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Preclinical models of Wilson's disease: from pathogenesis elucidation to clinical translation

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Preclinical models of Wilson's disease

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30 Z.W.Z. conducted the literature search, analyzed the literature, and drafted the
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ABSTRACT

Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism caused by pathogenic *ATP7B* variants, resulting primarily in hepatic and neurological injury. The marked clinical and genetic heterogeneity of WD complicates diagnosis and treatment, highlighting the need for reliable preclinical models. This review summarizes animal, cell-based, induced pluripotent stem cell, and organoid models of WD and compares their phenotypic characteristics, strengths, limitations, and translational applications. Rodent models remain central to studies of hepatic copper toxicity and therapeutic validation, whereas currently available models inadequately reproduce the characteristic neurological manifestations of human WD. Patient-derived cells and organoids provide complementary platforms for investigating mutation-specific mechanisms, drug responses, and genome-editing approaches, although their maturation, cellular complexity, and standardization remain limited. Future priorities include improving neurological and humanized models, developing physiologically relevant organoid systems, establishing standardized phenotyping criteria, and integrating complementary models across the preclinical development pipeline. This review provides a reference for selecting appropriate models to advance WD precision medicine.

KEYWORDS

Wilson's disease; *ATP7B*; Preclinical model; Clinical translation; Gene therapy; Drug development

ABBREVIATIONS

Bis-choline tetrathiomolybdate (ALXN1840)

D-penicillamine (DPA)

Food and Drug Administration (FDA)



60 Induced pluripotent stem cells (iPSCs)
 61 Kayser-Fleischer ring (K-F ring)
 62 Atp7b gene knockout mice (Atp7b^{-/-})
 63 Long-Evans Cinnamon (LEC)
 64 Long-Evans Cinnamon × PVG (LPP)
 65 triethylenetetramine (TETA)
 66 Jackson Laboratory toxic milk mouse (TX-J)
 67 toxic milk mouse (TX)
 68 tetrathiomolybdate (TTM)
 69 Wilson's disease (WD)
 70 three-dimensional (3D)
 71 copper metabolism MURR1 domain containing 1 (COMMD1)
 72 adeno-associated virus (AAV)
 73 hepatocellular carcinoma (HCC)
 74 extracellular matrix (ECM)

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INTRODUCTION

Wilson's disease (WD), also known as hepatolenticular degeneration (HLD), is an autosomal recessive disorder of copper metabolism caused by pathogenic mutations in the *ATP7B* gene, which encodes a copper-transporting P-type ATPase highly expressed in the liver and moderately expressed in the brain, kidney, and other organs (Bull et al., 1993). To date, more than 1 400 pathogenic variants have been identified in *ATP7B*, including substitutions, deletions, insertions, and duplications, resulting in significant genetic heterogeneity. ATP7B dysfunction impairs biliary copper excretion, resulting in progressive copper accumulation in the liver, brain, kidney, and eye, which manifests clinically as liver damage (from hepatitis to cirrhosis and liver failure), neuropsychiatric symptoms (tremors, dystonia, psychiatric disturbance), kidney injury, osteoarthropathy, and Kayser-Fleischer ring (K-F ring). The global prevalence of WD ranges from 1/2 600 – 1/30 000, with a carrier frequency of approximately 1/90 (European Association for the Study of the Liver, 2012). The clinical heterogeneity of WD—attributed to differences in mutation types, age of onset, and organ involvement—poses significant challenges to accurate diagnosis and individualized treatment in clinical practice.

Without treatment, WD is invariably fatal. However, as one of the few neurological genetic disorders that can be diagnosed and treated, the main treatment strategies for WD at present are to promote copper excretion and inhibit copper absorption. The former mainly includes metal chelators such as D-penicillamine (DPA) and triethylenetetramine (TETA); notably, TETA exerts a dual effect by both chelating copper and inhibiting intestinal copper absorption (Kirk et al., 2024). The latter mainly includes various zinc agents (European Association for the Study of the Liver, 2012). Although the current treatment is effective, it does not correct the underlying



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ATP7B defect by restoring copper homeostasis. In addition, due to the complex clinical manifestations and genetic heterogeneity of WD patients, low compliance, limited improvement of brain symptoms and certain side effects of current drugs, patients require more precise and individualized treatment regimens (Dong et al., 2021). When liver function cannot be restored, liver transplantation remains the only viable option for patient survival. In recent years, gene therapy, novel copper chelators and personalized cell therapy have emerged as promising therapeutic directions, and preclinical models have become the essential platforms for the development of these new therapies (Penning et al., 2023; Padula et al., 2023).

Elucidating the pathogenesis of WD and developing novel therapeutic approaches are critical to addressing unmet clinical needs. Preclinical models serve as indispensable bridges between basic research and clinical practice, enabling the simulation of human disease phenotypes, validation of therapeutic targets, and prediction of clinical treatment responses (Table 1) (Zhao et al., 2019).

The current models of WD mainly include: (1) invertebrates and fish: roundworms (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*) (Norgate et al., 2006), and zebrafish (*Danio rerio*); (2) large mammals: such as Labrador dogs, Bedlington terriers, and North Ronaldsay sheep; (3) rats: such as Long-Evans Cinnamon (LEC) rats and LPP rats; (4) mice: such as toxic milk (TX) mice, the Jackson Laboratory toxic milk (TX-J) mice, and *Atp7b* knockout (*Atp7b*^{-/-}) mice, and gene-edited *Atp7b* point-mutated mice; (5) induced pluripotent stem cells (iPSCs): hepatocyte models; neuronal models; (6) organoids: hepatobiliary organoids, intrahepatic cholangiocyte organoids. Each model has unique translational potential in addressing specific clinical challenges (Figure 1), and the comparative analysis of their characteristics is the key to rational model selection for WD research.



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Small animal models (*C. elegans*, *Drosophila*, zebrafish) are cost-effective for high-throughput screening of potential therapeutic agents, laying the foundation for early clinical drug development (Reed et al., 2018). Mammalian models, including mice, rats, and dogs, permit systemic assessment of copper metabolism, organ injury, therapeutic efficacy, and safety, making them important intermediate platforms between cell-based studies and clinical trials. However, significant discrepancies exist between these models and human WD. Although some mutant mice display subtle behavioral alterations or neuroinflammatory markers, all current rodent models critically lack phenotypic fidelity regarding the hallmarks of human neurological WD. Specifically, they fail to recapitulate the typical extrapyramidal symptoms (such as progressive tremors and dystonia) and Kayser-Fleischer (K-F) rings driven by severe copper accumulation in the basal ganglia, which severely hinders the translation of neuroprotective therapies. LEC rats that survive acute hepatitis may subsequently develop spontaneous hepatic tumors. This phenotype is useful for investigating copper-associated hepatocarcinogenesis, although its relevance to human WD should be interpreted cautiously (Medici & Huster, 2017). The advent of CRISPR/Cas9 technology has revolutionized the generation of WD models, enabling the construction of patient-specific gene-edited models that better recapitulate clinical genetic heterogeneity, thereby enhancing the precision of preclinical translational research.

Organoid culture systems have emerged as valuable tools in liver and biliary research. These three-dimensional cultures, derived from pluripotent stem cells or adult hepatobiliary cells, provide experimentally accessible platforms for modeling selected aspects of tissue structure and function. Integration with microfluidic or perfusable chip systems may further improve nutrient exchange, cellular maturation,



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and simulation of the in vivo microenvironment (Wang et al., 2018).

This review critically examines experimental model systems for ATP7B-deficient Wilson's disease, compares their strengths and limitations, and evaluates the challenges associated with translating preclinical findings into clinical applications. In addition, this review summarizes the application of preclinical models in WD drug development and gene therapy research, and outlines the future perspectives of translational research based on multi-model integration.

ANIMAL MODELS

Animal models are the most widely used preclinical tools in WD research, which reproduce the core pathological characteristics of WD by simulating ATP7B deficiency or copper metabolism disorders. According to species differences and construction strategies, they can be mainly divided into rodent models (mice, rats), large mammal models, and invertebrates/fish models. Each type of model has unique characteristics in pathological phenotype and research applicability, and this section highlights the comparative analysis of their translational value rather than detailed construction methods.

Mouse models

The construction of mouse models is mainly based on targeted modification of the *ATP7B* gene, covering three categories: gene knockout, gene-edited point-mutated mice, and humanized models. This section integrates gene-edited *Atp7b* mouse models, which were previously discussed independently, to form a unified and systematic overview of mouse models.

***Atp7b*^{-/-} mouse:** *Atp7b* gene knockout mice (*Atp7b*^{-/-}) are the earliest WD models



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constructed by ES cell targeting technology. The classical *Atp7b*^{-/-} mice were generated by targeted disruption of exon 2, resulting in loss of functional Atp7b expression (Buiakova et al., 1999). These mice develop severe hepatic copper accumulation and early liver disease, including steatosis, inflammation, and fibrosis (Huster, 2014). Furthermore, the *Atp7b*^{-/-} mouse model has provided complementary insights into WD liver pathology, including altered lipid metabolism and dysregulation of LXR/RXR signaling (Hamilton et al., 2016). Unlike other models, *Atp7b*^{-/-} mice survive severe hepatitis, allowing the study of liver regeneration—an important clinical endpoint for evaluating therapies targeting liver repair. Although brain copper levels increase moderately without overt neurological symptoms, ultrastructural abnormalities in the basal ganglia (particularly in mitochondria and microtubules) have been observed (Dong et al., 2015), providing insights into the early mechanisms of WD neurotoxicity. Therapeutic studies, including hepatocyte transplantation and gene therapy, have significantly alleviated liver copper deposition (Murillo et al., 2016; Roybal et al., 2012), validating these strategies for clinical translation.

CRISPR/Cas9 technology has been used to construct *Atp7b*-deficient mice. By specifically removing the exon 2 region of the *Atp7b* gene to cause frameshift, gene function loss is achieved. Compared with traditional ES cell targeting technology, this approach offers high editing efficiency, a simplified generation procedure, and better control of the genetic background (such as C57BL/6J background), which is more conducive to the standardized verification of experimental results. *Atp7b*^{-/-} mice are widely used in the study of WD liver injury mechanisms (such as mitochondrial dysfunction and oxidative stress) and gene therapy validation. For example, AAV8-mediated *Atp7b* gene delivery can provide long-term correction of copper



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metabolism defects in this model (Greig et al., 2019), and the translation of advanced gene-editing tools, such as prime editors, holds great potential for precisely repairing specific mutations like p.His1069Gln (p.H1069Q) in vivo (Chen & Liu, 2023).

TX mouse: The toxic milk mouse arose from a spontaneous mutation in *Atp7b* and exhibits impaired copper transport, reduced copper secretion into milk, and progressive copper accumulation in the liver and other tissues (Rauch, 1983; Hadrian & Przybyłowski, 2021). TX mice have been instrumental in translating therapeutic strategies to clinical practice, including evaluating the efficacy of tetrathiomolybdate (TTM) and bis-choline tetrathiomolybdate (ALXN1840). ALXN1840 has undergone clinical evaluation in patients with WD, although its eventual therapeutic role remains to be established. Preclinical studies in TX mice supported the copper-lowering effects of tetrathiomolybdate-based therapies. Bis-choline tetrathiomolybdate was subsequently evaluated clinically in patients with WD (Weiss et al., 2017). Additionally, AAV8-mediated *Atp7b* delivery substantially lowered hepatic copper levels (Greig et al., 2019), providing proof-of-concept evidence in WD. However, their genetic background contains additional incompletely characterized variants, and their hepatic and neurological phenotypes do not fully reproduce those of human WD. Therefore, findings obtained in this model should be validated in targeted *Atp7b*-deficient or variant-specific models before clinical translation.

TX-J mouse: Developed from a recessive point mutation at Jackson Laboratory, TX-J mice exhibit a phenotype resembling WD (Coronado et al., 2001). TX-J pups require cross-fostering due to copper-deficient milk, and their liver pathology progresses to cirrhosis. By five months, histological changes include hepatocyte nuclear enlargement, inflammatory infiltration, and fibrosis (Mazi et al., 2024). Neuroinflammation markers and behavior analyses indicate mild abnormalities,



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suggesting relevance for neurocognitive aspects of WD (Terwel et al., 2011; Hadrian & Przybyłkowski, 2021). Behavioral tests show subtle motor deficits and memory impairment, supporting the use of TX-J mice for preliminary neurobehavioral studies in WD (Przybyłkowski et al., 2013). Nevertheless, these mild neurobehavioral alterations remain far from the profound extrapyramidal movement disorders observed in human patients, underscoring the ongoing challenge of achieving full neurological disease replication in mice. AAV8 gene therapy corrected copper metabolism, demonstrating the model's utility for therapeutic research.

Gene-edited *Atp7b* mice: Targeted gene editing has been applied to create *Atp7b* mice models mimicking the most common mutation found in Chinese WD patients, specifically the p.Arg778Leu (p.R778L) mutation (Dong et al., 2024; Zeng et al., 2025). Through microinjection of Cas9 mRNA into one-cell-stage mouse embryos, researchers successfully generated mouse models harboring the p.R778L point mutation. Mice with the p.R778L mutation exhibited significantly lower serum ceruloplasmin-bound copper levels compared to wild-type mice, while having markedly higher non-ceruloplasmin-bound copper levels in serum, liver, and brain tissues. The p.R778L mice also exhibited behavioral abnormalities and pathological liver damage (Dong et al., 2024). Furthermore, a recently established *Atp7b* R780L knock-in mouse model (corresponding to the human p.R778L variant) has proven highly valuable for evaluating liver-targeted AAV8-mediated *ATP7B* gene therapy, demonstrating successful rescue of impaired copper metabolism and reversal of liver histopathology (Zeng et al., 2025). For WD gene therapy, functional correction of approximately 10 – 20% of hepatocytes via gene editing is sufficient to reverse copper metabolism disorders and improve the phenotypic abnormalities of WD mice (Padula et al., 2023), which provides a critical reference for the clinical design of gene



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therapy. These models provide a foundation for subsequent gene therapy research.

Hepatocyte-specific *Atp7b* knockout mice: A specific hepatocyte-specific *Atp7b* knockout mouse model has been constructed by Cre-LoxP system (Muchenditsi et al., 2017), which specifically knocks out the *Atp7b* gene in hepatocytes while retaining its expression in other tissues and organs. This model is valuable for studying the specific function of Atp7b in hepatocytes and the impact of copper overload in hepatocytes on non-parenchymal liver cells (such as hepatic stellate cells, Kupffer cells). The model exhibits liver steatosis, obesity, and mild copper accumulation, which reveals the specific role of hepatocyte Atp7b deficiency in the occurrence of WD liver metabolic disorders, and provides a unique tool for studying the crosstalk between hepatocytes and non-parenchymal liver cells in WD.

ATP7B-humanized and variant-specific mouse models: The development of mouse models carrying clinically prevalent human ATP7B variants remains an important goal for WD research. Such models may facilitate the evaluation of mutation-specific genome-editing strategies and help clarify genotype – phenotype relationships. However, their translational relevance will still be influenced by the murine genetic background, immune system, and copper metabolism. Further validation is therefore required before these models can be used to predict long-term therapeutic efficacy and safety in humans.

Rat models

Rats share over 80% genetic homology with humans, and the *Atp7b* gene in rats was cloned in 1994 (Wu et al., 1994). Long-Evans Cinnamon (LEC) rats, a widely used model for liver cancer studies, carry a significant deletion in the *Atp7b* gene, causing spontaneous copper accumulation in the liver, low serum copper, and abnormal iron



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levels. By eight weeks, hepatic copper exceeds 1 000 μ g/g, leading to acute hepatitis and a mortality rate of 30 – 40% by four months (Medici et al., 2005). Survivors often develop hepatocellular carcinoma (HCC) by 12 months, linked to reduced apoptosis, DNA strand breaks, and cell proliferation (Jia et al., 2006). Clinically, acute liver failure is a life-threatening complication of WD, and LEC rats are valuable for evaluating emergency therapeutic interventions (e.g., novel chelators, liver support therapies) that can support further translational evaluation. However, they do not fully mimic WD pathology, particularly neurological manifestations, limiting their utility for neurotherapeutic research.

Due to its rapid liver disease progression, LEC rats are particularly useful for intervention studies, such as those involving therapeutic agents like DPA and zinc (Klein et al., 2000; Weiss et al., 2011). To eliminate confounding genetic influences, LEC rats were crossed with PVG rats to generate the LPP strain, which retains the *Atp7b* mutation but has normal N-acetyltransferase activity and fur color (Ahmed et al., 2005). LPP rats exhibit progressive hepatic copper accumulation, fibrosis, and mitochondrial abnormalities, closely mimicking chronic WD liver disease in humans. Methanobactin, a long-acting copper chelator, has been shown to effectively treat acute liver failure in these rats by restoring copper homeostasis and liver function, highlighting the model's value in translating novel long-acting chelators to clinical practice for patients with chronic WD (Lichtmannegger et al., 2016). Unlike most rodent models of WD, LEC rats that survive the acute hepatitis phase may spontaneously develop hepatic tumors, particularly HCC. This feature makes them useful for studying copper-associated hepatocarcinogenesis, but it should not be interpreted as evidence that HCC is a common complication of human WD.

Non-rodent mammalian models



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Hereditary copper toxicity has been observed at a high incidence in several purebred dog breeds, including Dalmatians, West Highland White Terriers, Dobermans, Briards, and Labrador Retrievers. The use of canine models for WD research abides by the 3R ethical principles (Replacement, Reduction, Refinement), and all animal experiments must obtain the approval of the institutional animal ethics committee (Kruitwagen & Penning, 2019). These conditions share certain phenotypic features with WD, such as the accumulation of copper in the liver, which leads to progressive liver fibrosis and cirrhosis, typically manifesting from adolescence to middle age, along with symptomatic improvement following treatment with DPA (Fieten et al., 2012, 2014). In Bedlington terriers, hereditary copper toxicosis is associated with pathogenic variants in *COMMD1* (formerly known as *MURR1*), whereas the genetic basis differs among other affected breeds (van de Sluis et al., 2002; Favier et al., 2012). While liver copper accumulation and related pathology resemble those found in WD patients, affected dogs do not exhibit significant neurological defects, and their copper-bound ceruloplasmin levels remain within normal ranges (Wu et al., 2006).

Non-rodent mammalian models, including dogs and rabbits, offer distinct advantages for WD translational research: (1) Their physiological metabolism, organ size and anatomical structure are closer to humans, which is suitable for evaluating the long-term safety and efficacy of therapeutic schemes such as gene therapy and cell transplantation; (2) Canine models are suitable for studying the progression of chronic liver fibrosis caused by copper overload, which is consistent with the clinical course of human WD; (3) Gene-edited rabbit models can simulate human WD mutations and exhibit severe liver damage, which is suitable for the preclinical verification of novel drug delivery systems and surgical intervention strategies (Jiang et al., 2018).

Another intriguing large mammalian model of copper accumulation is the North



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Ronaldsay sheep, which, like the canine model, presents a clinical and genetic mechanism distinct from WD (Haywood et al., 2004). Although the exact genetic defect in this model is currently unknown, it holds potential for investigating copper accumulation in the brain, the role of the blood-brain barrier, and the effects of environmental copper exposure (Haywood et al., 2008, 2014).

Several other mammalian disorders of copper metabolism, characterized by inherited defects and resulting in copper accumulation and liver toxicity, have also been identified. However, the underlying genetic mechanisms in these models differ from those in human WD, limiting their applicability as direct models for the study of WD.

Gene-edited *Atp7b* rabbits: Using the CRISPR/Cas9 system in combination with single-stranded DNA oligonucleotides (ssODNs), researchers have generated an *Atp7b* point mutation rabbit model to simulate the most common mutation observed in East Asian populations with WD, the p.R778L mutation. Rabbits carrying this point mutation in the *Atp7b* allele die at approximately three months of age. At disease onset, liver copper levels in these rabbits increase by ninefold, closely mirroring the fivefold increase observed in some symptomatic WD patients (Jiang et al., 2018). By injecting RNA into zygotes at early developmental stages, the precision of point mutations has been enhanced, making *Atp7b* gene-edited rabbits a promising model for WD. This model provides a potentially useful platform for investigating severe hepatic copper toxicity and for evaluating therapeutic strategies in a larger mammalian species. However, its early mortality and limited neurological characterization may restrict its applicability to chronic and neurological WD.

CELL-BASED MODELS



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In vitro cell-based models have long been a cornerstone of WD research, providing an accessible and controlled environment to study the disease at the cellular level. Their translational value lies in elucidating the molecular mechanisms underlying copper dysregulation and ATP7B dysfunction, identifying potential therapeutic targets, and performing preliminary drug screening—critical steps for advancing therapies to clinical trials (Cai et al., 2022). These models are typically categorized by tissue type (hepatic, neuronal, etc.) to investigate copper overload effects on different organs, aligning with the multi-organ involvement observed in clinical WD (Członkowska et al., 2018).

Hepatocyte models

Liver dysfunction is a hallmark of WD, and hepatocytes are central to copper metabolism. The human hepatoma cell line HepG2 is commonly used to study hepatic copper accumulation and *ATP7B* mutations, as it expresses key copper transporters including ATP7A and ATP7B (Petters et al., 2020). Introducing *ATP7B* mutations into HepG2 cells allows modeling of impaired copper efflux—a core pathological feature of clinical WD—facilitating research on dysfunctional ATP7B trafficking from the Golgi apparatus to the plasma membrane (critical for copper excretion) (Bartee & Lutsenko, 2007; Braiterman et al., 2009). Translationally, this model is used for high-throughput screening of potential copper chelators and evaluating their efficacy in restoring copper efflux, laying the foundation for preclinical drug development. Primary human hepatocytes offer a more physiologically relevant model, retaining many liver biochemical properties, but their limited long-term culture viability restricts their use in long-term drug toxicity studies—an important consideration for clinical drug development (Malakhova et al., 2020).

Neuronal models



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Copper accumulation in the brain (especially the basal ganglia) causes WD neurological symptoms (tremors, dystonia, psychiatric disturbances)—a major source of disability in clinical WD. Neuronal cell lines such as SH-SY5Y (human neuroblastoma cells) are used to investigate copper-induced neurotoxicity, exhibiting oxidative stress, mitochondrial dysfunction, and apoptosis under copper exposure—key mechanisms of WD neurodegeneration (Xiang et al., 2021). Translationally, these models are valuable for screening neuroprotective agents that can prevent or reverse copper-induced neuronal damage, addressing an unmet clinical need. Patient-derived iPSC-derived neurons are increasingly used to study neuronal pathophysiology, providing a more accurate representation of copper dysregulation effects in the context of patient-specific *ATP7B* mutations. This enables personalized preclinical testing of neuroprotective therapies, advancing precision medicine in WD clinical practice.

Despite their translational value, cell-based models have significant limitations that affect their clinical relevance. Cell lines like HepG2 and SH-SY5Y cannot replicate the systemic nature of WD or multi-organ interactions observed in clinical practice, limiting their ability to predict whole-body therapeutic responses. Cultured cells lack the complexity of in vivo tissues, failing to recapitulate the full spectrum of WD tissue damage (especially neuropsychiatric aspects). Additionally, inadequate cellular differentiation/maturation environments may reduce the relevance of findings to human pathophysiology, potentially leading to discrepancies between preclinical and clinical results. Addressing these limitations (e.g., via 3D co-culture systems) is critical to enhancing their translational value (Liu et al., 2019).

Induced pluripotent stem cells (iPSCs)

Induced pluripotent stem cells (iPSCs) technology has revolutionized disease



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modeling by enabling the generation of patient-specific in vitro models. iPSCs derived from WD patients (reprogrammed from adult somatic cells such as skin fibroblasts) can differentiate into various cell types (hepatocytes, neurons), providing a human-specific, patient-derived platform that closely mimics clinical genetic and phenotypic heterogeneity (Yi et al., 2012; Rowe & Daley, 2019). Translationally, this technology facilitates personalized medicine by enabling the prediction of individual treatment responses, screening of personalized therapeutic agents, and validation of mutation-specific therapies—critical for advancing precision medicine in WD clinical practice.

Studies using WD patient-derived iPSCs have established hepatic and neural differentiation platforms, providing a foundation for investigating multi-organ involvement in WD. These platforms enabled the differentiation of WD iPSCs into hepatocyte-like cells and neuron-like cells, which exhibited the core pathological features of WD such as copper accumulation and impaired copper efflux. The study further optimized the differentiation protocol of WD iPSC-derived hepatocytes, and found that the differentiated hepatocytes have more mature liver function and can better simulate the copper metabolism disorder of human WD liver, which is suitable for the high-throughput screening of novel copper chelators (Yi et al., 2012; Wei et al., 2022).

WD patient-derived iPSCs closely mirror the genetic and phenotypic heterogeneity of clinical WD (Rowe & Daley, 2019). Differentiated liver or brain cells carry the same mutations as patients, facilitating investigation of copper dysregulation in different tissues and comprehensive understanding of disease mechanisms that underlie clinical variability. These models enable studies of disease onset and progression that are unavailable with traditional cell lines, providing insights into



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early pathological processes that can guide the development of early intervention strategies in clinical practice. iPSCs recapitulate key WD cellular features (e.g., impaired copper transport) and aid in biomarker identification—critical for improving early diagnosis of WD, a major clinical challenge. Additionally, iPSC-derived models can be used to evaluate the safety and efficacy of novel therapies (e.g., gene editing) in a patient-specific manner, reducing the risk of adverse events in clinical trials.

Despite their significant translational potential, iPSC-derived models face challenges that hinder their clinical application (Rashid et al., 2010). Complex and variable differentiation protocols for generating functional hepatocytes/neurons from iPSCs lead to differences in cellular identity/function between lines, complicating data interpretation and reproducibility—critical for reliable preclinical translation. While iPSCs can differentiate into specific cell types, they do not fully recapitulate in vivo tissue architecture or systemic interactions, limiting their ability to capture multi-organ interplay (liver-brain-kidney) observed in clinical WD. Additionally, iPSC-derived models are time-consuming and expensive to generate, limiting their scalability for large-scale therapeutic screening. Overcoming these challenges (e.g., standardized differentiation protocols, establishment of iPSC biobanks for WD patients) will enhance their utility in clinical translational research.

ORGANOID MODELS

Organoids are three-dimensional cell aggregates with organ-specific structures and functions formed by stem cells [embryonic stem cells, induced pluripotent stem cells (iPSCs), or adult stem cells] under in vitro three-dimensional culture conditions, which can highly simulate the physiological microenvironment and pathological characteristics of in vivo tissues. In recent years, the establishment of WD organoid models has provided a new in vitro platform for WD research, making up for the



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deficiencies of traditional cell models and animal models (Yang et al., 2023).

When liver function cannot be restored, the only definitive treatment for patients with irreversible liver failure is transplantation (Pascher et al., 2013; Ahmad et al., 2017). There is a vigorous search for cell-based technologies that will provide alternatives for liver transplantation. Furthermore, there is a need for better cell culture models of human hepatobiliary tissues to study normal tissue function and mechanisms of disease. In humans, following a 60% liver resection, the organ regenerates to approximately 74% of its original size within 10 days, which is sufficient to restore normal liver biochemical function (Nagino et al., 2001; Yagi et al., 2020).

Human organoid cultures offer a powerful platform for studying human development and modeling disease processes, while providing experimentally accessible human tissue models. By recapitulating the complexity of actual tissues or organs, patient-derived human organoids hold great promise for accelerating medical research. They create new opportunities in tissue engineering and regenerative medicine while generating valuable insights and tools for preclinical studies, including drug development and testing.

Organoids derived from pluripotent stem cells or from adult organ-specific stem/progenitor cells (Dutta et al., 2017) can develop into structures that closely mimic the anatomy and physiology of intact organs in vivo (Figure 2). Specifically, researchers have obtained tissue biopsies from the livers of WD patients during liver transplant procedures and successfully derived human iPSCs from patients' dermal fibroblasts (Tang et al., 2016).

Hepatocyte-like organoids derived from COMMD1-deficient Bedlington Terriers (non-ATP7B mediated copper toxicosis model), first reported in 2015, exhibited



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increased intracellular copper accumulation, which was reversible upon infection with a lentiviral construct encoding *COMMD1* (Dutta et al., 2017). When autologous organoids with the corrected gene defect were transplanted into the liver via the portal vein, they successfully engrafted and survived for up to two years (Kruitwagen et al., 2020). These findings highlight the potential of gene therapy in treating genetic liver diseases by correcting underlying genetic defects in autologous organoids (Asai et al., 2017). Moreover, prime editing functionally recovers disease-causing mutations in liver organoids from patients with WD (Schene et al., 2020).

A key challenge in the organoid field is that epithelial monocultures lack the tissue complexity and heterogeneous cell – cell interactions found in native tissues. For hepatobiliary organoid co-cultures, incorporating mesenchymal cells, such as fibroblasts derived from liver or biliary tissue from the same patient, could enhance hepatocyte maturation, as seen in human fetal cell co-cultures (Wang et al., 2019). Additionally, the inclusion of endothelial cells alongside mesenchymal stem cells has been shown to promote hepatocyte differentiation and 3D spatial organization in induced pluripotent stem cell-derived organoids (Asai et al., 2017). Another challenge is identifying suitable extracellular matrices (ECMs) to support organoid growth. Matrigel, the most widely used matrix, is derived from mouse sarcoma cells and is complex, undefined, and variable between batches (Hughes et al., 2010; Ea et al., 2020). Clinically relevant, standardized ECMs that meet FDA Good Manufacturing Practices are needed for preclinical and drug-testing applications. Lastly, standardization in organoid research remains lacking, particularly regarding publication requirements. Factors such as patient variability and passage number, which affect organoid growth and disease phenotype, should be considered to accurately interpret findings (Hu et al., 2018).



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511 **TRANSLATIONAL APPLICATIONS OF PRECLINICAL MODELS**

512 Preclinical models are indispensable for translating basic research into clinical
513 practice, with direct applications in guiding WD diagnosis, optimizing therapeutic
514 regimens, developing novel therapies, and predicting treatment responses. This
515 section focuses on the translational impact of preclinical models on clinical practice,
516 highlighting their role in addressing unmet clinical needs, and summarizes the latest
517 progress in drug development and gene therapy validation based on preclinical
518 models.

519 **Pathological mechanism analysis**

520 Preclinical models facilitate the elucidation of WD pathogenesis, providing critical
521 insights that guide clinical diagnosis and treatment. In liver injury research, *Atp7b*^{-/-}
522 mouse models have revealed that copper overload induces mitochondrial respiratory
523 chain dysfunction and cholesterol metabolism disorders, leading to hepatocyte
524 apoptosis and fibrosis—mechanisms that underlie clinical WD liver disease
525 progression (Huster, 2014). These findings have guided the development of clinical
526 biomarkers for liver fibrosis progression (e.g., mitochondrial function markers) and
527 the identification of potential therapeutic targets (e.g., cholesterol metabolism
528 regulators). Zebrafish models have identified HIF-1 signaling pathway activation as a
529 potential intervention target for copper-induced liver steatosis, a common clinical
530 manifestation of WD (Mi et al., 2020). In neurological injury research, TX-J mouse
531 models (with mild neurological symptoms) have been used to explore brain copper
532 distribution and neurotoxicity, showing that copper accumulation enhances oxidative
533 stress and synaptic dysfunction—key mechanisms of clinical WD neuropsychiatric
534 symptoms (Terwel et al., 2011).



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These insights have guided the development of clinical neuroprotective strategies targeting oxidative stress. As highlighted in recent comprehensive reviews, combining organoid models with multi-omics technologies provides a path toward systematic screening of copper-related differential genes, proteins, and metabolites, thereby constructing WD pathological regulatory networks to identify novel clinical biomarkers for early diagnosis (Wolska et al., 2025).

Drug development and clinical regimen optimization

Zebrafish and organoid models are used for high-throughput screening of potential therapeutics (new copper chelators, antioxidants, traditional Chinese medicine active components) that can be translated to clinical practice. For example, high-throughput screening using zebrafish models has identified novel copper chelators with improved efficacy and reduced toxicity compared to existing drugs (Krishnan et al., 2018). Rodent models (*Atp7b*^{-/-} mice, LEC rats) validate in vivo drug efficacy and safety, evaluating improvements in hepatic copper content, liver function, and pathological injury—key clinical endpoints. These models have been used to optimize clinical dosing regimens for existing drugs (e.g., zinc agents) to maximize efficacy while minimizing side effects. These preclinical findings have guided clinical trials evaluating the efficacy of traditional Chinese medicine in combination with Western medicine for WD, such as Gandou Decoction and Gandou Bushen Decoction, which have been shown to reduce copper levels and alleviate hepatic and neurological damage in WD animal models (Wang et al., 2020).

In recent years, the development of WD drugs has achieved important progress: (1) Novel long-acting copper chelators (e.g., methanobactin) have been developed, which have higher copper chelating efficiency and lower side effects than traditional chelators (Summer et al., 2011); (2) The optimized zinc preparation (e.g., zinc acetate



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sustained-release tablets) has improved oral bioavailability and patient compliance (Camarata et al., 2019); (3) Targeted drugs regulating copper metabolism-related signaling pathways (e.g., HIF-1 inhibitors) have entered preclinical research, providing a new direction for the treatment of WD liver steatosis (Mariño et al., 2025). Preclinical models play a key role in the screening, optimization and efficacy verification of these novel drugs.

Gene therapy validation for clinical application

Organoid models and humanized mouse models are critical for evaluating the efficacy and safety of gene editing technologies before clinical trials. Advanced genome manipulation tools, such as Prime Editing, have demonstrated high versatility for precise mutation repair (Chen & Liu, 2023), supporting the development of clinical gene-editing strategies for patients harboring the H1069Q mutation. CRISPR/Cas9-mediated correction of pathogenic *ATP7B* variants has been evaluated in cell-based WD models, demonstrating the feasibility of restoring ATP7B expression or copper-transport function (Pöhler et al., 2020), providing personalized preclinical data for clinical gene therapy. Gene-edited rabbit models may provide a useful large-animal platform for future evaluation of gene-delivery efficiency, therapeutic efficacy, dose selection, and long-term safety. However, additional studies are required before their translational value for AAV-mediated ATP7B therapy can be established.

FUTURE DIRECTIONS

WD has been studied for over a century since its initial description. Over the past 30 years, the discovery and research of WD experimental models have greatly advanced our understanding of the disease's etiology and pathogenesis. With the development



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of CRISPR/Cas9 technology and RNA-based drug delivery systems, the creation of new models, as well as advancements in cell-based therapies, gene therapies, and drug development, are rapidly progressing. However, several challenges remain. First, existing models cannot completely reproduce the clinical heterogeneity of human WD, especially the insufficient simulation of neurological symptoms. Most animal models lack typical extrapyramidal symptoms and K-F rings. Second, the maturity and microenvironment simulation of organoid models need to be improved, and they cannot completely replace in vivo research (Caiazza et al., 2021). Third, the standardization of models is low, and the construction methods and evaluation indicators of different laboratories are different, affecting the comparability of research results. Fourth, the construction cost of large animal models (such as canines and pigs) is high and the cycle is long, limiting their wide application (Ferreira et al., 2019; Kruitwagen & Penning, 2019).

The future development direction of preclinical models for WD can focus on the following points. First, optimize humanized models, develop and validate variant-specific or humanized mouse models carrying clinically prevalent ATP7B variants, such as p.H1069Q and p.R778L, and introduce nerve injury-related pathways through genetic modification to enhance the capacity to reproduce neurological symptoms, and integrate the immune system and gut-liver axis to make the model more in line with human physiological characteristics. Second, promote the upgrading of organoid models, construct an "organoid-on-a-chip" system by integrating bioengineering technologies (such as microfluidic chips), and introduce blood vessels, immune cells and other components to improve the microenvironment simulation, and establish standardized organoid culture and identification protocols. Third, establish a model standardization system, standardize the construction process



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and phenotypic evaluation indicators (such as hepatic copper content, liver function, pathological score) of various models, and improve the repeatability and comparability of research results, and formulate international consensus on WD preclinical model evaluation. Fourth, develop new large animal models (such as pig models), and use their advantage of closer physiological metabolism to humans to provide a more reliable platform for the long-term safety and efficacy evaluation of preclinical therapeutic schemes, and construct gene-edited pig models carrying human *ATP7B* mutations. Last, promote the combined application of multiple models, such as initial drug screening through organoids, in vivo validation using animal models, and individual therapeutic effect evaluation through organoid-on-a-chip systems, to achieve efficient transformation from basic research to precision medicine, and establish a multi-model integrated translational research platform for WD.

The preclinical models of WD have formed a diversified system with animal models as the core and organoid/iPSC models as the supplement. Various models have unique advantages in pathological phenotype simulation and research applicability, providing key tools for the analysis of WD pathological mechanisms and the development of therapeutic schemes. Animal models are suitable for in vivo mechanism research and preclinical therapeutic validation, while organoid models have unique value in high-throughput screening and patient-specific studies. However, existing models still have many limitations, such as insufficient simulation of neurological symptoms and limited maturity of organoids. In the future, through model optimization, establishment of a standardized system, and combined application of multiple models, the reliability and translational efficiency of preclinical research will be further improved, providing strong support for the development of potentially curative therapies for WD such as gene therapy and



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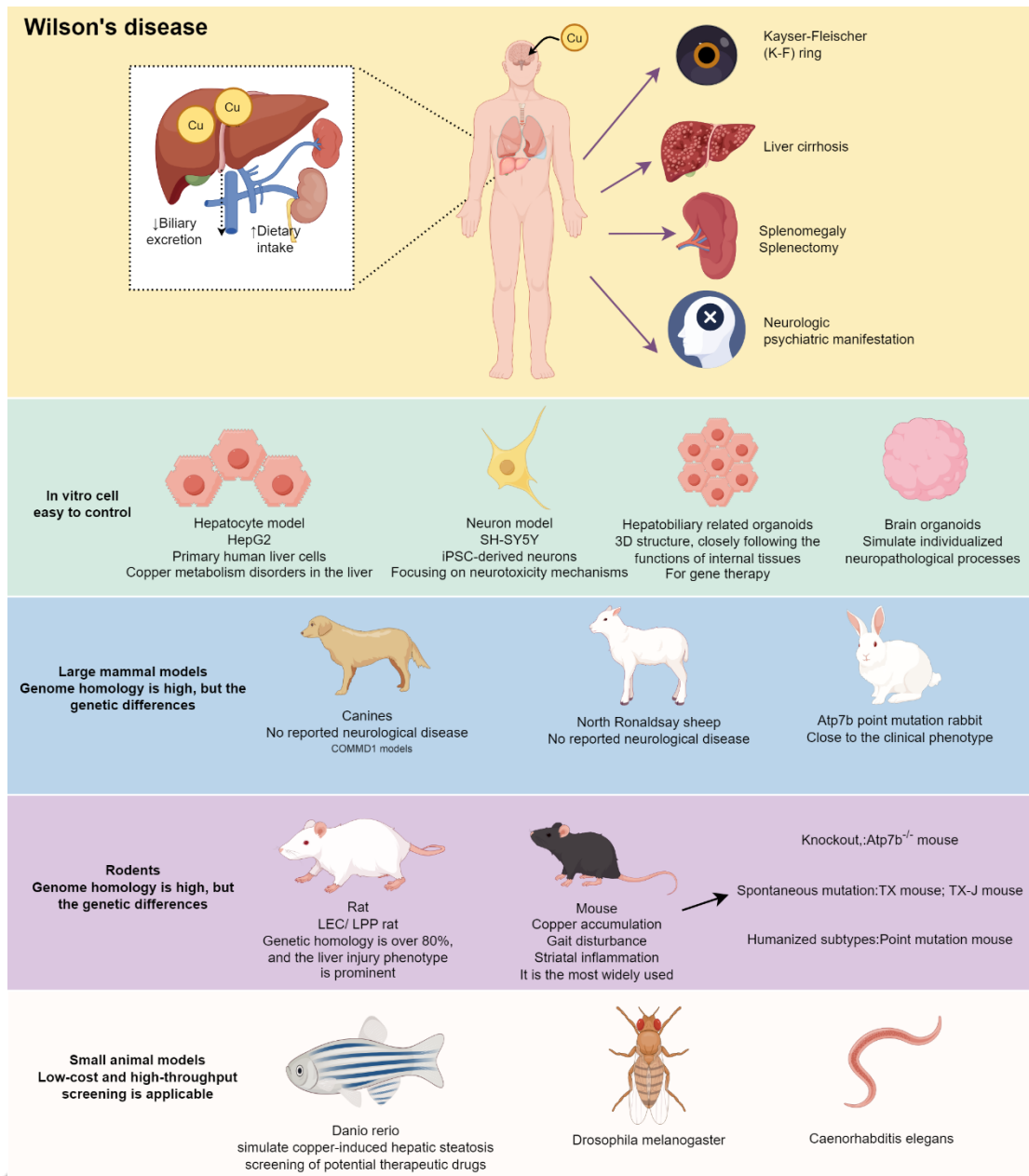


Figure 1 Overview of experimental models for studying Wilson's disease. The figure summarizes various model systems used in WD research, categorized into in vitro cell models, organoids, and animal models. In vitro systems include hepatocyte models (e.g., HepG2, primary human liver cells) and neuronal models (e.g., iPSC-derived neurons), which allow controlled study of copper metabolism and neurotoxicity. Organoid models comprise hepatobiliary and brain organoids, enabling 3D tissue simulation and individualized disease modeling for gene therapy and neuropathology studies. Animal models are grouped into large mammals (canines,

sheep, rabbits), rodents (rats, mice with spontaneous or engineered *ATP7B* mutations), and small animals (zebrafish, *Drosophila*, *C. elegans*), highlighting their utility in phenotypic replication, high-throughput screening, and mechanistic investigation. The rabbit model carrying an *Atp7b* point mutation reproduces severe hepatic copper accumulation and selected hepatic features of Wilson's disease. Mouse models are widely used to investigate hepatic copper accumulation, metabolic injury, and selected neuroinflammatory or behavioral alterations, although they generally fail to reproduce the severe extrapyramidal manifestations of human neurological WD.

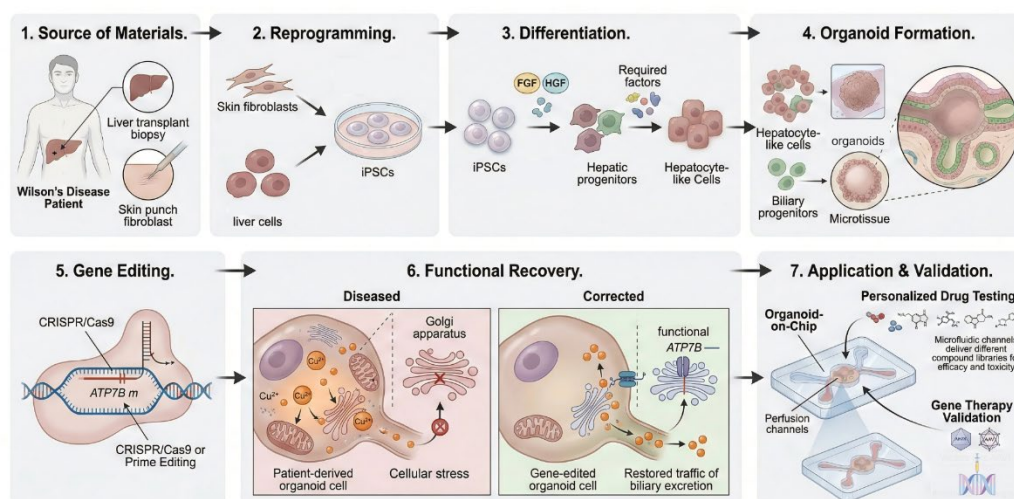


Figure 2 Potential translational workflow for patient-derived hepatobiliary organoids in Wilson's disease. Patient somatic cells or liver tissue can be used to generate iPSC-derived or adult stem cell-derived organoids. These models permit disease characterization, therapeutic screening, genome correction, functional validation, and, potentially, autologous cell-based applications.

Table 1 Comparison of representative experimental models of Wilson's disease

Model	Mutation type	Mutation details	Genetic background	ATP7B protein synthesis and function	Limitations	Strengths	Related references
LEC rats	Deletion mutation	Approximately 900-bp deletion affecting the 3' region of <i>Atp7b</i>	LEA rats, also carrying two other gene mutations	Abnormal or truncated Atp7b protein with severe loss of copper-transport function	Homology differences between rat and human genes	Excellent for studying acute liver failure and rapid interventions	Klein et al., 2000; Weiss et al., 2011
TX mice	Spontaneous missense mutation	Met1356Val	DL mouse strain, with additional unknown mutations	Normal protein synthesis with reduced copper transport	Presence of unknown mutations, liver phenotype and histopathology differ significantly from WD patients	Useful for historical chelation therapy translation and behavioral studies	Rauch, 1983
TX-J mice	Point mutation	Gly712Asp mutation	C3H/HeJ mouse strain, with additional unknown mutations	Not fully characterized	Presence of unknown mutations	Useful for investigating mechanisms of brain copper neurotoxicity and evaluating neuroprotective therapies	Przybyłkowski et al., 2013
<i>Atp7b</i> ^{-/-} mice	Targeted null mutation	Targeted disruption of exon 2	C57BL/6 mouse strain	No functional Atp7b expression; complete loss of copper-transport function	Does not replicate patient-specific mutations; lacks a clear neurological phenotype	Robust for studying chronic liver fibrosis, lipid metabolism, and AAV gene therapy	Buiakova et al., 1999
Gene-Edited mice	Point mutation	p.Arg778Leu mutation	C57BL/6J or standard inbred strain	Abnormal full-length protein expression	The model does not carry the full human ATP7B sequence	Useful for evaluating patient-specific genetic therapies (e.g., Prime Editing)	Dong et al., 2024; Zeng et al., 2025
Organoids	Patient-specific ATP7B variants	Patient-derived pathogenic <i>ATP7B</i> variants	Human patient-derived iPSCs or adult stem/progenitor cells	Variant-dependent ATP7B expression, localization, and copper-transport function	Do not fully reflect tissue complexity and heterogeneous cell-cell interactions; furthermore, an appropriate ECM is required to support growth	Potentially useful for personalized drug screening and 3D architectural studies	Schene et al., 2020; Wei et al., 2022; Yang et al., 2023

Abbreviations: AAV, adeno-associated virus; ECM, extracellular matrix; LEC, Long-Evans Cinnamon; TX, toxic milk; TX-J, Jackson Laboratory toxic milk; WD, Wilson's disease; 3D, three-dimensional.