

Review

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Metabolic mysteries of copper dysregulation in Wilson disease

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Abstract

Wilson disease, a well-established genetic disorder characterized by impaired copper excretion and toxic copper accumulation in the liver, has clear clinical presentations and diagnostic criteria. However, the metabolic and molecular pathways leading to liver injury - a critical hallmark of the disease - remain largely cryptic and require new analytical approaches to elucidate underlying mechanisms. In Wilson disease, supraphysiological and toxic amounts of hepatic copper arise due to genetically reduced biliary excretion. Interestingly, hepatic iron accumulation of unknown etiology is also observed. Both metals contribute to the production of reactive oxygen species (ROS), including singlet oxygen, superoxide anions, and highly disruptive hydroxyl radicals generated via the Haber-Weiss and Fenton reactions. Historically, liver injury has been attributed to copper-induced toxicity (cuproptosis) without sufficient consideration of ROS involvement, neglecting the significant ROS burden in hepatic tissue. A revised concept of cuproptosis incorporates ROS, particularly hydroxyl radicals, which convert copper ions into reactive intermediates such as copper peroxy, copper hydroperoxy, and copper superoxy species. These intermediates induce mitochondrial oxidative stress by covalently binding to mitochondrial constituents including lipoylated dihydrolipoamide S-acetyltransferase (DLAT), leading to its aggregation and triggering regulated cell death via cuproptosis. Thus, copper ions must first be converted into reactive intermediates to initiate cuproptosis effectively. Furthermore, singlet oxygen and superoxide anion hydroxyl radicals generated by hepatic iron ions may promote regulated cell death via ferroptosis. This process involves the accumulation of lipid peroxides derived from polyunsaturated fatty acids in mitochondrial membranes, with malondialdehyde (MDA) serving as a diagnostic marker. Consequences include enhanced mitochondrial



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membrane rigidity, disruption of plasma membrane integrity, and ultimately cell death. This mechanistic pathway requires the activity of iron-dependent enzymes such as lipoxygenases, ferroptosis suppressor protein 1, glutathione peroxidase 4, dihydroorotate dehydrogenase, and lysosomal iron release from ferritin stores. To sum up, the definition of cuproptosis requires refinement to incorporate its mechanistic dependence on ROS, and its quantitative contribution to liver injury should be reassessed alongside hepatic iron and ferroptosis.

Keywords: Copper, Wilson disease, cuproptosis, ferroptosis, reactive oxygen species, immune reactions

INTRODUCTION

Heavy metals are metallic elements with a high atomic weight of > 20 and are commonly found in the environment^[1-9]. At low concentrations, copper and iron are essential for human survival^[2,10]; however, they can become harmful at higher concentrations^[10]. Even when ingested in normal amounts, copper and iron may become toxic in certain individuals with genetic predispositions that lead to hepatic overload - iron overload in hemochromatosis^[11-17] or copper overload in Wilson disease^[18-27]. In these patient cohorts, copper and iron can have deleterious effects^[17,27]. Moreover, in patients with Wilson disease, the liver not only accumulates excess copper but also contains some iron. These metals may act synergistically to exacerbate liver injury through regulated cell death (RCD) pathways: copper via cuproptosis and iron via ferroptosis^[27].

This review aims to clarify how environmental copper intake can lead to hepatic copper dysregulation due to impaired metabolic pathways, ultimately causing Wilson disease - a clinically complex and still poorly understood disorder. It discusses the role of inflammatory cytokines, reactive oxygen species (ROS) generated from copper (cuproptosis) or iron (ferroptosis), and immunological observations in selected cases that may be classified as copper-induced autoimmune hepatitis (CIAIH). These processes contribute to severe liver disease in Wilson disease patients, which may progress to acute liver failure and life-threatening outcomes, occasionally necessitating liver transplantation when conventional drug therapy fails.

ENVIRONMENTAL COPPER

All heavy metals, including copper, originated in the universe, produced from helium and hydrogen through nuclear fusion in stars and supernova explosions before arriving on Earth around 13.7 billion years ago^[10]. Copper and other heavy metals were present on our planet long before the emergence and evolution of humans^[28-30], with copper detected throughout the environment - in soil, water, the atmosphere^[28-31], and the food chain^[27,32] - thereby entering the human body^[27]. Copper is an essential trace element for various physiological functions. Copper ions are involved in mitochondrial energy production and maintenance, redox homeostasis, biosynthesis of biomolecules, signaling pathways, and extracellular matrix remodeling^[27]. More specifically, physiological levels of copper are necessary for hemoglobin synthesis, drug and xenobiotic metabolism, carbohydrate intermediary metabolism, catecholamine biosynthesis, collagen cross-linking, hair keratin formation, and metabolic processes enabling antioxidant defense^[27,33]. Copper also serves as a cofactor for various copper-dependent enzymes, such as oxygenases and oxidoreductases, which facilitates electron transfer processes. These include hydroxylases, transferases like Cu/Zn superoxide dismutase, cytochrome c oxidase, hephaestin, ferroxidase, ceruloplasmin, monoamine oxidase, lysyl oxidase, and dopamine β -monooxygenase, which catalyzes the conversion of dopamine to epinephrine^[27]. The beneficial effects of environmental copper are well documented in individuals without genetic defects affecting copper metabolism^[27,32]. In contrast, patients with Wilson disease experience copper dysregulation due to mutations in the *ATP7B* gene, which encodes a copper-transporting ATPase^[18-27].

COPPER DYSREGULATION RELATED TO *ATP7B* GENETIC ISSUES IN WILSON DISEASE

Excessive hepatic copper deposition in Wilson disease results from impaired biliary excretion of excess copper due to dysfunction of the *ATP7B* gene^[20,21,24,27]. This leads to disrupted hepatocellular copper homeostasis. When hepatocytes can no longer store copper adequately, toxic copper ions are released into the bloodstream and accumulate in other organs, increasing the risk of cell injury^[27]. Consequently, copper accumulation outside the liver can cause damage to many clinically important organs^[19,25,27].

METABOLIC COPPER DISTURBANCE IN WILSON DISEASE

The metabolic pathways underlying Wilson disease remain poorly understood, partly because the liver is a “cryptic” organ that tends to conceal metabolic disturbances, limiting insight unless specific biomarkers are released into the bloodstream for analysis by physicians and scientists^[27]. Animal models that mimic Wilson disease^[34], as well as studies using zebrafish^[35], are expected to help elucidate pathogenetic mechanisms driving this disorder. After acute copper intoxication, the pathogenetic steps in animals resemble those in healthy humans^[36,37]; however, they differ from those in Wilson disease patients, who have hereditary risk factors. Consequently, these animal models are of limited value for fully understanding the disease^[27].

The excessive hepatic copper deposition in Wilson disease greatly exceeds physiological levels and causes significant liver injury in affected patients^[38]. The cascade of events causing liver injury begins with the physiological dietary intake and intestinal absorption of copper, which cannot be adequately managed by patients with Wilson disease, ultimately resulting in severe copper dysregulation. The key steps are summarized for quick reference in [Table 1](#)^[39-68].

Different mechanistic steps, as summarized above [[Table 1](#)], ultimately result in liver injury in patients with Wilson disease^[39-68]. The cascade starts with the ingestion of food-derived copper, present in its monovalent (Cu^{1+})^[69] and divalent (Cu^{2+}) forms^[59,69]. Dietary Cu^{2+} is readily reduced to Cu^{1+} by duodenal cytochrome *b*₅₆₁ (DCYTB), a transplasma membrane oxidoreductase^[70]. In healthy individuals, excess copper is effectively excreted via bile, maintaining homeostasis^[59]. By contrast, Wilson disease patients continuously absorb copper from their diet but lack effective mechanisms to regulate intestinal copper uptake or excrete excess copper^[19,20,27,44,59,70]. Dietary copper restriction alone is not evidence-based or effective in these patients^[71], as intestinal copper absorption is genetically controlled through specific enterocyte transporters^[72] and tightly regulated to meet the body's needs^[27,72-76]. Hepatocellular copper uptake proceeds continuously via membrane transporters, irrespective of the defective biliary excretion of surplus copper^[77].

Consequently, various hypotheses have been proposed regarding the metabolic mechanisms by which copper causes liver injury in Wilson disease, ranging from strongly evidence-based models to those relying on circumstantial evidence. These diverse perspectives highlight the need for detailed discussion of this orphan disease and its complex metabolic disturbances in copper dysregulation^[59].

Critical role of ROS and vicious cycles

Convincing evidence shows that copper generates ROS, with hydroxyl radicals as the main toxic product. This has been demonstrated using electron paramagnetic resonance (EPR) spectroscopy measurements^[77] and electron spin resonance (ESR) spin-trapping studies^[46], approaches often combined with hepatic copper measurements in Wilson disease patients using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)^[21]. However, it remains unclear whether copper alone, or in combination with iron, triggers this process, as both metals are present in the liver of Wilson disease patients^[21,27,45,47,50,52]. Both metals contribute to ROS production via the Haber-Weiss and Fenton reactions, ultimately generating radical species identical to those produced by iron alone [[Table 2](#)].

Table 1. Cascade of events leading to liver injury in Wilson disease

Cascade of events	Short description	References
1. Normal intestinal uptake of copper	In healthy individuals, copper from food is absorbed in normal amounts under tight genetic control	Chen et al., 2022 ^[39] Doguer et al., 2018 ^[40]
2. Transfer of copper to the liver	In both healthy individuals and Wilson disease patients, copper is transferred from enterocytes to hepatocytes via the portal circulation. In Wilson disease, hepatocellular uptake remains unchanged despite ongoing accumulation, as there is no mechanism to regulate uptake based on intracellular copper levels	Chen et al., 2022 ^[39] La Fontaine et al., 2010 ^[41]
3. Impaired biliary excretion in Wilson disease	Mutations in the <i>ATP7B</i> gene, encoding the ATP7B transporter, impair both biliary copper excretion and the synthesis of ceruloplasmin, a copper-carrying protein	Chang et al., 2017 ^[42] Chen et al., 2022 ^[39] La Fontaine et al., 2010 ^[41] Lutsenko, 2016 ^[43] Yu et al., 2018 ^[44]
4. Copper-induced ROS generation	Excess intracellular copper promotes the generation of ROS, similar to iron in hemochromatosis. In Wilson disease, hepatic iron accumulation occurs secondarily, likely due to hemolysis	Hayashi et al., 2006 ^[45] Kadiiska et al., 2002 ^[46] Shiono et al., 2001 ^[47] Tsang et al., 2021 ^[48] Xue et al., 2023 ^[49]
5. Cuproptosis and ferroptosis	Liver injury is primarily caused by copper binding to lipoylated enzymes in the TCA cycle, triggering cuproptosis. Iron contributes minimally via ferroptosis. Both processes are initiated by ROS derived from the Haber-Weiss and Fenton reactions	Chen et al., 2022 ^[39] Gromadzka et al., 2022 ^[50] Li et al., 2023 ^[51] Pak et al., 2021 ^[52] Tang et al., 2022 ^[53] Tsvetkov et al., 2022 ^[54] Wang et al., 2022 ^[55] Xie et al., 2023 ^[56]
6. Autoimmunity	In rare cases, CIAIH can occur, requiring immunosuppressive therapy	Antczak-Kowalska et al., 2022 ^[57] Jańczyk et al., 2023 ^[58] Penning et al., 2023 ^[59] Stremmel et al., 2021 ^[60]
7. Inflammatory cytokine cross-talk	Copper accumulation triggers inflammation in various organs and tissues. Antibody microarray analysis reveals dysregulated cytokine profiles, including increased interleukin and chemokine expression	Goyal et al., 2008 ^[61] Hopkins et al., 1997 ^[62] Hopkins et al., 1999 ^[63] Kalita et al., 2014 ^[64] Kelley et al., 1995 ^[65] Kisseleva et al., 2021 ^[66] Wu et al., 2019 ^[67]
8. Gut microbiome	The gut microbiome may influence the clinical course of Wilson disease	Cai et al., 2020 ^[68]

The table is modified and updated from a previous publication in an open access journal^[27]. ROS: Reactive oxygen species; TCA: tricarboxylic acid; CIAIH: copper-induced autoimmune hepatitis.

Table 2. Cascade of events triggered by the Fenton and/or Haber-Weiss reactions

Reaction type	Reaction
Copper-based Haber-Weiss reaction	$\text{Cu}^{2+} + \bullet\text{O}_2^- \rightarrow \text{Cu}^{1+} + \text{O}_2$
Copper-based Fenton reaction	$\text{Cu}^{1+} + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \text{OH}^- + \bullet\text{OH}$
Iron-based Haber-Weiss reaction	$\text{Fe}^{3+} + \bullet\text{O}_2^- \rightarrow \text{Fe}^{2+} + \text{O}_2$
Iron-based Fenton reaction	$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \bullet\text{OH}$
Copper-based Net reaction	$\bullet\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \bullet\text{OH} + \text{O}_2$
Iron-based Net reaction	$\bullet\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \bullet\text{OH} + \text{O}_2$

This table was derived from an open access article^[27]. The Net reaction represents the overall result of the combined Haber-Weiss and Fenton reactions.

Moreover, these metals can establish self-perpetuating vicious cycles [Table 2]^[27]: Cu^{2+} is reduced to Cu^{1+} , which is then re-oxidized to Cu^{2+} , while Fe^{3+} is reduced to Fe^{2+} and then oxidized back to Fe^{3+} . As a consequence, both cycles sustain liver injury through continuous, irreversible chain reactions, with copper posing a greater risk due to its higher hepatic levels compared to iron^[27]. Quantitatively, the livers of Wilson disease patients contain not only excessive copper^[21,45,52] but also substantial quantities of iron^[21,45,47,50,52].

The hepatic iron deposition is primarily attributed to low levels of ceruloplasmin^[21], a ferroxidase that facilitates iron storage in tissues, similar to what occurs in hemochromatosis^[52]. Additionally, iron released from erythrocytes due to hemolysis, observed in some Wilson disease patients, may further contribute to hepatic iron deposition^[27]. Considering the critical roles of these two metals in liver injury [Table 2], the mechanistic processes known as cuproptosis (copper-induced cell death) and ferroptosis (iron-induced cell death) merit intensive discussion [Figure 1].

Copper-related cuproptosis and iron-mediated ferroptosis

In addition to the close interaction between copper and iron in the Haber-Weiss and Fenton reactions [Table 2], copper-mediated cuproptosis, copper itself, ferroptosis, and iron itself all interact and are strongly associated with Wilson disease^[27]. As a key example, copper and iron are both absorbed by duodenal enterocytes^[40,76]. They share a common mechanistic pathway involving duodenal cytochrome b (DCYTB) for transfer from the intestinal lumen into enterocytes, which ultimately release both metals into the bloodstream^[76].

Cuproptosis

Cuproptosis contributes to the toxicity of copper ions in Wilson disease^[27,78], driven by their redox properties that generate ROS [Table 2]^[27,59,79-82]. ROS-induced cellular oxidative stress is considered a major cause of liver injury in Wilson disease^[27,51,59,78-89]. However, this ROS-related concept has been challenged by the proposal of cuproptosis as a novel, ROS-independent, copper-related form of RCD^[54], which plays a pathogenic role under copper overload^[90]. A hallmark of cuproptosis is the aggregation of lipoylated dihydrolipoamide S-acetyltransferase (DLAT), implicated in Wilson disease^[54]. These aggregates are associated with the mitochondrial tricarboxylic acid (TCA) cycle, resulting in proteotoxic stress consistent with the cuproptosis mechanism^[51,54]. Cuproptosis, as a causal mechanism in Wilson disease, differs from other RCD types such as apoptosis, ferroptosis, necroptosis, and pyroptosis. Nevertheless, conflicting views persist regarding the precise mechanistic pathways driving liver injury, with earlier reports emphasizing apoptosis, caspase-independent cell death, the ubiquitin-proteasome system, or ROS involvement^[54]. Despite initial enthusiasm for cuproptosis as a novel pathogenetic mechanism^[27,51,54], the molecular and metabolic complexities underlying copper-dependent hepatocellular injury in Wilson disease remain unresolved, especially since the role of ROS and reactive copper intermediates was not originally included in the cuproptosis framework^[51,54]. Therefore, further studies are needed to clarify these pathogenetic mechanisms and confirm the contribution of cuproptosis.

To integrate possible mechanistic events, a unified proposal for the role of cuproptosis in Wilson disease is suggested: (1) Published data support that copper ions induce oxidative stress by generating ROS, including hydroxyl radicals and H_2O_2 , via the Haber-Weiss and Fenton reactions^[21,27,45-49,59,77,90-92]. These ROS, particularly hydroxyl radicals, directly damage subcellular membranes, especially mitochondrial membranes, representing a “first hit”^[27,51,54]; (2) ROS also promote the conversion of copper ions into reactive intermediates such as copper peroxy, hydroperoxy, and superoxy radicals^[93-95], which further attack mitochondrial membranes, leading to mitochondrial dysfunction - considered a “second hit”; (3) Finally, as a “third hit”, damaged mitochondria^[51,83] become susceptible to adduct formation with these copper intermediates^[93-95], leading to irreversible binding to mitochondrial phospholipids and proteins, including DLAT. This results in DLAT aggregation and ultimately cell death due to impaired mitochondrial respiration^[51]. Whether DLAT aggregation can also occur through iron and ferroptosis remains to be determined. Lipoylated protein aggregation is associated with the destruction of iron-sulfur cluster proteins, commonly initiated by ROS^[54]. This process releases iron, which may further enhance ROS generation via ferroptosis, especially in Wilson disease livers already rich in iron.

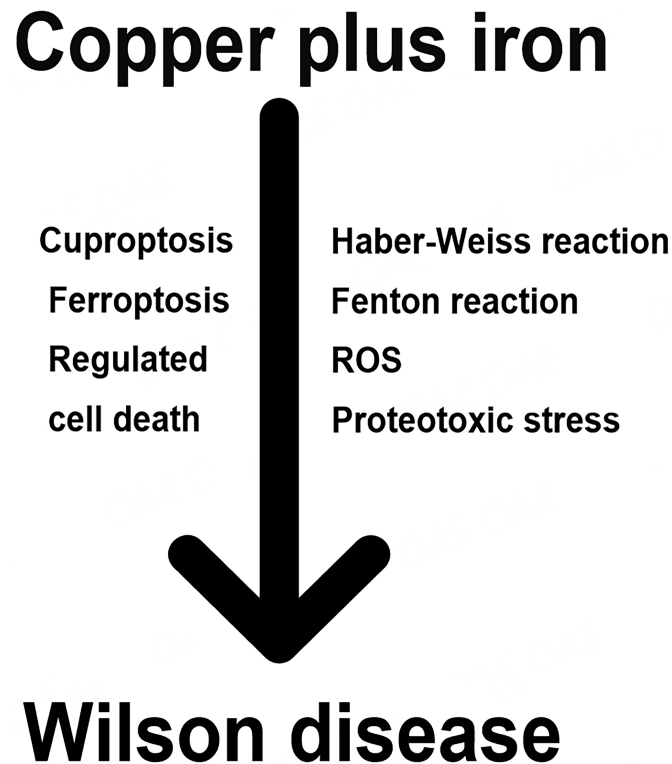


Figure 1. Flow chart illustrating the mechanistic steps by which copper and iron contribute to the pathogenesis of Wilson disease. This figure was modified from a previously published open access article^[27]. ROS: Reactive oxygen species.

Multiple mechanistic pathways involving excessive copper ions are under investigation, yet robust quantitative data and reliable biomarkers are still lacking, leaving cuproptosis as a molecular and metabolic enigma^[96]. Driven by the Haber-Weiss and Fenton reactions [Table 2], excess copper ions initially generate hydroxyl radicals, which subsequently convert copper into highly reactive intermediates (peroxyl, hydroperoxyl, and superoxyl species). While hydroxyl radicals induce mitochondrial oxidative stress, the reactive copper intermediates covalently bind to DLAT, triggering its aggregation and thereby initiating cuproptosis. Thus, copper ions require conversion to reactive intermediates before they can drive cuproptosis.

Ferroptosis

Hepatic iron deposits have been observed in both patients with Wilson disease and in animal studies^[21,27,45,47,50,52,97-99]. These iron accumulations resemble those seen in secondary iron overload (hemosiderosis) or primary genetic iron storage disorders (hemochromatosis)^[12-17]. However, contrary to earlier reports suggesting an association with *HFE* gene polymorphisms, later clinical studies demonstrated that allele frequencies in Wilson disease patients are comparable to those in the general population^[100]. The mechanisms underlying hepatic iron accumulation and the roles of iron and ferroptosis in liver injury alongside cuproptosis in Wilson disease remain unclear^[27].

Iron-dependent ferroptosis is another form of RCD, potentially contributing to liver injury in Wilson disease together with cuproptosis^[27]. Ferroptosis is characterized by the following key molecular events: (1) Elevated hepatic iron levels promote ROS generation (including hydroxyl radicals, singlet oxygen, and superoxide anions) via the Haber-Weiss and Fenton reactions [Table 2]^[27,51,59,101-103]; (2) Iron-derived ROS induces oxidative stress and lipid peroxidation of polyunsaturated fatty acids within mitochondrial membranes, marked by increased malondialdehyde (MDA) levels^[104,105]. This leads to mitochondrial membrane stiffening^[104], disruption of plasma membrane integrity^[105], and ultimately cell death^[104,105]. This process involves iron-dependent enzymes such as lipoxygenases, ferroptosis suppressor protein 1, glutathione peroxidase 4, dihydroorotate dehydrogenase, and lysosomal iron release from ferritin^[104]. The regulatory roles of iron and ferroptosis in autoimmune and inflammatory diseases are also under scientific discussion^[105].

Wilson disease remains a complex disorder, with the exact contributions of ferroptosis and cuproptosis to liver injury yet to be fully established. This uncertainty requires further research, including precise comparative measurements of hepatic iron and copper overload using advanced methods such as LA-ICP-MS^[21,106,107]. Although technically complex, LA-ICP-MS allows highly sensitive, quantitative, and spatially resolved visualization of metal distribution in liver tissue with excellent reproducibility^[21].

Autoimmunity

For the sake of completeness, autoimmunity in rare cases of Wilson disease should be discussed due to its therapeutic implications. In one patient with Wilson disease, elevated serum titers of anti-nuclear antibodies (ANA) were detected, accompanied by increased transaminases despite normal serum free copper levels after continuous treatment with D-penicillamine. This laboratory and clinical constellation was interpreted as hyperimmunity, which was successfully treated with steroids^[60], with additional data published subsequently^[59]. Alternatively, this case may be classified as CIAIH, analogous to drug-induced autoimmune hepatitis (DIAIH)^[108-112]. The diagnosis is based on two separate scoring algorithms: the updated Roussel Uclaf Causality Assessment Method (RUCAM) to evaluate the drug-induced liver injury (DILI) component^[113], and the simplified autoimmune hepatitis (AIH) score to assess the autoimmune component^[114]. To apply these considerations to the Wilson disease case with hyperimmunity features and to establish CIAIH as a definitive diagnosis^[60], two scoring algorithms are essential: the Modified Leipzig Scoring System for Wilson Disease to confirm the copper-related component^[27,115,116], and the simplified AIH score to verify the autoimmune component^[114]. The advantage of using these tools lies in their quantitative approach, as they use key elements that are individually scored and summed to achieve a final score indicating the probability of causality. However, both methods require liver histology data^[114,115,116]. In the case under discussion, the simplified AIH score was not applied, but the effective response to steroids supports the diagnosis of CIAIH^[60].

In another case of liver disease, increased serum titers of ANA and anti-smooth muscle antibodies (ASMA) led to the erroneous diagnosis of AIH, and steroid treatment was unsuccessful^[59]. This failure prompted further diagnostic evaluation, which ultimately resulted in the correct diagnosis of Wilson disease, successfully treated with copper chelating drugs^[59]. This case underscores that increased serum autoimmune makers alone do not justify a firm AIH diagnosis, especially given the high prevalence of elevated ANA titers in the general white population in the US, reported to be up to 17.8%^[117].

Autoantibody seropositivity is a common finding in Wilson disease and remains a clinical issue^[50,57,58,118]. For example, ANA was detected in 11.0% of a control group without Wilson disease, compared to 21.6% of patients with Wilson disease. However, this Wilson disease cohort was heterogeneous, as it included

patients receiving D-penicillamine or zinc sulfate, which may have confounded the results^[57]. Therefore, it is recommended to assess serum autoimmune markers in all patients with suspected Wilson disease before initiating chelation treatment. Repeated measurement is also advised if chelation therapy fails, to rule out the interim development of CIAIH, which may require treatment with steroids.

Immune cells and cross-talk of inflammatory cytokines

Inflammation is a self-defensive process that helps eliminate or neutralize injurious stimuli with the aim of restoring tissue integrity^[67,119]. In this context, hepatic immune cells play a role in Wilson disease, as evidenced by the presence of cytokines and other inflammatory mediators in the plasma of affected patients^[61,64,67]. This observation transforms the liver from a quiescent organ into an active source of information, providing insights into the molecular and mechanistic changes occurring during hepatic injury^[67]. Studies analyzing cytokines and chemokines in the plasma of patients with Wilson disease have enhanced our understanding of the underlying mechanisms and confirmed that the disease is driven by dysregulated inflammatory mediators^[61,64,67]. In one study, a cohort of 99 patients with Wilson disease was compared to 32 healthy controls. Unlike the controls, patients with Wilson disease showed significantly increased plasma levels of T helper (Th) 1 cytokines (IL-2, TNF- α , and TNF- β), Th2 cytokines (IL-5, IL-10, and IL-13), and Th17 cytokines (IL-23). Moreover, neurological patients exhibited significantly higher plasma levels of Th 1 cytokines (IL-2, TNF- α , and TNF- β), Th 2 cytokines (IL-13), and Th 17 cytokines (TGF- β 1, IL-23) compared to control groups. In hepatic and neurological patients, Th 1 (TNF- α and TNF- β), Th 3 (TGF- β 1), and Th 17 (IL-23) levels were also significantly elevated. These findings suggest that higher levels of Th1 (IL-2, TNF- α , and TNF- β), Th2 (IL-13), and Th17 (TGF- β 1, IL-23) cytokines are associated with the severity of neurological symptoms in Wilson disease patients^[67]. Similar patterns of inflammatory mediator changes have been reported in other studies on Wilson disease^[61,64]. The diversity of circulating mediators suggests dynamic cross-talk among them, potentially leading to mutual stimulation or inhibition^[61,64,67,119]. Prolonged hepatic exposure to excessive copper activates resident hepatic stellate cells, transforming them into myofibroblasts that secrete extracellular matrix proteins, including collagen^[66,120]. This process can lead to liver fibrosis and cirrhosis^[66,121]. Molecular, mechanistic, and metabolic uncertainties in Wilson disease may be further clarified through animal models^[34,122,123], including goldfish^[124] and zebrafish models^[125].

Gut microbiome and dysbiosis

Gut microbiome dysbiosis is closely related to many disorders^[68,126-129]. For example, such a relationship has been proposed in alcoholic liver disease^[127], iron-based hemochromatosis^[128,129], and Wilson disease^[68]. In the study on Wilson disease, 16S rRNA sequencing of fecal samples from 14 patients was compared to samples from 16 healthy individuals. Overall, the gut microbiome composition and diversity in the Wilson disease group differed significantly from those in healthy controls. These and other results implied dysbiosis of gut microbiota in Wilson disease. Moreover, gut dysbiosis contributes to enhanced intestinal permeability through disruption of the epithelial barrier, alteration of tight junctions, and bacterial translocation. This leads to endotoxemia due to increased generation of endotoxins by bacteria, which can leave the intestinal tract and reach the liver via the portal system, potentially causing liver injury^[68,126,127].

Animal models

Animal models are valuable for clarifying specific pathogenic steps leading to human Wilson disease [Tables 1 and 2], although species differences must be considered. Several models have been comprehensively listed^[130]: (1) zebrafish *Atp7b*^{-/-} knockout; (2) mouse *Atp7b*^{-/-} knockout; (3) hepatocyte-specific *Atp*^{-/-} mice; (4) toxic milk mice; and (5) Long-Evans Cinnamon rats.

Ongoing considerations on mechanistic and molecular developments

A new conceptual approach in Wilson disease has been proposed, focusing on the effects of copper on hepatocellular nuclear receptors, which function as transcription factors coordinating hepatic metabolic pathways in both normal and diseased livers^[70,130,131]. In experimental models of Wilson disease, copper accumulates in the nuclear region of hepatocytes, where these metabolic nuclear receptors are primarily located^[130]. The activity of several hepatic nuclear receptors is reduced in patients with Wilson disease and in Atp7b-/- animal models, though it remains unclear how this contributes to the disease's pathogenesis^[130].

Genome-wide screening has recently identified cellular prion protein (PrP) as a critical mediator of copper toxicity in Wilson disease^[123]. This increased toxicity is ascribed to the loss or malfunction of ATP7B, which induces hepatic PrP expression, thereby enhancing endocytic copper uptake and contributing to hepatic copper overload and toxicity.

Recent studies have also explored the issue of iron deposition in the liver of Wilson disease patients, proposing a unifying conceptual framework^[132]. Previous analyses suggested that hepatic iron accumulation might result from iron release during hemolysis^[27]. The new concept implicates reduced ceruloplasmin ferroxidase activity, which is typically low in Wilson disease, resulting in decreased circulating iron and increased hepatic iron storage^[132].

Mechanistic studies of cuproptosis have also advanced, using both animal models and patient data. Evidence suggests that various genes participate in cuproptosis, with ferredoxin 1 (FDX1) emerging as a pivotal regulatory gene^[78]. FDX1 encodes a small iron-sulfur protein that is involved in the electron transport chain, iron-sulfur cluster biogenesis, and regulation of lipoic acid acylation. However, the precise mechanisms by which it triggers cell death via cuproptosis remain largely unknown.

ORGAN MANIFESTATION IN WILSON DISEASE

In Wilson disease, copper deposition is found not only in the liver but also in other organs, including the brain, kidneys, eyes, heart, female reproductive tissues, bones, and erythrocytes^[132]. This widespread distribution explains the broad range of clinical features: acute liver failure, cirrhosis, neuropsychiatric manifestations, renal insufficiency, corneal Kaiser-Fleischer rings, cardiomyopathy, infertility, arthropathy, and Coombs-negative hemolysis^[27,133].

DIAGNOSTIC STRATEGIES

Diagnostic approaches for Wilson disease, including the modified Leipzig Scoring System, are well established and generally considered robust^[27,133]. Nevertheless, diagnosis can be challenging due to the variability of clinical presentations and the absence of a single gold-standard test. Traditional diagnostic markers such as serum ceruloplasmin, urinary copper excretion, and liver biopsy often lack sufficient specificity and sensitivity^[134]. To overcome these limitations, newer diagnostic techniques have been explored^[133-138]. Promising advances include novel biomarkers such as relative exchangeable copper (REC), defined as the ratio of exchangeable copper to total serum copper^[133-135], and ATP7B protein quantification in dried blood spots^[134-136], both of which have demonstrated improved diagnostic accuracy^[134]. Other proposed methods include advanced imaging modalities, such as anterior segment optical coherence tomography (AS-OCT), quantitative susceptibility mapping (QSM), and copper-64 positron emission tomography, which offer noninvasive tools for detecting early disease changes. Additionally, next-generation sequencing (NGS) may enhance genetic screening^[134]. Validation studies are still required for all suggested diagnostic methods.

THERAPEUTIC OPTIONS

Treatment for Wilson disease is lifelong and typically includes drugs such as D-penicillamine, a copper chelator that facilitates urinary excretion of copper bound to albumin, and zinc sulfate, which inhibits intestinal copper absorption. In cases where these medical therapies fail, liver transplantation may be necessary^[27,133]. Treatment efficacy can also be monitored by evaluating markers of cuproptosis, which are upregulated before treatment and downregulated following successful therapy^[137], including FDX1, DLAT, lipoic acid synthetase (LIAS), aconitase 2 (ACO-2), succinate dehydrogenase complex iron-sulfur subunit B (SDHB), procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (PLOD1), and dihydropyrimidine dehydrogenase (DPYD).

Experimental therapies include methanobactin, a peptide produced by *Methylosinus trichosporium*, which facilitates copper removal via enhanced biliary excretion^[27], and *ATP* gene therapy using autologous reprogrammed hepatocytes, which has reduced hepatic copper accumulation in a mouse model of Wilson disease^[138]. Additional approaches under investigation include Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-targeted genome editing in human induced pluripotent stem cell-derived hepatocytes^[139]. Furthermore, potential therapeutic components may be evaluated using cultured rat astrocytes that show oxidative injury due to copper^[140].

CONCLUSIONS

New approaches are needed to clarify mechanistic uncertainties and improve the treatment of patients with Wilson disease. Existing animal models that mimic Wilson disease may help solve current challenges. Currently, there is significant interest in the mechanistic and clinical roles of cuproptosis and ferroptosis in the pathogenesis of Wilson disease, particularly in relation to genetic alterations involving the *ATPB7* gene. In the livers of patients with Wilson disease, as well as in corresponding animal models, both copper accumulation leading to cuproptosis and iron overload causing ferroptosis have been well documented. These processes are characterized by vicious cycles that generate ROS, which contribute to liver injury. Additional challenges relate to autoimmune and immune mechanisms, as evidenced by the presence of serum autoimmune markers such as ANA and increased plasma cytokine levels in patients with Wilson disease. Specifically, the occurrence of ANA may suggest an autoimmune-mediated liver injury in Wilson disease, best termed CIAIH, analogous to drug-induced autoimmune hepatitis (DIAIH). The diagnosis of CIAIH requires the use of two different diagnostic algorithms: the Wilson disease component should be assessed using the Modified Leipzig Scoring System for Wilson Disease, while the autoimmune component is best evaluated using the simplified AIH score. Recognizing CIAIH as a subtype of Wilson disease is clinically important, as it can guide treatment strategies, particularly the effective use of steroids.

DECLARATIONS

Authors' contributions

Conceived the idea for this review: Teschke R

Provided the outline, literature, and figure: Eickhoff A

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