subpopulations and glia.<sup>25</sup>
- ▶ Copper can directly modulate neurotransmission in the basal ganglia; it reduces tonic inhibition mediated by extrasynaptic GABA<sub>A</sub> receptors in striatal neurons.<sup>30</sup>

## BIOMARKERS

### Diagnostic biomarkers

Diagnostic delays are common and represent a missed opportunity to start chelation therapy and prevent neurological disability. In a cohort of 163 patients from Germany, neurological presentations were associated with a significantly longer time from onset of symptoms to diagnosis than hepatic presentations (44 vs 14 months).<sup>3</sup> The mean interval in patients diagnosed in Ireland between 1990 and 2011 was 21 months and had not improved over the preceding four decades.<sup>31</sup> The longest delays are often in patients who initially present with psychiatric symptoms.<sup>32</sup>

Delays usually arise because the clinical features associated with neurological presentation, which are outlined in table 1, are highly variable and initially misattributed to more prevalent disorders. In our experience, some patients are incidentally found to have deranged liver function tests or an isolated thrombocytopenia indicative of cirrhosis or hepatomegaly in the years before presenting with neurological or psychiatric symptoms.

**Table 1** Clinical features in patients with neurological presentations

Clinical feature	Frequency	Comments
<b>Neurological</b>		
Dysarthria	52%–91%	Often associated with drooling and/or dysphagia
Postural tremor	55%–72%	May be worse with arms flexed (wing-beating tremor)
Dystonia	20%–69%	Facial/oromandibular involvement common (risus sardonicus)
Parkinsonism	45%–58%	
Ataxia	28%–54%	Cerebellar eye signs may be seen
Chorea	11%–16%	
Seizures	4%–15%	Usually younger patients
Executive dysfunction	–	Often manifests as decline in performance at school or work
<b>Psychiatric</b>		
Incongruous behaviour	13%–25%	
Irritability	13%–18%	May be misattributed to puberty in adolescent patients
Personality change	8%–26%	
Depression	4%–21%	
Elation/hypomania	5%–13%	Sometimes misdiagnosed as bipolar affective disorder
Anxiety	2%–7%	
Delusions	1%–2%	
<b>Hepatic</b>		
Transaminitis	30%	Alanine transaminase >40 IU/L
Thrombocytopenia	50%	Platelet count <140×10 <sup>9</sup> /L
Coagulopathy	65%	International normalised ratio >1.2
Splenomegaly	30%	Identified on ultrasound
Oesophageal varices	48%	Identified on endoscopy
<b>Ophthalmological</b>		
Kayser-Fleischer rings	85%–90%	Confirmed on slit lamp examination
Sunflower cataracts	<5%	

Frequency refers to the number of patients with a given clinical feature relative to patients with neurological presentations. References are provided in the online supplemental material.

However, the lack of a simple diagnostic test that can confirm or exclude Wilson’s disease compounds the issue. Here, we discuss the current approach to making a diagnosis and two novel diagnostic biomarkers.

### Current approach

A combination of tests is usually required to make the diagnosis. A working group at an international meeting on Wilson’s disease in 2001 proposed a diagnostic scoring system that integrates clinical features with serum caeruloplasmin, 24-hour urinary copper, slit lamp examination (for Kayser-Fleischer (KF) rings), ATP7B genotyping and hepatic copper content (on liver biopsy) to determine whether Wilson’s disease is highly likely, probable or unlikely.<sup>33</sup> However, in our experience, most neurologists and hepatologists initially arrange a full blood count, liver function tests and serum caeruloplasmin and investigate more thoroughly with a 24-hour urine collection and slit lamp examination when the serum caeruloplasmin is low, neuroimaging is abnormal or they already suspect Wilson’s disease.

Neurologists should, however, be aware of the pitfalls of this approach. While a very low caeruloplasmin (<0.1 g/L) is highly

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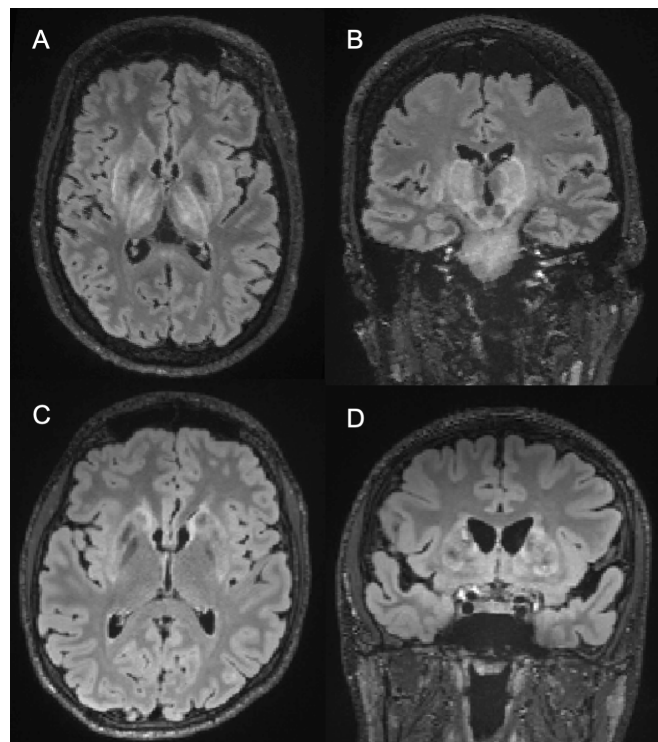
suggestive of Wilson's disease, intermediate concentrations (0.1–0.2 g/L) are less specific, and up to 15% of patients with neurological presentations have normal concentrations (>0.2 g/L).<sup>34</sup> There are also methodological concerns about the widely used immunological assay for caeruloplasmin, which also detects apo-caeruloplasmin and may overestimate results. We therefore advocate a low threshold for arranging a urine collection and slit lamp examination with a serum caeruloplasmin at initial assessment, particularly in patients with suspected liver disease or a positive family, and promptly arranging further investigations in patients found to have a serum caeruloplasmin less than 0.2 g/L or specific neuroimaging abnormalities, discussed further.

The urinary copper output is increased (>0.64  $\mu\text{mol}/24$  hours) and KF rings are present in 78% and 90% of neurological presentations, respectively.<sup>34</sup> The diagnosis of Wilson's disease can therefore be established using a combination of serum caeruloplasmin, 24-hour urinary copper and slit lamp examination in the vast majority of patients with neurological presentations. *ATP7B* sequencing, which may take several months, may be required to confirm the diagnosis in a minority of cases. Liver biopsy is very rarely needed for diagnostic purposes in patients with neurological presentations.<sup>35</sup> Copper incorporation tests that involve administering a radioactive <sup>64</sup>Cu isotope (intravenously) or non-radioactive <sup>65</sup>Cu isotope (orally) and indirectly measuring incorporation of exogenous copper into caeruloplasmin over 48–72 hours may be helpful if other tests are inconclusive but, to our knowledge, are only available in a few countries.<sup>36 37</sup>

### Novel diagnostic biomarkers

An alternative approach to measuring serum copper has been proposed in the last decade. The *exchangeable* copper reflects the labile fraction of copper bound to albumin and other peptides and is measured by adding EDTA to a serum sample and performing ultrafiltration prior to copper quantification. Levels are higher in patients with extrahepatic involvement (2.75 vs 1.26,  $p < 0.001$ ) and correlate with neurological severity ( $r = 0.45$ ,  $p = 0.016$ ), semiquantitative scores for KF rings ( $r = 0.46$ ,  $p = 0.014$ ) and neuroradiological involvement ( $r = 0.46$ ,  $p = 0.048$ ).<sup>38</sup> Expressed as percentage of total serum copper, relative exchangeable copper (REC) appears to be a valuable diagnostic test with a high sensitivity and specificity. With a cut-off of 15%, REC discriminates patients with Wilson's disease from heterozygous *ATP7B* carriers and healthy controls.<sup>39</sup> While this test can differentiate Wilson's disease from other causes of liver disease in children and adults, data on its ability to differentiate Wilson's disease from other neurological and psychiatric disorders are not yet available.

Collins *et al* recently proposed direct measurement of *ATP7B* peptides on dried blood spots using immunoaffinity-enriched mass spectrometry as a novel diagnostic test for Wilson's disease.<sup>40</sup> In a retrospective cohort of 264 patients and 150 healthy controls, they report an overall sensitivity of 92% and specificity of 98%. *ATP7B* peptide concentrations were below diagnostic cut-off values in 171/184 (93%) with a low serum caeruloplasmin (<0.2 g/L) and 14/16 (88%) with a normal serum caeruloplasmin (>0.2 g/L). This suggests that a combination of *ATP7B* peptide quantification and serum caeruloplasmin is likely to improve diagnostic accuracy. Nonetheless, the ability to confirm or exclude Wilson's disease on a dried blood spot has the potential to reshape our approach to investigating Wilson's disease, enabling more widespread testing of patients presenting to primary and secondary care services with neurological or



**Figure 1** Axial and coronal FLAIR images demonstrating signal abnormality, which appears heterogeneously hyperintense, in the basal ganglia, thalami and brainstem of a patient presenting with hypomania, tremor and ataxia (A,B) and in the basal ganglia of a patient presenting with dysarthria, dystonia and parkinsonism (C,D). FLAIR, fluid-attenuated inversion recovery.

psychiatric symptoms. It also revives the possibility of newborn screening programmes for Wilson's disease after previous attempts, based on caeruloplasmin, were largely disappointing.<sup>41</sup> These findings have important implications but need to be validated in larger, prospective cohorts that include disease controls.

### Biomarkers for neurological involvement

The urinary copper output and non-caeruloplasmin-bound ('free') copper, which is calculated from the serum caeruloplasmin and serum copper, are currently used to monitor treatment response with chelation therapy. However, these conventional copper indices do not differ between patients with hepatic and neurological presentations or correlate with clinical neurological severity, measured using the Unified Wilson's Disease Rating Scale (UWDRS).<sup>42</sup> Significant progress has been made in understanding and developing end-organ biomarkers that directly measure neurological involvement in recent years. These could potentially be used to guide chelation therapy or predict response to treatment and, in a research setting, for cohort stratification or as secondary outcomes measures in clinical trials. Here, we discuss recent advances in neuroimaging and the use of wet and ophthalmological biomarkers for measuring neurological involvement in Wilson's literature.

### Neuroimaging

Hyperintense signal abnormality in the basal ganglia, thalamus and/or brainstem on T2-weighted (or fluid-attenuated inversion recovery) sequences is characteristic of Wilson's disease and seen in 90% of patients with neurological presentations (figure 1).<sup>43</sup> Those in the putamen may be associated with dystonic features,

whereas those in the thalamus may be associated with tremor or ataxia and in the midbrain with parkinsonism. However, these clinicoradiological correlations have not been systematically tested. The *face of the giant panda* sign, where hyperintense signal abnormality surrounds the red nucleus and substantia nigra, is highly specific for Wilson's disease but only seen in a minority of patients.

Dusek *et al* recently proposed a semiquantitative MRI scale for measuring neuroradiological abnormalities in Wilson's disease that includes an acute toxicity subscore based on the distribution and severity of these hyperintense signal abnormalities. In a validation study that included 39 treatment-naïve patients, the acute toxicity subscore improved with treatment ( $p=0.002$ ) but did not correlate with UWDRS scores at baseline or after 2 years.<sup>44</sup> A chronic damage subscore, based on brain atrophy and hypointense signal abnormalities on T2/T2\*/SWI, correlated with UWDRS scores at baseline ( $r=0.59$ ,  $p=0.005$ ) and 24 months ( $r=0.68$ ,  $p<0.001$ ).

Quantitative MRI analyses have provided further insights on brain atrophy, susceptibility-related changes and diffusion abnormalities in Wilson's disease. Relevant studies are listed in [table 2](#) (with references in online supplemental material), some of which were used to inform the aforementioned semiquantitative MRI scale. Volumetric studies demonstrate that patients with neurological presentations have atrophy and increased susceptibility in regions of interest including the caudate, putamen, pallidum and thalamus at diagnosis. Dusek *et al* recently confirmed Wilson's disease causes a predominantly central pattern of atrophy using deformation and surface-based morphometry. The overall degree of brain atrophy also correlates with neurological severity. Smolinski *et al* calculated the volume of deep grey matter ( $r=-0.46$ ,  $p=0.001$ ), peripheral grey matter ( $r=-0.52$ ,  $p<0.001$ ) and white matter ( $r=-0.60$ ,  $p<0.001$ ) in treatment-naïve patients and found that each correlated with UWDRS scores. In addition, they identified an association between brain volume and serum non-caeruloplasmin-bound copper ( $r=-0.39$ ,  $p=0.008$ ). Diffusion tensor imaging studies demonstrate decreases in fractional anisotropy and increases in mean diffusivity in the white matter of patients with neurological presentations. In a prospective, longitudinal study, Lawrence *et al* showed that these indices improve over the first 2 years of treatment.

Importantly, the cognitive and psychiatric aspects of Wilson's disease have received relatively little attention in the quantitative MRI studies. Executive dysfunction associated with Wilson's disease is assumed to be related to basal ganglia dysfunction, but evidence to demonstrate this is so far limited. Neuroimaging correlates for psychiatric symptoms, which are a major source of morbidity for patients with Wilson's disease, have not yet been described.

Several other neuroimaging modalities have been used to study neurological involvement in Wilson's disease ([table 3](#)). Magnetic resonance spectroscopy (MRS) demonstrates that N-acetylaspartate to creatinine ratios are consistently reduced in the basal ganglia of patients with neurological involvement and that myoinositol is increased in patients with portosystemic shunting. Single-photon emission CT (SPECT) confirm presynaptic and postsynaptic dopaminergic dysfunction is common in Wilson's disease and correlates with motor function and that cerebral hypoperfusion is seen in the striatum and scattered cortical areas. Transcranial sonography (TCS) studies have shown that hyperechogenicity of the putamen is seen in the vast majority, if not all, patients with neurological presentation. This can accurately differentiate patients with Wilson's disease from healthy

controls and patients with early-onset Parkinson's disease and correlates with neurological examination scores but does not improve with treatment. Third ventricular width measured using TCS correlates with UWDRS and Addenbrooke's Cognitive Examination scores.

Overall, these findings suggest that repeat MRIs are justified to determine whether initial signal changes improve or worsen after initiation of chelation therapy. However, questions remain regarding the mismatch between T2-weighted hyperintensities and clinical severity, the ability of measures of brain atrophy to predict clinical outcomes and the role for diffusion and SWI in monitoring response to treatment. Further studies exploring how imaging biomarkers correlate with clinical and biochemical characteristics in the initial stages of treatment and with clinical outcomes in chronically treated patients are needed.

### Other biomarkers

Neuronal and glial-derived proteins that are promising biomarkers for other neurodegenerative diseases have recently been tested in Wilson's disease. Neurofilament light (NfL), a non-specific marker of neuroaxonal injury that can be measured in plasma samples using single-molecule array technology, is higher in patients with neurological than hepatic presentations (8.7 vs 7.0,  $p=0.005$ ) and correlates with UWDRS scores in chronically treated, stable patients ( $\beta=0.1$ ,  $p=0.003$ ).<sup>45</sup> Levels of glial-fibrillary acidic protein (GFAP) and tau have also been reported to be higher in serum/plasma samples from patients with neurological presentations.<sup>46</sup> Further studies investigating NfL, GFAP and tau in patients starting treatment for Wilson's disease and age-specific reference ranges are needed; however, these preliminary findings provide proof-of-concept that non-invasive wet biomarkers could be used to measure neurological involvement in Wilson's disease in the future.<sup>45</sup>

Serial slit lamp examination to confirm disappearance, or exclude recurrence, of KF rings is helpful in some cases. Anterior segment optical coherence tomography (OCT) has recently been proposed as an alternative technique for detecting KF rings and monitoring treatment response that can provide a quantitative measure of copper deposition and may be more sensitive than slit lamp examination.<sup>47</sup> In a study that included 29 chronically treated patients with Wilson's disease, 15 had KF rings on anterior segment OCT that were not visible with slit lamp examination highlighting the potential for OCT to supersede slit lamp examination for the detection of KF rings in a clinical setting. Retinal OCT has also been proposed as a biomarker for neurodegeneration in Wilson's disease. Thinning of the retinal nerve fibre layer (RNFL) was greater in patients with neurological involvement (88.1 vs 94.8,  $p=0.002$ ) and negatively correlated with UWDRS scores in chronically treated patients ( $\beta=-0.95$ ,  $p=0.008$ ).<sup>48</sup> The ability of changes in the RNFL or macula to predict outcome in newly diagnosed patients and associations with neuroimaging abnormalities are so far untested.

## TREATMENT

### First-line treatments

Chelating agents increase urinary copper excretion and are used to 'decopper' patients, and then continued lifelong. Zinc salts inhibit intestinal absorption of copper by upregulation of metallothionein and can also be used to treat Wilson's disease. Treatment strategies and dosing regimens vary. Adverse effects, including paradoxical neurological worsening, are relatively common and outcomes are mixed: in a retrospective study of 137 patients presenting with neurological symptoms, 42%

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**Table 2** Quantitative MRI studies on Wilson's disease

Study	Participants	Methods	Key findings
<b>T1-weighted (structural) imaging</b>			
Stein <i>et al</i>	10 WD, 11 HC	Voxel-based morphometry	Volume reduced in striatum, pallidum, thalamus, cerebellum and some cortical areas
Smolinski <i>et al</i> *	48 WD	Tissue-type segmentation	UWDRS negatively correlates with volume of deep GM, peripheral GM and WM Non-caeruloplasmin-bound copper negatively correlates with total brain volume
Zou <i>et al</i>	27 WD, 24 HC	Volumetric/ROI Morphometric	Volume reduced in caudate, putamen, pallidum, thalamus, RN and SN Modified Young Scale negatively correlates with volume of putamen and pallidum
Song <i>et al</i>	30 WD, 25 HC	Volumetric/ROI Cortical thickness	Volume reduced in subcortical nuclei, diffuse WM and several other GM regions Cortical thickness reduced in left precentral gyrus and left insula Global Assessment Scale negatively correlates with volume of several ROI, including putamen
Tinaz <i>et al</i>	30 WD, 30 HC	Volumetric/ROI Cortical thickness	Volume reduced in striatum and WM; volume of striatum also lower in nWD than in hWD Cortical thickness reduced in bilateral superior frontal gyrus
Viveiros <i>et al</i>	20 WD, 20 HC	Volumetric/ROI	Volume reduced in basal ganglia, amygdala, hippocampus, cerebellum, WM, MCP and SCP Volume of striatum lower in nWD than hWD
Hu <i>et al</i>	22 WD, 26 HC	Volumetric/ROI	Volume reduced in basal ganglia and occipital fusiform cortex and increased in visual association cortex
Dusek <i>et al</i>	29 WD, 26 HC	Deformation and surface-based morphometry	Volume decreased in basal ganglia, brainstem, internal capsule, corticospinal tract, motor and visual cortex UWDRS negatively correlates with volume of putamen, pallidum, RN and SN
<b>Susceptibility-weighted imaging</b>			
Fritzsche <i>et al</i>	11 WD, 10 HC	QSM values/ROI	QSM values increased in basal ganglia, RN and SN; also higher in SN and RN of hWD than controls
Bai <i>et al</i>	23 WD, 23 HC	Phase values/ROI	Phase values reduced in caudate, putamen, thalamus, RN and SN
Zhou <i>et al</i>	30 WD, 20 HC	Phase values/ROI	Phase values reduced in caudate, pallidum and SN Tremor score negatively correlated with phase value in caudate
Yang <i>et al</i>	33 WD, 18 HC	Phase values/ROI	Phase values reduced in caudate, putamen, pallidum, thalamus, RN and SN
Dezortova <i>et al</i>	38 WD, 26 HC	QSM values/ROI	QSM values increased in caudate, putamen, pallidum and thalamus in nWD compared with hWD and controls
Dusek <i>et al</i>	29 WD, 26 HC	QSM values/ROI	UWDRS did not correlate with mean QSM values in deep GM nuclei
<b>Diffusion-weighted imaging</b>			
Jadav <i>et al</i>	15 WD, 15 HC	DTI/ROI	MD increased and FA decreased in WM Neurological severity correlates with MD in internal capsule and frontal and occipital WM
Lawrence <i>et al</i> *†	35 WD	DTI/TBSS	Decreases in FA and increases in MD, AD and RD improve over first 2 years of treatment
Zhou <i>et al</i>	70 WD, 20 HC	DTI/ROI	FA and fibre volumes lower in nWD than hWD
Wang <i>et al</i>	35 WD, 30 HC	DTI/ROI	FA increased in subcortical GM nuclei and decreased in thalamus and WM
Song <i>et al</i>	24 WD, 25 HC	NODDI/ROI	Vic and ODI reduced and Viso increased in basal ganglia and thalamus Clinical severity negatively correlates with Vic in putamen and ODI in pallidum
Dong <i>et al</i>	30 WD, 35 HC	DTI/ROI	FA increased in subcortical GM nuclei and decreased in right cerebellum and left middle frontal gyrus Event-based prospective memory and digit span correlate with FA in left thalamus and putamen
Hu <i>et al</i>	22 WD, 26 HC	DTI/ROI (tract-specific)	Increased MD, RD and AD and decreased FA in various association/limbic tracts Associations between AD and FA and event-based and time-based prospective memory tasks in some tracts

Studies are listed in chronological order under each subheading. References are provided in the online supplemental material.

\*Indicates study included newly diagnosed patients.

†Indicates study was longitudinal.

AD, axial diffusivity; DTI, diffusion tensor imaging; FA, fractional anisotropy; GM, grey matter; HC, healthy control; hWD, hepatic Wilson's disease; MCP, middle cerebellar peduncle; MD, mean diffusivity; NODDI, neurite orientation dispersion and density imaging; nWD, neurological Wilson's disease; ODI, orientation dispersion index; QSM, quantitative susceptibility mapping; RD, radial diffusivity; RN, red nucleus; ROI, region of interest; SCP, superior cerebellar peduncle; SN, substantia nigra; UWDRS, Unified Wilson's Disease Rating Scale; Vic, intracellular volume; Viso, isotropic volume; WD, Wilson's disease; WM, white matter.

became asymptomatic; 26% improved but had residual symptoms; 18% had significant ongoing disability; and 8% died despite seemingly adequate chelation therapy.<sup>2</sup> In a separate study on long-term outcomes, 20% of patients with hepatic presentations developed neurological symptoms during the course of their treatment.<sup>3</sup>

### Choice of agent

Penicillamine, trientine and zinc are currently the most widely used treatments in Europe, and their use is described in detail in the European Association for the Study of the Liver clinical practice guidelines on Wilson's disease. Retrospective studies suggest rates of neurological recovery and paradoxical worsening are

**Table 3** MRS, SPECT and TCS studies on Wilson's disease

Study	Participants	Key findings
Magnetic resonance spectroscopy		
Van Den Heuvel	22 WD, 13 HC	Decreases in NAA/Cr and Cho/Cr in pallidum. Decreases in ml/Cr in pallidum of patients with porto-systemic shunting.
Kraft <i>et al</i>	13 WD, 12 HC	No differences in putamen, GM or WM.
Lucato <i>et al</i>	36 WD, 37 HC	Decreases in NAA/Cr in basal ganglia, WM and GM. Increases ml/Cr in basal ganglia.
Tarnacka <i>et al</i> *	37 WD	Increases in Lip/Cr and decreases in ml/Cr and NAA/Cr in pallidum. Increases in Glx/Cr and Lip/Cr in thalamus.
Tarnacka <i>et al</i> *†	17 WD	NAA/Cr in pallidum increases with neurological recovery and decreases with deterioration ml/Cr and Glx/Cr in pallidum decrease with liver failure.
Tarnacka <i>et al</i>	12 HZ, 21 HC	Increases in Glx/Cr and Lip/Cr in pallidum and thalamus of heterozygous carriers.
Pulai <i>et al</i>	38 WD, 32 HC	Decreases in NAA/Cr and Cho/Cr in basal ganglia.
Alkhalik Basha <i>et al</i> *	26 WD, 26 HC	Decreases in NAA, Cho, Cr, NAA/Cho, NAA/Cr, Cho/Cr in pallidum.
Single photon emission CT		
Barthel <i>et al</i>	46 WD, 10 HC	Neurological severity correlates with <sup>123</sup> I-β-CIT and <sup>123</sup> I-BZM binding in striatum.
Hermann <i>et al</i>	37 WD	Fine motor ability correlates with <sup>123</sup> I-β-CIT binding in putamen.
Eggers <i>et al</i>	23 WD	Hamilton Rating Scale for depression correlates with <sup>123</sup> I-β-CIT binding in thalamus–hypothalamus.
Wang <i>et al</i>	31 WD, 12 HC	Decreases in <sup>99m</sup> Tc-TRODAT-1 binding in posterior putamina.
Piga <i>et al</i> *	25 WD, 24 HC	Decreases in <sup>99m</sup> Tc-ECD uptake in the caudate, putamen and scattered cortical areas.
Eo <i>et al</i>	9 WD, 17 HC	Decreases in <sup>123</sup> I-β-CIT binding in striatum.
Transcranial sonography		
Walter <i>et al</i>	21 WD	HE in putamen seen in all 18 nWD cases. HE in putamen and thalamus and third ventricular width correlate with disease severity.
Svetel <i>et al</i>	54 WD	HE in SN and third ventricular width correlate with disease severity.
Maskova <i>et al</i>	22 WD, HC, 16 EOPD	HE higher in putamen in WD than HC and EOPD. HE higher in SN in EOPD than WD and HC.
Tribl <i>et al</i>	40 WD, 49 HC	HE in putamen differentiates WD and HC with area under the curve 0.95. HE in putamen correlates with dystonia and dysarthria scores. Third ventricular width correlates with UWDRS, ACE-R, MMSE scores.
Skowronska <i>et al</i> *†	41 WD	HE in SN but not putamen improves with treatment.
Skowronska <i>et al</i>	34 HZ, 18 HC	HE in putamen seen in 75% of heterozygous carriers.
Skoloudik <i>et al</i>	22 WD, 24 HC	HE in insula in WD and correlates with HE in putamen.

Studies are listed in chronological order under each subheading. References are provided in the online supplemental material.

\*Indicates study included newly diagnosed patients.

†Indicates study was longitudinal.

ACE-R, Addenbrooke's Cognitive Examination; Cho, choline; Cr, creatinine; EOPD, early-onset Parkinson's disease; Glx, glutamate–glutamine; HC, healthy control; HE, hyperechogenicity; hWD, hepatic Wilson's disease; HZ, heterozygous carrier; Lip, lipid; ml, myo-inositol; MMSE, Mini-Mental State Examination; MRS, magnetic resonance spectroscopy; NAA, N-acetyl-aspartate; nWD, neurological Wilson's disease; SN, substantia nigra; SPECT, single-photon emission CT; TCS, transcranial sonography; UWDRS, Unified Wilson's Disease Rating Scale; WD, Wilson's disease.

broadly similar between these three treatments, but discontinuation is more likely with penicillamine.<sup>49</sup> The relationship between the dose of penicillamine or trientine in the initial decoppering phase of treatment and neurological outcomes is largely unknown, but there is a consensus that clinicians should *start low and go slow* in patients with neurological presentations. Zinc is considered second or third line in some countries, given reports that liver disease can progress despite monotherapy.

The role of tetrathiomolybdate, a chelating agent that can also increase biliary excretion and prevent intestinal absorption of copper, is currently being re-evaluated. Brewer *et al* compared ammonium tetrathiomolybdate to trientine in 48 patients with neurological presentations also treated with zinc in a randomised controlled trial in 2006. Worsening was less common in the tetrathiomolybdate group (4% vs 26%,  $p < 0.05$ ).<sup>50</sup> In an open-label phase II trial reported in 2017, Weiss *et al* treated 28 patients with bis-choline tetrathiomolybdate. At 24 weeks, 71% achieved the primary endpoint, based on improvement in the non-caeruloplasmin-bound copper, with a mean reduction of 72% from baseline ( $-2.4 \mu\text{mol/L}$ ,  $p < 0.001$ ).<sup>51</sup> Mean UWDRS neurological subscores improved from 22.8 to 16.6 ( $p < 0.001$ )

but increased by at least 5 points in two patients. A phase III trial comparing bis-choline tetrathiomolybdate to standard care (NCT03403205) is ongoing.

Dimercaptosuccinic acid (DMSA) is a chelating agent widely used in China. A recent retrospective study comparing DMSA, in combination with zinc, to penicillamine in 158 patients with neurological presentations showed the proportion of patients with neurological improvements was higher (88% vs 59%) and neurological worsening was lower (9% vs 26%) in the DMSA group.<sup>52</sup> There are also reports of successfully using the parent compound, dimercaprol (British anti-Lewisite), as a rescue therapy for severe neurological worsening.<sup>53</sup> It is lipid soluble and therefore needs to be administered intramuscularly but, unlike other chelating agents, can cross the blood–brain barrier.

### Paradoxical neurological worsening

This unusual phenomenon is a major concern when initiating treatment. In a retrospective study that included 70 patients with neurological presentations, Litwin *et al* reported that 23% developed early neurological worsening with a mean delay of

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2.3±1.9 months.<sup>54</sup> The deterioration was reversible in half of cases and higher baseline neurological severity (UWDRS 38.4 vs 22.3,  $p<0.01$ ), more thalamic or brainstem lesions on MRI (73% vs 33%,  $p<0.01$ ) and concurrent use of antipsychotic medication (46% vs 5%,  $p<0.01$ ) were more common in patients who deteriorated. Brewer *et al* initially proposed the widely cited theory that paradoxical worsening is caused by transient elevation in blood and brain copper levels secondary to mobilisation of hepatic copper. However, supporting evidence for this is limited. Hartard *et al* found that cerebrospinal fluid and non-caeruloplasmin-bound copper levels continued to decrease in a series of patients with paradoxical worsening.<sup>55</sup> An alternative hypothesis that mobilisation of copper from a harmless, sequestered form (incorporated into metallothioneins) to other cellular compartments where it can cause oxidative damage has also been proposed.

The management of this challenging clinical scenario varies between clinicians, and decisions on whether to increase or decrease doses, switch treatment or add another agent are usually guided by personal experience. Liver transplantation, usually reserved for patients with acute liver failure or decompensated liver disease, was recently evaluated in 18 patients with neurological worsening on chelation therapy.<sup>56</sup> These patients had severe neurological disability, with a median modified Rankin scale (mRS) score of 5 and a median UWDRS score of 105 at baseline. At the last follow-up, these had decreased to a median mRS score 1.5 and a median UWDRS score 36 ( $p<0.001$ ). Eight patients had major improvement; four patients had moderate improvement; two were stable; and four died. The mechanism by which liver transplantation can ameliorate neurological involvement is currently unclear, as is the role for this treatment in clinical practice.

### Experimental therapies

Novel copper chelators, including DPM-1001, a protein-tyrosine phosphatase inhibitor with high copper affinity, Chel2, a hepatocyte-directed prochelator that releases a trithiolate chelator intracellularly, and  $\alpha$ -lipoic acid, an endogenous sulfur-containing compound derived from caprylic acid, have been tested in animal or cell-based models of Wilson's disease with promising results. Methanobactin, a bacterial peptide with an exceptionally high affinity for copper, has been shown to prevent liver failure in *ATP7B*-deficient rats.<sup>57</sup> An alternative strategy where a chelating agent, 8-hydroxyquinolone, is covalently bound to an indigestible biopolymer has also been shown to prevent intestinal absorption of copper in rodents.<sup>58</sup>

Targeted molecular therapies that restore the localisation and/or function of *ATP7B* are also being developed. A 20-residue peptide derived from  $\alpha$ B-crystallin, a heat shock protein, has been shown to restore localisation of *ATP7B* to the TGN in cells transfected with *ATP7B*-H1069Q.<sup>59</sup> Concilli *et al* recently interrogated the interactome of *ATP7B* and identified that the H1069Q mutation increases interactions with HSP70, thereby accelerating *ATP7B* degradation. Using a bioinformatic screen for drugs approved by the Food and Drug Administration, they found that domperidone inhibits the function of HSP70 and improved localisation of *ATP7B* to the TGN and tolerance to exogenous copper in transfected cells.<sup>60</sup> Whether this approach translates into a clinically meaningful restoration of *ATP7B* function remains to be seen.

Finally, gene therapy using an adeno-associated viral (AAV) vector has shown encouraging results in animal models of Wilson's disease. Murillo *et al* transduced the liver of *ATP7B*

knockout mice with AAV8 encoding *ATP7B* cDNA under the control of a liver-specific promoter. They found a dose-dependent restoration of biliary copper excretion with normalisation of urinary copper output, hepatic copper content and liver function.<sup>61</sup> Vectors containing a truncated form of the gene have shown similar results, and a subsequent study has confirmed that AAV8-based therapy reduces cerebral copper content in knockout mice.<sup>62</sup> An open-label phase I/II trial is due to start this year (NCT04537377).

### FUTURE DIRECTIONS

Significant progress has been made with our understanding of the genetics and cell biology of *ATP7B* dysfunction; however, the fundamental question of why only some patients primarily manifest with neurological disease remains. Novel approaches to exploring the genetic, epigenetic and wider metabolic influences that determine the initial presentation may be required. The lack of convincing neurological phenotype in animal models has also hindered our ability to characterise the pathogenesis of neurodegeneration and paradoxical neurological worsening in Wilson's disease. The use of induced pluripotent stem cells to study copper toxicity and *ATP7B* dysfunction in neurons and glia may provide novel insights here.

Delayed diagnoses remain an issue, and confirming the sensitivity and specificity of novel diagnostic tests, including their potential use in newborn screening programmes, is a priority. Strategies to prevent and manage paradoxical worsening using novel treatments, existing chelating agents and liver transplantation also need to be clarified. We anticipate that imaging and wet biomarkers for neurological involvement will be increasingly used to guide chelation therapy. In an age of targeted molecular and gene therapies, we expect these will also play an important role in measuring neurological outcomes for these treatments in clinical trials.

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