



# Wilson disease: more complex than just simply a copper overload condition? – a narrative review

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**Background and Objective:** The therapeutic arsenal in Wilson disease is limited, but effective. It focuses on copper depletion, although this has never been defined nor measured. Accordingly, disorder adapted long term dosing strategies are missing. Goal of the analysis was the elucidation of copper metabolism in Wilson disease to detect critical mechanisms inducing cell injury and strategies for prevention.

**Methods:** We sought the literature between years 1980–2022 under the following key words: mechanism of liver injury in Wilson disease, therapeutic strategies, monitoring and outcome.

**Key Content and Findings:** Copper is absorbed by intestinal mucosa depending on its content in diet. Release in bile controls the copper balance. In Wilson disease biliary copper excretion is genetically diminished and copper accumulates in hepatocytes mainly bound to metallothionein. Hepatocellular copper overload leads to release in blood for excretion in urine and distribution to other organs, preferentially to the basal ganglia of the brain. Excess of intracellular free copper bears the risk of parenchymal damage, e.g., hepatic and neurologic manifestation. One therapeutic approach intends the reduction of free copper in blood by chelation and consequent enhanced urinary excretion. As alternative therapy the oral application of zinc is possible. Zinc is a very potent metallothionein inducer operative mainly in mucosal cell, but also in hepatocytes. Metallothionein copper complexes caught in mucosal cells are lost with their desquamation and, thus, intestinal copper absorption is reduced.

**Conclusions:** It is the free copper which induces the organ manifestation in Wilson disease and represents the target of therapy.

**Keywords:** Wilson disease; cholestasis; copper metabolism; liver; brain

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## Introduction

The present review collects data from literature and combines them with an own perspective of the disease obtained by long standing personal experience with Wilson disease patients.

It should not be considered as a complete textbook-

like collection about all the facts on Wilson disease which would burst the allowed space for the contribution. For this we refer to a respective representative publication (1). The subject is not even a systematic review of which so many already exist or a meta-analysis. Intention of the

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article is the collection of data to support the view that Wilson disease is a complex multifaceted and unique copper overload condition, where intracellular accumulation of toxic free copper, but not total copper, causes organ damage. Therapy should therefore be directed to reduce copper toxicity. The paper is to stimulate scientists to drill further into the pathophysiology of this obscure disease with the intention to help affected patients. We present the following article in accordance with the Narrative Review reporting checklist (available at <https://amj.amegroups.com/article/view/10.21037/amj-22-24/rc>).

## Methods

### *Search process for relevant papers about studies reporting critical mechanisms inducing cell injury and strategies for prevention*

For this narrative review, we searched the PubMed database for studies published between years 1980–2022 using the search terms “mechanism of liver injury in Wilson disease”, “therapeutic strategies”, and “monitoring and outcome”. Papers identified by these search terms with no further inclusion or exclusion criteria were screened for information providing new aspects about the biochemical disease process and the role of copper in the pathogenesis of Wilson disease.

## Key Content and Findings

### *Physiology of copper metabolism*

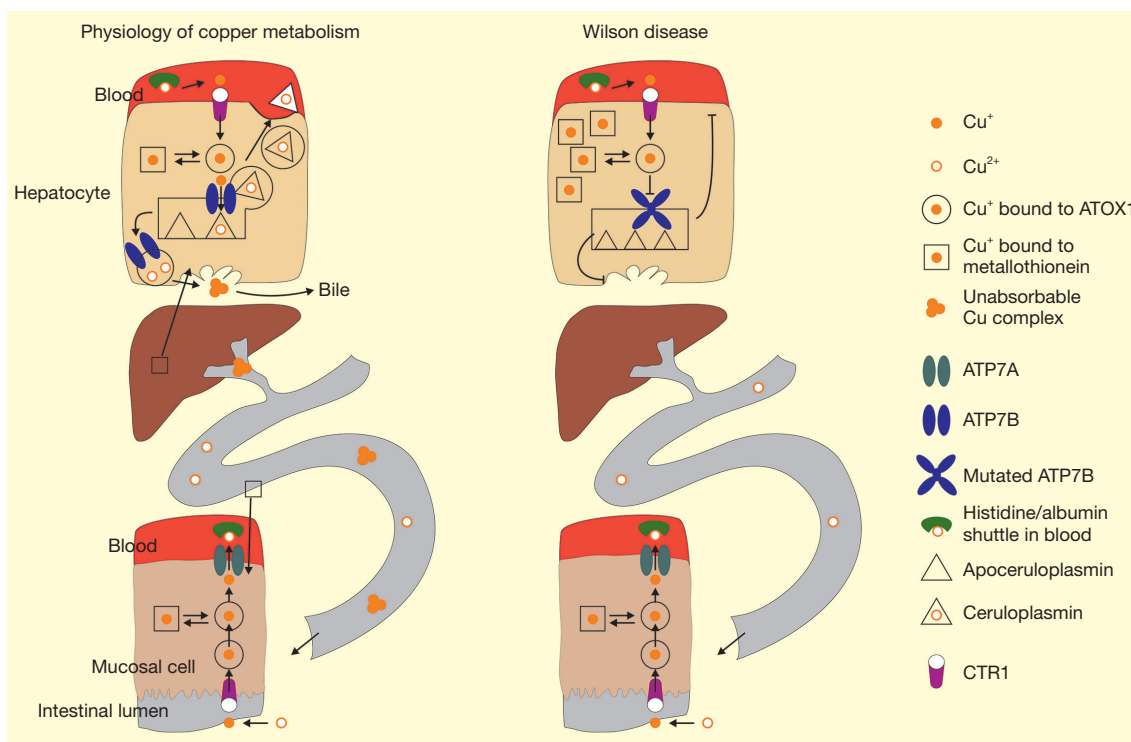
Copper (Cu) is a trace element essential in electron transfer reaction which is required for cellular respiration including handling of oxygen and free radicals (2). In food it is present mostly in its cupric form ( $\text{Cu}^{2+}$ ) and the recommended daily allowance is age-dependent, e.g., in adults 900  $\mu\text{g}/\text{day}$  (3). About 50% is absorbed for which it is reduced to  $\text{Cu}^+$  (cuprous form) outside of mucosal cells (4). The mechanistic of copper physiology is depicted in the cartoon of *Figure 1*. Only free  $\text{Cu}^+$  can be translated across membranes. However, it is cell toxic due to free radical formation and therefore must be bound to proteins for cellular protection (5). Uptake into cells is mediated by a specific, high affinity transport system, the Cu transporter 1 (CTR1) (6-9). Within cells it is handled to the antioxidant Cu chaperone (ATOX1), which directs  $\text{Cu}^+$  to Cu-transporting P-type ATPases (10,11). In intestine the predominant Menkes disease protein, the ATPase copper transporter alpha (ATP7A) translocates  $\text{Cu}^+$  out of mucosal

cells (*Figure 1*). The external loop of ATP7A is rich in histidine and methionine residues which are essential for dephosphorylation to release copper (12). At the basolateral side of mucosal cells transported  $\text{Cu}^+$  is reduced to  $\text{Cu}^{2+}$  and taken up by the amino acid histidine. It is reported that  $\text{Cu}^{2+}$  in blood is shuttled between histidine for cellular uptake and release and on the other hand albumin for transportation, both constituting the non-ceruloplasmin bound “free” Cu fraction (13-15). It means that  $\text{Cu}^{2+}$  bound to albumin for transport in blood can be released at cellular uptake sites by accumulated histidines or histidine residues at the transporter CTR1. Free copper also represents the Cu, which is secreted in urine because histidine bound  $\text{Cu}^{2+}$  can be filtered by the glomerula and accounts for increased urinary Cu excretion in Wilson disease.

The liver demands most of the absorbed Cu for incorporation in enzymes controlling metabolism, for ceruloplasmin synthesis and for biliary excretion which is the control mechanism to maintain a balanced Cu level (16-18). Uptake in hepatocytes occurs via CTR1. Its external loop allows binding of  $\text{Cu}^{2+}$  by histidine containing domains followed by oxidation to  $\text{Cu}^+$  for transportation into the cell (15). Intracellularly  $\text{Cu}^+$  is handled to ATOX1 for delivery to the Wilson disease protein ATP7B located on the trans-Golgi network for transmembrane transfer and release to the Golgi lumen. Similar as with ATP7A in mucosal cells, release is mediated by dephosphorylation utilizing histidine and methionine rich domains (19,20).

Translocated  $\text{Cu}^{2+}$  is soluble in the acid milieu of the Golgi lumen and therefore accessible for  $\text{Cu}^{2+}$  requiring enzymes, in particular ceruloplasmin (*Figure 1*). Step by step apoceruloplasmin is loaded with 6 atoms of  $\text{Cu}^{2+}$  (21,22). Holoceruloplasmin is functional and packed into vesicles, the content of which is released at the basolateral side to the circulation, representing the predominant Cu-binding protein in blood (95% of Cu found in plasma) (21-23). It is required in the organism for oxidation and cellular iron excretion (21,23). Non-Cu-loaded apoceruloplasmin is also secreted in blood. Its function needs further exploration (22). ATOX1 bound  $\text{Cu}^+$  can also be transferred to other cytosolic enzymes requiring  $\text{Cu}^+$  as well as metallothionein, a cysteine rich protein which can store to a certain extent excessive  $\text{Cu}^+$  (*Figure 1*) (24-26).

In situation of an increase in hepatocellular cytosolic Cu, ATP7B localizes to an acidified vesicular compartment near the canalicular membrane to accumulate  $\text{Cu}^{2+}$  for subsequent excretion into bile (*Figure 1*) (27-30). The mechanism of biliary release is not completely elucidated.



**Figure 1** Illustration of copper metabolism under physiological condition and in Wilson disease. Copper balance operates between absorption in the upper gastrointestinal tract and excretion by hepatocytes into bile as an unabsorbable complex. The two essential players are the mucosal cell and the hepatocyte. Cellular uptake, intracellular transport, storage, metabolic processing in the liver and cellular excretion is depicted in the cartoon. In Wilson disease the *ATP7B* gene is mutated resulting in a malfunctioning copper transporter at the trans-Golgi network. It prohibits proper copper loading of apoceruloplasmin leading to low serum copper level. Due to inhibition of biliary copper excretion it accumulates in hepatocytes, initially bound to metallothionein.  $\text{Cu}^+$ , cuprous form;  $\text{Cu}^{2+}$ , cupric form; ATOX1, antioxidant copper chaperone; ATP7A, ATPase copper transporter alpha; ATP7B, ATPase copper transporter beta; CTR1, copper transporter 1.

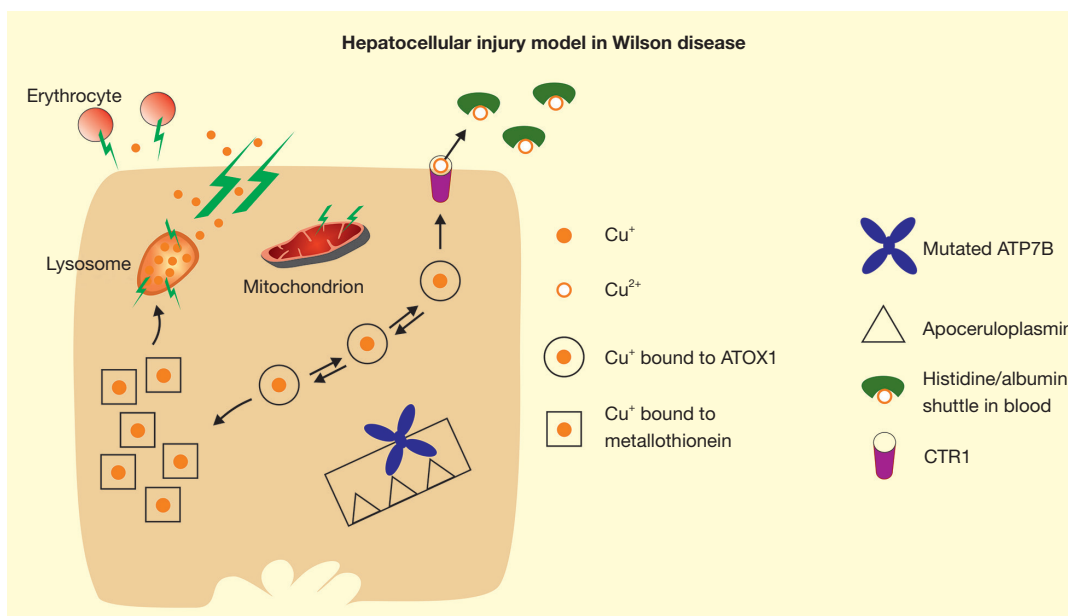
The presence of the Cu metabolism gene MURR1 also known as COMM domain-containing protein 1 (COMMD1) plays a catalytic roll in this process (30,31). Once Cu is in bile it forms an unabsorbable complex, possibly with certain bile acids, which is eliminated in stool (Figure 1) (30).

**Pathophysiology of Wilson disease and its impact of disease manifestation**

Cu overload is most impressively seen in Wilson disease where mutations only of the *ATP7B* gene are causative (about 1,400 reported mutations) (31-33). Mutations result in malfunction of *ATP7B* (Figure 1). Due to impaired influx in the trans-Golgi network Cu is not loaded to apoceruloplasmin. Consequently, holoceruloplasmin is not or only marginally produced disturbing its metabolic responsibilities. In contrast to low ceruloplasmin levels

observed in Wilson disease, the very rare genetic disorder of aceruloplasminemia presents with a more severe neurodegenerative phenotype including ataxia, unvoluntary movements, dystonia, tremor and chorea (34). Underlying defect is the disturbance of cellular iron release with intracellular iron accumulation, but iron deficiency in blood (21,23).

The other pathophysiologically more relevant consequence of *ATP7B* malfunction is impairment of Cu excretion in bile resulting in hepatocellular accumulation, although bile flow is maintained. Low biliary Cu excretion results in view of the negligible urinary excretion of free Cu in a positive Cu balance. In early stages the liver deposits the Cu burden within metallothionein, the synthesis of which is stimulated by Cu (Figure 1) (35). At this stage liver injury is not expected although hepatic Cu content may be highest over the course of the disease. When the metallothionein storage capacity is exhausted, the hepatocyte has the option



**Figure 2** Illustration of the potential hepatotoxic effects of copper overload in Wilson disease. Initially excess copper is stored in cytosolic metallothionein. When stores are full, copper can reversely be excreted via CTR1 back to blood where it is transported within the histidine/albumin shuttle complex as non-ceruloplasmin free copper through the organism for uptake in other organs, e.g., brain and release in urine. Within hepatocytes further accumulation leads to deposition in lysosomes (Rhodamine positive staining). The aggressiveness of  $\text{Cu}^+$  destroys the lysosomal membrane with release of  $\text{Cu}^+$  to cytoplasm and consequent hepatocellular injury, furthermore the plasma membrane becomes leaky with loss of  $\text{Cu}^+$  to blood and eventually destruction of erythrocyte membranes (hemolysis).  $\text{Cu}^+$ , cuprous form;  $\text{Cu}^{2+}$ , cupric form; ATOX1, antioxidant copper chaperone; ATP7B, ATPase copper transporter beta; CTR1, copper transporter 1.

to stop further influx and to export  $\text{Cu}^+$  through CTR1 back to the systemic circulation. This  $\text{Cu}^{2+}$  bound to histidine or albumin, the free Cu pool, remains in the circulation for delivery to other organs.

Later in the course of the disease excessive hepatic copper is distributed to intracellular Cu storing organelles, which could be lysosomes (Figure 2) (36). With time the lysosomal storage capacity is also exhausted, and the organelle membrane may disrupt by free radical attack (Figure 2) (37). Liver injury with elevated transaminases occurs. The process is a slowly progressing disorder involving repair mechanisms eventually leading to fibrosis and cirrhosis. If the content of bursted lysosomes containing  $\text{Cu}^+$  and acid is rapidly released to cytoplasm, fulminant hepatic failure may evolve, triggered by an apoptotic shut down (38,39). The consequent cell destruction leads to massive release of  $\text{Cu}^+$  to the circulation which may not even be bound anymore to the histidine/albumin complexes, at least not with high affinity. Excessive free  $\text{Cu}^+$  release also causes attack of red cell membranes and, thus, hemolysis (40). Cu in urine increases dramatically. However, increased urinary Cu excretion may

not be seen in fulminant hepatic failure due to a concomitant hepatorenal syndrome with kidney failure and anuria.

The free Cu is distributed via the histidine-albumin shuttle throughout the organism. In brain uptake of excess Cu leads to neuronal degeneration, mostly in basal ganglia. It affects the fine motoric movements and coordination, impairing speech, swallowing and hand writing (41). Moreover, psychiatric disturbances are recognized (42). It is discussed whether this is only due to copper overload or in part also to iron accumulation, in particular in cases with severe neurologic manifestation, because ceruloplasmin—the iron exporter—is decreased (23,43). Indeed, brain iron was found to be increased, however, it was argued not to be primary, but secondary to tissue damage (44).

From kidneys the free Cu is excreted in urine. Under physiologic conditions where 95% of serum Cu is tightly bound to ceruloplasmin, only trace amounts appear in urine. However, in untreated Wilson disease cases more than 60  $\mu\text{g}/\text{day}$  are released. The amount can be much higher depending on the free Cu load in blood.

Other rare manifestations of Wilson disease concern

the heart revealing cardiomyopathy, joints revealing arthritis and kidneys revealing renal tubular acidosis with predisposition for stone formation (45). The accumulation of Cu in the cornea leads to the pathognomonic Kayser-Fleischer rings (17).

The presentation of Wilson disease reveals a predominant hepatic, predominant neurologic, a mixed or asymptomatic phenotype. The correlation to a certain *ATP7B* genotype failed at first sight (46). However, recent reports challenged this view and it not excluded that some associations may be found in near future (47-49). Therefore, investigators also sought for disease amplifying conditions, but, unfortunately, without any success until today. Candidates of the involved known proteins of Cu metabolism could not be identified to modify symptomatology and disease progression (46). In particular, asymptomatic patients or those who developed symptoms rather late in life are of interest. If failure of biliary excretion is essential for pathogenesis, the question arises whether there are *ATP7B* independent routes of biliary copper excretion. Alternatively, it is likely that Wilson disease mutations cause only a less functional or lower quantity of *ATP7B*, which slow down metabolism without extinction of the responsible pathways completely. It may be similar as observed in newborns where the *ATP7B*-dependent Cu excretion pathway is not operative due to lack of bile flow, but no hepatocellular injury occurs (50,51). The switch of just Cu overload, where Cu<sup>+</sup> is bound to metallothionein, to Cu-induced hepatocellular injury is not well defined. However, the answer to this question will be of help for therapy of Wilson disease. In this context it is of interest that a gender effect is recognized in Wilson disease (52,53). There is a slight male predominance in disease manifestation with little difference in neuropsychiatric symptoms, but females tend to have more hepatic manifestation (53).

It is not excluded that other liver tackled disorders have impact on presentation of Wilson disease, e.g., primary hemochromatosis,  $\alpha$ 1-antitrypsin deficiency or alcoholic and non-alcoholic steatohepatitis (54). As an example, we recently identified two siblings with Wilson disease who had an autoimmune disorder disposition with elevation of anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies, although the IgG level was in both patients in the normal range (55). Antibodies were already elevated at time of diagnosis. Both showed a hepatic type of Wilson disease. Treatment with D-penicillamine resulted in decoppering with normal urinary Cu excretion after one year. Over the next following 10 years,

transaminases fluctuated between 50 and 500 U/L. At peak of transaminases a short-term (4 weeks) application of 20 mg prednisolone reduced these by 80% which indicates that hyperimmunity but not Cu overload induced the inflammatory phenotype. Although such case reports are not conclusive evidence for a new disease entity, it may open the treating physician for the option that underlying conditions may modulate disease presentation.

### ***Cholestasis and idiopathic childhood cirrhosis, two other copper overload conditions***

When biliary Cu excretion is impaired as under cholestatic conditions, Cu accumulates in hepatocytes (30,56-58). However, the degree of Cu overload is less severe and ceruloplasmin metabolism remains unaltered (59). Moreover, the copper chelator D-penicillamine, which is helpful in Wilson disease, does not have therapeutic impact in reversing cholestasis induced liver injury (60). Intra- and extrahepatic cholestasis can be complicated by inflammation, the cholangitis. In this case laboratory evaluation shows beside cholestasis (elevation of bilirubin, alkaline phosphatase,  $\gamma$ -glutamyltransferase) also elevation of transaminases (i.e., alanine aminotransferase, aspartate aminotransferase).

Cholangitis is attributed to accumulation of too many and toxic bile acids in hepatocytes. Whether the inflammatory phenotype of hepatocytes could also be due to accumulation of non-biliary secreted Cu, reflects an interesting hypothesis. However, intrahepatocellular Cu levels are not excessive and neutralizing metallothionein is operative.

As mentioned before, dietary Cu overload may accelerate liver injury in Wilson disease. As another—even more dramatic example—the idiopathic childhood cirrhosis is a rapid and progressive disorder with a marked increase in hepatic Cu. It was initially described in India in children already by 2 years of age (61,62). Serum ceruloplasmin is normal or even elevated. Epidemiologic studies revealed an increase of Cu content in the diet of affected children. However, in some families an autosomal recessive inheritance with incomplete penetrance was found and genetic abnormalities, also in *ATP7B* gene, were registered (31,63). The pathogenesis of the disease remains obscure. Therapy with D-penicillamine is effective in many cases and liver transplantation can be curative.

### ***Diagnosis***

Diagnosis of Wilson disease is often a challenge. Therefore,



**Table 1** Diagnostic criteria for Wilson disease\*

Criteria/clinical symptoms	Data/score
A: Threshold laboratory values for Wilson disease	
Serum copper	<70 µg/dL (12 µmol/L)
Ceruloplasmin	<0.2 g/L
Urinary copper	>60 µg/day (>0.94 µmol/day)
Free copper (calculated)	>15 µg/dL (>0.23 µmol/dL)
D-penicillamine 500-induced urinary copper	>1,600 µg/day
Liver copper	>250 µg/g dry weight (>4 µmol/g)
B: Wilson disease scoring system (Leipzig score)	
Kayser-Fleischer rings	Absent: 0 Present: 2
Neurological symptoms	Absent: 0 Present: 1 Severe: 2
Ceruloplasmin	Normal (>0.2 g/L): 0 0.1–0.2 (g/L): 1 <0.1 (g/L): 2
Hemolysis	Absent: 0 Present: 1
Liver copper	<0.8 µmol/g: –1 0.8–4 µmol/g: 1 >4 µmol/g: 2 Rhodanine-positive granules (if no quantitative liver copper available): 1
Urinary copper	Normal: 0 60–120 µg/day: 1 >120 µg/day: 2 Normal, but >300 µg/day after 2×0.5 g D-penicillamine: 2
Mutation analysis	No mutation: 0 On one chromosome: 1 On both chromosomes: 4
Evaluation of total score	2 or less: diagnosis very unlikely 3: diagnosis possible 4 or more: diagnosis established

\*, all data were taken from (39).

a diagnostic algorithm has been developed (Leipzig Score) which is highly reliable and applicable in daily practice (39). In *Table 1* the threshold laboratory parameters and the probability of Wilson disease according to the clinical and laboratory findings is summarized. According to pathophysiology, key laboratory parameters for Wilson disease are low ceruloplasmin and, thus, total Cu in blood (*Table 1*) (39).

The direct measurement of pathognomonic elevated

free Cu (60–80% of serum Cu in Wilson disease!) is nowadays possible as non-ceruloplasmin bound copper (NCC) or relative exchangeable copper (64,65). It is of high diagnostic value, also for control of therapy. However, test performance is still a technical challenge for some routine laboratories and, thus, unfortunately not widely available (66).

However, at low ceruloplasmin concentration (<0.1 g/L) there is a simple formula by which free Cu is approximately estimated (39,67):

$$\text{Free Cu}(\mu\text{g/dL}) = \text{total Cu}(\mu\text{g/dL}) - \text{ceruloplasmin}(\text{mg/dL}) \times 3.15 \quad [1]$$

The value is useful, but not 100% reliable (67). At ceruloplasmin >0.2 g/L it fails. Due to the high rate of false positive results is not generally recommended (39).

When kidney function is normal, the amount of Cu in urine (24 h collection) is very sensitive and specific to diagnose Wilson disease (>60  $\mu\text{g}$  Cu/day). It represents the filterable free Cu fraction. Thus, urinary Cu reflects the pathogenetic player of organ manifestation in Wilson disease. It is also very useful to control efficacy of the executed therapy removing free Cu from blood. Accordingly, aim of therapy is to suppress urinary Cu excretion below 60  $\mu\text{g}$ /day. For measurement chelator therapy must be paused for about 48 h because they induce artificially excessive copper excretion. Therefore, in case chelator therapy is continued, values of 200–500  $\mu\text{g}$ /day are desirable (68).

Clinically the Kayser-Fleischer ring is a robust criterion for Wilson disease. It is present in almost 100% of neurologic Wilson disease. This is also true for the combination of neurologic symptoms showing motoric disturbances (mostly tremor, speaking and swallowing difficulties, micrographia with small or cramped handwriting) and signs of liver disease (clinical/ultrasonographic features and laboratory elevation of transaminases and parameters of liver dysfunction). It is less frequent in hepatic and pre-/asymptomatic patients (39).

In unclear cases it is still recommended to perform a liver biopsy and to determine the Cu content in dry weight, accounting in normal liver 15–55  $\mu\text{g/g}$ . A measurement of >250  $\mu\text{g/g}$  is pathognomonic for Wilson disease. It has been reported that already a Cu content of 75  $\mu\text{g/g}$  is suggestive for Wilson disease with a specificity of 95.4% and a sensitivity of 96.5% (69).

Another diagnostic criterion is the identification of disease-causing mutations in the *ATP7B* gene. Due to the autosomal recessive trait compound heterozygous patients are predominant. The number of newly discovered mutations constantly increases up to about 1,400 at present, located in coding, intronic and regulatory sequences, which all affect the function of *ATP7B* or its abundance in hepatocytes (31–33). The global incidence is 1:30,000 with symptomatic presentation mostly between ages of 5 and 35 (70–73). In case just one mutation is detectable, a heterozygous carrier is suspected with an incidence of 1:5,000 (70–73). They often have slightly depressed serum Cu and ceruloplasmin levels and a mild increase in urinary

Cu. However, cohort studies showed no statistical differences between healthy controls and heterozygous carriers, meaning that they do not develop Wilson disease (74).

Currently also the ATP7B peptide assessment is proposed for diagnosis (75). Moreover, the radiocopper test is very reliable to detect homozygous and heterozygous mutation carriers for the Wilson disease gene (76). It is frequently applied in Poland, but due to logistic shortcomings not widely available elsewhere. In addition, the evaluation of Wilson disease with  $^{18}\text{F}$ -FDG PET/CT is of high relevance to detect cerebral copper deposition (77).

### Therapy

Biliary Cu excretion is the only regulative mechanism to maintain a balanced Cu level in the organism (*Figure 1*). Therefore, dietary Cu overload should be avoided in Wilson disease, because biliary Cu excretion is impaired. Cu rich foods are cacao, chocolate, nuts, beans, mushrooms, shellfish, raisins and innards like liver, brain and kidney (78). Even drinking excessive tap water from Cu tube installation can increase the Cu burden.

The ultimate goal of the therapy is to reverse Cu induced cellular injury, mainly improvement of hepatic and neurologic manifestation. One may think that it is the removal of Cu from the organism. However, this may only partially be achievable due to the underlying pathophysiology of the disease.

At present there are three, almost equally effective therapies available: zinc, D-penicillamine and trientine. The latter should only be chosen when zinc and D-penicillamine therapy failed. D-penicillamine and trientine are chelators, which operate in blood to bind free Cu for excretion in urine. Thus, they reduce the harmful free Cu to avoid its distribution to organs, including liver and brain. However, a calculation of their capacity to remove Cu from the organism failed to show a significant effect (67). On the other hand, it was shown that both chelators induce metallothionein synthesis, which is known to act as a  $\text{Cu}^+$  binding protein neutralizing its cellular toxicity (79–82). In intestine, where the chelators must pass the mucosal cells, it is likely that this intracellular induction can occur. Then mucosal cell  $\text{Cu}^+$  bound to metallothionein is lost in stool with shedding of these cells after 1.4 days. Since both chelators cannot easily enter parenchymal cells in liver and brain, it is questionable whether induction of metallothionein occurs there.

In comparison, zinc acts as strong metallothionein

inducer in mucosal cells inhibiting absorption but is also taken up by hepatocytes for further metallothionein synthesis stimulation (67,83). Induction of urinary release of free Cu seems not to be its mode of action. However, by increasing the intracellular pool of Cu binding proteins, it reduces the free Cu pool in blood as effective as chelators and urinary Cu excretion becomes normal. Thus, in addition it would be helpful to measure free Cu in blood for monitoring therapeutic efficacy directly.

A new drug is presently under investigation in clinical trials: bis-choline-tetrathiomolybdate (TTM). It acts as intracellular Cu chelator (84,85). It was shown to improve neurologic symptoms (84). It is also reported to bind free Cu in blood (86). Although the exact mechanism of its action needs more exploration, within hepatocytes it removes Cu bound to proteins and binds it in stable complexes which are cleared into bile (85). At least in high doses it often shows elevation of transaminases indicating cell injury (84–86). Apparently, there is an intracellular redistribution of Cu with concomitant mitochondrial damage (87). This clinically important issue needs attention.

Finally, there is the option of liver transplantation (67). However, it is only chosen in end stage liver disease with poor prognosis due to hepatic failure. In symptomatic Wilson disease the therapeutic goal is to stop progression of liver disease, reversal or improvement of extrahepatic manifestation, and allow an almost normal life expectancy, which in most cases is achievable by the conventional medical therapy (88). Only in some patients, mainly those treated with chelators, neurologic symptoms deteriorate (89). Occasionally disease exacerbation is such severe that patients even become handicapped. The phenomenon is not explained yet. It could be the increase in chelated free Cu, which may enter brain parenchymal cells. Intracellular Cu chelators like TTM or the very old drug British Anti Lewisite (Dimercaprol—painful intramuscular injections!) may theoretically help although it is not systematically investigated in these cases. The metabolic defect in Wilson disease resides in the liver. Correction of the genetic defect would most likely cure the disease. Since gene therapy is still being investigated in two phase I/II clinical trials (39), liver transplantation is established and would at present serve the same purpose. Thus, liver transplantation was suggested also to be applied in patients with a fulminant neurologic course. Indeed, positive outcomes about this approach have been reported (90). However, critics argue since the neurologic disorder is manifested, it is an irreversible damage and also liver transplantation would be

of no cure (91).

However, liver transplantation is an unequivocal therapeutic consideration in end stage liver disease which occurs in a cirrhotic liver concomitant with hepatic failure. In Wilson disease it is observed in too late diagnosed and, thus, mainly untreated cases. Liver transplantation also has to be applied in fulminant hepatic failure. It is the rarely observed first manifestation of Wilson disease, mostly in young, predominantly female patients without previous signs of liver disease. It occurs unexpectedly with a very rapid course of deterioration ending in death within a few days. Mechanistically it is an apoptotic process affecting the total liver at once (38). Hepatic failure, coagulopathy, kidney failure and hepatic coma are the sequel of events. In this case liver transplantation with utmost urgency is required. If successful, Wilson disease appears to be history because the genetic defect is corrected and requires no further Cu controlling therapy. The only question which remains open, is whether the mutated *ATP7B* itself may still induce cell injury in organs other than the liver, e.g., brain.

In addition, several experimental strategies have suggested other possibilities to cure Wilson disease-induced hepatic Cu overload. In one approach, the transduction of an adeno-associated vector serotype 8 (AAV8) encoding the human *ATP7B* cDNA placed under control of the liver specific  $\alpha$ 1-antitrypsin promoter allowed long-term correction of copper metabolism in *Atp7b* null mice (92,93). The gene correction was also associated with significant lowering of cerebral Cu concentrations, most prominently in the cerebellum, cerebellar white matter, corpus callosum, and in some ventricles (94). Another study showed that the blood-brain barrier that complicates the treatment with chelating agents in the setting of Wilson disease can be successfully passed by vectorized liposomes in which chelating agents such as triethylenetetramine (TETA) can be encapsulated. In rats such a strategy resulted in an up to 16-fold higher brain uptake of TETA compared to free TETA (95).

## Conclusions

It is established that malfunctional *ATP7B* is the trigger of liver injury. There is already an exceptional knowledge about the mechanisms by which copper and mutations within the *ATP7B* gene develop their pathogenic properties during onset and progression of Wilson disease (96,97). Nevertheless, the narrative review presented here shows that all this knowledge is not suitable to prevent the onset of serious symptoms in many cases or to explain the variability



in the type and severity of disease-associated symptoms. Furthermore, the efficacy of chelating agents and other therapies are still controversially discussed and there are still many questions unanswered.

In particular, key questions that need to be elucidated are:

- ❖ Is it indeed the intracellular free fraction of  $\text{Cu}^+$  which causes membrane destruction?
- ❖ How is the free Cu fraction structurally defined and where exactly is it generated?
- ❖ How  $\text{Cu}^+$  is translocated across intracellular membranes?

In this regard, the following observations are of interest:

- ❖ Newborns store excessive Cu without any harm to the liver.
- ❖ The years of Cu accumulation in Wilson disease pass without cell injury despite highest Cu levels.
- ❖ There are anecdotal reports that despite many years of effective therapy and improvement of liver function, intrahepatic Cu stores remain widely unaffected in comparison to time of diagnosis.

This may feed the hypothesis that it is not just Cu overload but the toxic form of cellular Cu which causes membrane destruction. Then the goal of therapy is (I) to reduce free copper in blood by chelators or zinc and (II) to switch cell toxic Cu into a harmless storage form, e.g., by binding to metallothionein. Whether these are the only protective mechanisms remains open. Uptake in cell organelles, i.e., mitochondria and lysosomes with consequent functional or structural damage requires further exploration.

### *Limitations of this study*

The applied literature research discovered basic findings relevant for pathophysiology of Wilson disease. Key findings were that elevation of free copper in blood is due to impaired biliary excretion in Wilson disease and that this free copper is responsible for copper accumulation in different organs. However, detailed mechanistic descriptions were often missing. Statements of key opinion leaders and commercial driven studies/trials limited the objectivity of published results. In addition, based on the high amount of studies dealing with topics of Wilson disease pathogenesis, it might be possible that several important studies were overseen in this analysis.

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