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Histological features of liver disease development in the Atp7b$^{-/-}$ mouse: a model of Wilson's disease

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ABSTRACT

Aims Wilson's disease (WD) is caused by mutations in the ATP7B gene, resulting in copper accumulation and toxicity in liver and brain tissues. Due to the initial asymptomatic liver involvement, the progression of liver injuries in WD stays primarily unknown. Atp7b−/− knockout mice have been shown to be an appropriate model of WD for liver involvement.

Methods A total of 138 Atp7b−/− mice were included and separated into five groups according to age as follows: 6, 20, 39 and 50 weeks without treatment, and 50 weeks with copper chelator treatment from 39 to 50 weeks of age and compared with 101 wild-type (WT) mice at the same stages. The evolution of histological liver lesions was analysed and compared between groups.

Results Significant changes were observed in Atp7b−/− mice compared with WT. Copper deposits in hepatocytes appeared as early as 6 weeks but no significant increase over time was observed. Inflammation appeared as early as 6 weeks and progressed henceforth. Lobular and periportal acidophilic bodies appeared after 20 weeks. Significant atypia was also observed at 20 weeks and increased over time to reach a severe stage at 39 weeks. Fibrosis also became apparent at 20 weeks, progressing subsequently to precirrhotic stages at 50 weeks. Copper content, inflammation and fibrosis scores were significantly reduced in the treated group. No bile duct lesions or dysplastic changes were noted.

Conclusions Copper accumulation leads to progressive changes in Atp7b−/− mice regarding inflammation, fibrosis and atypia. The severity of liver damage is lessened by chelation therapy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Atp7b−/− knockout mice are a suitable animal model for the study of Wilson's disease (WD).

WHAT THIS STUDY ADDS

⇒ Herein are reported the progressive stages of liver injury due to copper overload, including some differences from previous studies. Treatment with D-penicillamine allows the improvement of most histological features.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ A deeper understanding of the stages of liver involvement in WD could guide future studies in identifying key molecular mechanisms for a better therapeutic approach.

INTRODUCTION

Copper is an essential cofactor of many key metabolic enzymes participating in various physiological processes, including respiration, biosynthesis, radical detoxification, neurotransmission and iron uptake.[1] Wilson's disease (WD) is an autosomal recessive-inherited disorder of copper transport and homeostasis that leads to toxic intracellular accumulation of copper in the liver and copper deposits in other sites such as the brain. The prevalence is around 1:30 000 in most populations studied, and the frequency of heterozygote carriers varies from 1:40 to 1:90. The affected gene, coding for a copper-transporting protein, the ATPase 7B, is located on chromosome 13q14.3.[2–4] Mutations are responsible for the absence or dysfunction of ATP7B, which is localised in the trans-Golgi network's vesicles in the hepatocyte's canalicular area.[5] The dysfunction of the ATP7B protein leads to a defect of copper excretion in bile and its accumulation in hepatocytes.[6] Excessive intracellular accumulation of copper disrupts normal cell homeostasis causing protein malfunction and DNA damage.[7] Moreover, when the capacity for hepatocyte storage is exceeded, cooper is released to the plasma, leading to haemolysis and tissue deposits.

WD is phenotypically highly variable; patients with WD may suffer from liver disease and/or neurological or psychiatric symptoms.[8 9] More than 600 ATP7B gene mutations have been identified[10] and no genotype–phenotype correlation in WD has been clearly established.[8] Liver involvement is usually diagnosed in children during the second decade of life, and presentation may vary from asymptomatic elevation of serum transaminases to chronic hepatitis, cirrhosis or acute liver failure.[11 12] For diagnostic purposes, a liver biopsy is only required if the clinical signs and non-invasive tests do not allow a final diagnosis or if there is suspicion of other or additional liver diseases.[13 14] Recommendations to manage asymptomatic patients are based on laboratory tests and non-invasive methods for evaluating liver fibrosis. At the other end of the liver disease spectrum, most of the available human liver samples come from patients with advanced liver injuries when transplantation is needed; as a result, the first steps and disease progression of the liver involvement in the WD have been poorly described and understood. Therefore, animal models of copper

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overload are of great relevance for understanding the disease pathogenesis, particularly at early stages. Four animal models of WD have been established and have shown good similarity to human hepatic WD: the Long-Evans Cinnamon (LEC) rat, the toxic milk mouse, the Atp7b−/− knockout (KO) mouse and the Labrador retriever with mutations in the ATP7 proteins.[15 16] Our team has previously reported the longitudinal evolution of the liver function tests assays (Aspartate Aminotransferase - AST, Alanine Aminotransferease- ALT, total bilirubin) and copper tests (ceruloplasmin, serum exchangeable copper and relative exchangeable copper) in a cohort of Atp7b−/− mice.[17] This study aims to report, using the same cohort, the extensive longitudinal histological features of liver injury to support this model as a pertinent model of chronic liver WD.

MATERIALS AND METHODS

Mice, animal care and experimental procedure

The Atp7b−/− mouse model used in this study was developed by Buiakova *et al*.[18] The Atp7b−/− mice were bred as previously described in Heissat *et al*.[17] C57-BL/6J wild-type (WT) mice (Charles River Laboratories, France) were used as controls. Both male and female mice were included in this study. Breeding, genotyping, housing and all experimental procedures were performed according to a protocol. Conditions for breeding were the same for both groups: The mice were kept on a 12 hours/12 hours light/dark cycle and fed with maintenance dry food #3469 (Kliba Nafag CH), containing 14 mg/kg copper, 60 mg/kg zinc and 250 mg/kg iron. Food and water were provided ad libitum. Atp7b−/− mice did not receive any specific diet during gestation, lactation and after weaning.

A total of 138 Atp7b−/− mice were included in the study and separated into 5 groups according to age: 6 weeks (n=37), 20 weeks (n=26), 39 weeks (n=26), 50 weeks without treatment (n=25) and 50 weeks with copper chelator treatment (D-penicillamine) from 39 to 50 weeks of age (n=24). A total of 101 C57-BL/6J mice (WT mice) were included and separated into 4 groups according to age: 6 weeks (n=32), 20 weeks (n=29), 39 weeks (n=20) and 50 weeks (n=20). Animals were euthanised by CO_2 inhalation at the indicated ages. Livers were promptly removed after animals were euthanised and separated into two parts: one fixed in paraformaldehyde for histological analyses and the other immediately frozen at −80°C in dedicated trace elements tubes. The protocol was approved by the French Ministry of Research under reference #2015062510212698_v1(APAFIS#926).

Summarising biological results, as previously reported, serum ALT and AST regularly increased over time in the Atp7b−/− mice.[17] In addition, total serum copper (Cu), intrahepatic copper (CuIH) and serum exchangeable copper (CuEXC) varied over time, with maximum values at week 20 for CuIH and at week 39 for CuEXC and Cu. Finally, the treatment with a copper chelator (D-penicillamine) significantly reduced Cu, CuIH and CuEXC in Atp7b−/− mice.

Histological analysis

The histological analysis included a total of 184 livers; 120 from Atp7b−/− mice (6 weeks of age n=29, 20 weeks n=13, 39 weeks n=24, 50 weeks untreated n=27, 50 weeks treated n=27) and 64 from WT mice (20 weeks of age n=54, 39 weeks n=5, 50 weeks n=5). Liver tissue sections were prepared by fixation in 10% buffered formalin at pH 7.4 for 3 days, transferred into 80% ethanol and then tissue embedding in paraffin. Each section was stained with haematoxylin phloxine saffron, since it allows a better differentiation of collagen and fibrosis evaluation than H&E. Picro-Sirius red staining was used to investigate fibrosis. In each section, histological analysis investigated inflammation, activity, cellular atypia, fibrosis, steatosis, regenerative nodules and biliary abnormalities. Inflammation was assessed and graded from 0 (no inflammation) to 3 (significant inflammation). Inflammation corresponding to the evaluation of the lobular lymphoid infiltrate 0=no significant infiltrate, 1 = ≤ 1 focus per lobule, 2 = ≥ 2 focus per lobule, 3=diffuse lobular infiltrate. Activity (acidophilic bodies) was evaluated based on the METAVIR classification[19] corresponding to 0=A0, 1=A1, 2=A2 and 3=A3. Fibrosis of the liver parenchyma was evaluated using a score from 0 (absence of fibrosis) to 3 (severe fibrosis). Fibrosis score corresponding to 0=none, 1=mild (portal or pericellular fibrosis), 2=moderate (thin and diffuse or thick but occasional fibrosis) and 3=thick and diffuse fibrosis. Cellular atypia was evaluated and graduated from 0 (no atypia) to 3 (severe atypia), corresponding to 0=no atypia, 1=discreet enlargement of the hepatocytes, 2=ballooning degeneration and or giant nuclei and 3=pleomorphic cells with multinucleated cells and/or atypical nucleoli. Copper was detected using 5-p-dimethylaminobenzylidene rhodanine (ref 444678, Carlo Erba Reagents).

Statistics analysis

Statistical analyses were performed using GraphPad Prism V.9.0.0. The main characteristics were compared between groups using contingency analyses with a χ^2 test to compare two groups of mice or a χ^2 test for trend to compare groups over time. Statistical significance was set at a p value<0.05.

RESULTS

Histological analysis of Atp7b−/− mice found progressive development of chronic liver injury with significant lesions, homogeneously distributed across each age group (figures 1–3). No difference was noticed between male and female mice. No abnormality was noticed in the WT group at 20, 39 and 50 weeks. We decided to use the WT mice at 20 weeks as a control group.

Copper deposition

Over time, copper deposits in hepatocytes were observed in all Atp7b−/− mice and identified as granular deposits in the cytoplasm of hepatocytes, without lobular zonation, using rhodanine staining (figure 4). No significant increase over time was observed. No copper deposition was detected in WT. The changes in intrahepatic copper values were analysed in our previous publication about this mouse cohort[17] which went from 605 (172, 16) g/g dry liver at 39 weeks—mean values (±SD)—to 797 (402, 9) g/g at 50 weeks in the untreated groups to 486 (263, 11) g/g in the treated group (p 0.05).

Steatosis

No remarkable steatosis was observed at any age in both WT and Atp7b−/− groups (figure 2). Mild fat droplets were noticed in a few mice without significant differences between age groups or WT and Atp7b−/− mice.

Necroinflammation

There was a progressive inflammation increase in the liver parenchyma in Atp7b−/− mice (figure 1A,B). The inflammation was increased in Atp7b−/− mice at 6 weeks and significantly

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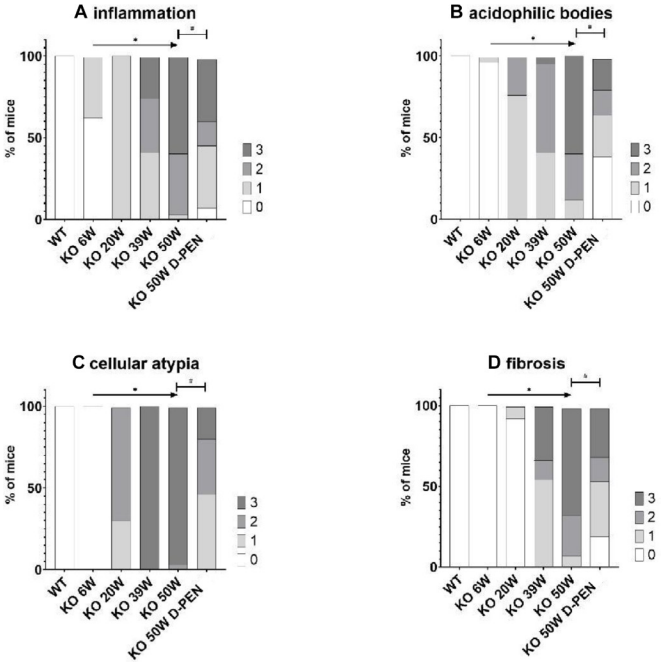

Figure 1 Liver histological evaluation in wild-type (WT) mice and over time in Atp7b$^{-/-}$ (KO) mice at 6 weeks (W), 20W, 39W and at 50W with or without D-penicillamine (D-Pen) treatment for the different features scored from 0 (absent) to 3 (intense). Inflammation (A): WT mice: 100%=0; KO mice 6W 62.07%=0 and 37.93%=1; KO mice 20W 100%=1; KO mice 39W 41.77%=1, 33.33%=2; 25%=3; KO mice 50W: 3.7%=1, 37.04%=2, 59.26%=3; KO mice 50W D-Pen: 7.70%=0, 38.46%=1, 15.38%=2 and 38.46%=3. Acidophilic bodies (B): WT mice: 100%=0; KO mice 6W: 96.55%=0; 3.45%=1. KO mice 20W: 76.92%=1; 23.08%=2. KO mice 39W: 41.67%=1; 54.17%=2. 4.17%=3. KO mice 50W: 12%=1; 28%=2; 60%=3. KO mice 50W D-Pen: 38.46%=0; 26.92%=1; 15.38%=2; 19.23%=3. Cellular atypia (C): WT mice: 100%=0; KO mice 6W: 100%=0. KO mice 20W: 30.77%=1; 69.23%=2. KO mice 39W: 100%=3. KO mice 50W: 3.7=2; 96.30%=3. KO mice 50W D-Pen: 46.15%=1; 34.62%=2; 19.23%=3. Fibrosis (D): WT mice: 100%=0; KO mice 6W: 100%=0. KO mice 20W: 92.31%=1; 7.69%=2. KO mice 39W: 54.16%=1; 12.5%=2. 33.33%=3. KO mice 50W: 7.41%=1; 25.93%=2; 66.67%=3. KO mice 50W D-Pen: 19.23%=0; 34.62%=1; 15.38%=2; 30.77%=3. *p for trend<0.0001 and #p<0.005.

progressed over time in Atp7b−/− mice (p <0.0001, χ^2) when compared with WT (p <0.0001, χ^2). This inflammation was a sinusoidal lymphocytosis consisting of linear arrays of lymphocytes around sinusoids; no periportal or perivenular preferential distribution was noticed. The inflammation was mainly chronic, with some rare grouped polymorphonuclear neutrophils. Inflammation was significantly lower in treated Atp7b−/− mice at 50 weeks compared with untreated Atp7b−/− mice at the same age (p <0.0003, χ^2); however, a significant degree of inflammation was still observed in most mice compared with WT.

No necrosis was observed in the WT mice (figures 1B and 2). Activity (lobular and periportal acidophilic bodies) was observed in all the Atp7b−/− groups but was statistically significant for Atp7b−/− mice after 20 weeks p <0.0001, χ^2). The activity significantly increased regularly over time in Atp7b−/− mice (p <0.0001, χ^2). The activity was significantly lower in treated Atp7b−/− mice at 50 weeks compared with untreated Atp7b−/− mice at the same age (p <0.0001, χ^2).

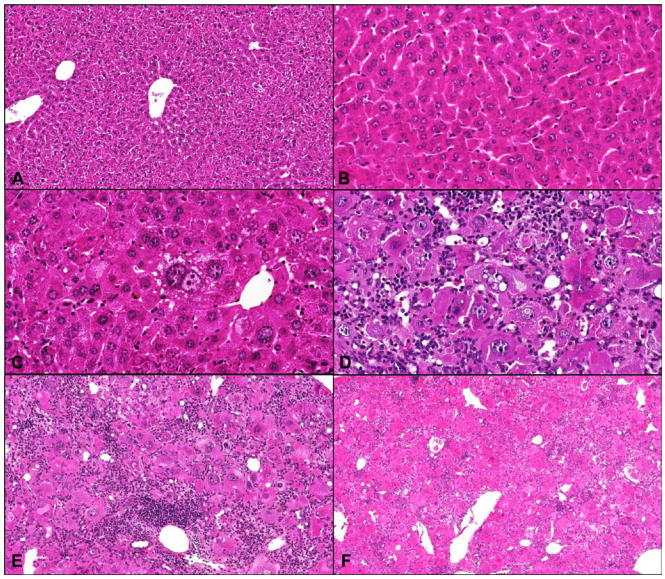

Figure 2 Histological evolution of the liver parenchyma over time in knockout mice at 6 weeks (W) (B) (×100), 20W (C) (×200), 39W (D) (×200), 50W untreated (E) (×100) and 50W treated (F). (×100) compared with wild-type mice (A) (×50). Haematoxylin phloxine saffron staining. Cellular atypia can be observed in (D) and (E).

Cellular atypia

No cellular atypia was noted in WT groups and in the 6-week-old Atp7b−/− mice (figures 1C and 2). In Atp7b−/− mice from 20 weeks of age, significant atypia was observed and increased over time to reach severe atypia at 39 weeks. It begins with nuclear enlargement and vacuolisation at 20 weeks, followed by significant dysmorphic changes in hepatocytes at 39 and 50 weeks (figure 4A,B). These changes were significant over time (p <0.0001, χ^2).

Cellular changes were relatively non-specific, with hepatocellular damages manifested by ballooning degeneration with some Mallory bodies and apoptotic hepatocytes throughout

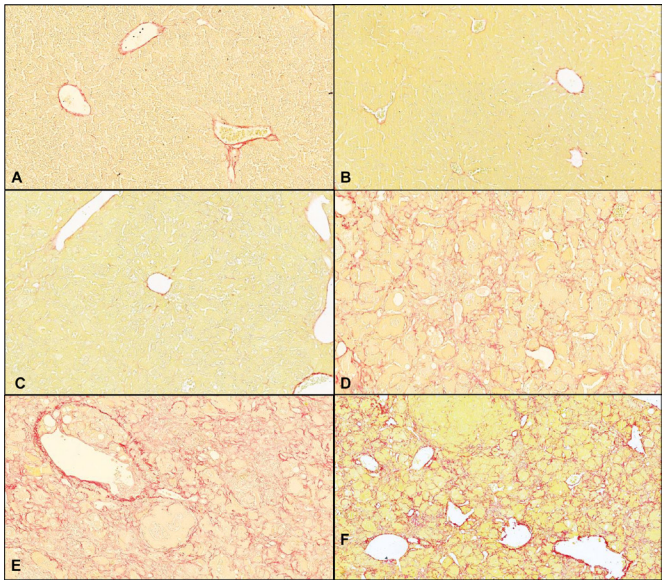

Figure 3 Fibrosis evaluation in wild-type mice (A), and untreated knock out (KO) mice at 6 weeks (W) (B), 20W (C), 39W (D) and 50W (E), as well as treated KO mice at 50W (F) Picro-Sirius red staining, ×200.

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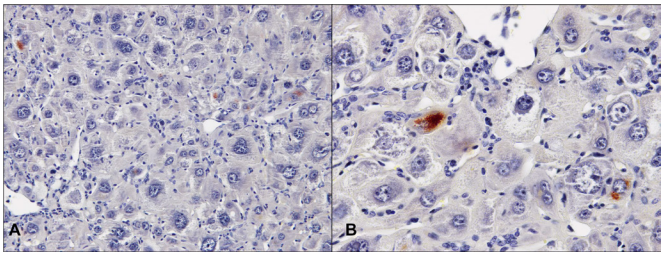

Figure 4 Copper deposits in knockout mice at 50 weeks identified by rhodanine staining (red-brown cytoplasmic granules), located in cytoplasm of hepatocytes. (A) ×100; (B) ×400.

the lobule with no preferential architectural distribution. At the late stages, hepatocytes showed a marked nuclear variability with giant nuclei, glycogen vacuolisation of the nucleus and multinucleated cells with atypical nucleoli. The Atp7b−/− treated group noticed a significant decrease in cellular atypia compared with the untreated Atp7b−/− mice at the same age ($p < 0.0001$, χ^2).

Fibrosis

The microscopic examination and fibrosis evaluation based on the Picro-Sirius red staining found fibrosis that appeared over time in Atp7b−/− mice (figures 1D and 3), while none of the WT mice livers showed any fibrosis. There was no fibrosis (score=0) in Atp7b−/− mice at 6 weeks. Mild fibrosis was significantly greater in each Atp7b−/− age group from 20 weeks compared with the WT group ($p < 0.0001$, χ^2). In Atp7b−/− mice, a significant progressive increase in fibrosis was observed over time ($p < 0.0001$, χ^2). The fibrosis starting at 20 weeks, was perisinusoidal without architectural alteration, showing fibrous tissue along sinusoids and extending progressively over time. At 39 weeks, fibrosis was more diffused, and defined central–central bridges at 50 weeks without reaching a mutilating stage defining the constituted cirrhosis (figure 3). Fibrosis score was significantly lower in treated Atp7b−/− mice at 50 weeks than in the untreated group at the same age ($p < 0.0002$, χ^2) (figure 1).

Others features

Nodular, well-circumscribed liver parenchymal areas, consisting of regenerating nodules, were seen in the Atp7b−/− mice from 39 weeks to the later stages with or without treatment. No nodule was noticed in the WT groups or before 39 weeks in the Atp7b−/− mice. Each liver contained 1–3 nodules, measuring from 1 mm (micronodules) to 13 mm (macronodules). Nodules, besides rare apoptotic bodies, contained no portal space and were free of fibrosis and activity (figure 5). Hepatocytes showed no cellular atypia, and few mitoses were noticed. Nodules were devoided of copper deposits (Rhodanine staining).

No bile duct lesion was noticed in any group, and no ductular proliferation or ductopenia was seen over time. Only one liver in the Atp7b−/− 50 weeks presented a mild ductular proliferation. Otherwise, no malignant feature or dysplastic change was noted in any groups of mice.

DISCUSSION

Our study confirms that the Atp7b−/− mouse represents a relevant and specific model for chronic hepatitis induced by copper overload, which helps understand the liver

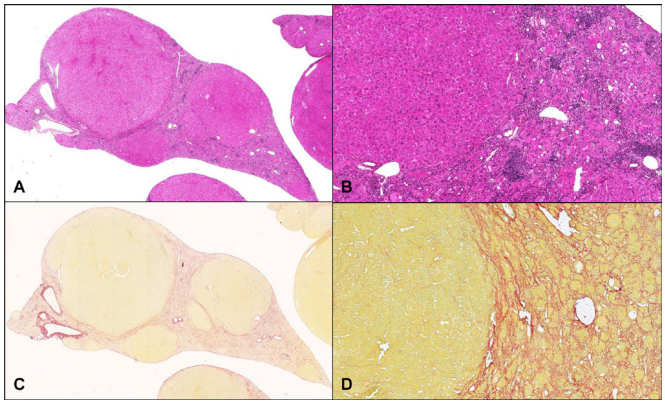

Figure 5 Regenerating nodules in the liver. (A) (×100) and (B) (×200) haematoxylin phloxine saffron staining at 39 weeks. (C) (×100) and (D) (×200) Picro-Sirius red staining at 39 weeks.

consequences of intrahepatic copper accumulation. Animal models of copper overload are essential to understand the disease pathogenesis and to improve the diagnosis, monitoring and treatment of patients with WD.

Several rodent models have been described. LEC rats, toxic milk mice and txJ mice are products of spontaneous mutation in the ATP7B gene that occurred during breeding.[20 21] However, the Atp7b−/− mouse, generated by Buiakova *et al*,[18] is the only genetically engineered rodent strain that can serve as an animal model of WD.[16] In these mice, multiple stop codons were inserted in exon 2, producing a truncated mRNA and suppressing ATP7B expression. Although copper accumulation can be detected in other organs, the highest copper concentration and the most striking phenotype is observed in the livers of Atp7b−/− mice.[16]

At birth, Atp7b−/− mice have copper deficiency, but soon after, copper accumulates in the liver and increases to peak levels between 6 and 20 weeks of age.[18 22] In our study, intrahepatic copper concentration was consistently higher in Atp7b−/− mice, and the peak level was reached at 20 weeks. This progressive copper overload leads to hepatic damage. Copper overload precedes and secondarily induces tissue damage initially with inflammation, followed by progressive fibrosis development. Hepatic lesions occur not only because of copper toxicity but also through several mechanisms dealing with lipid metabolism, mitochondrial injury and hepatic nuclear receptor dysfunction.[16 23] Acidophilic bodies score markedly increased over time in Atp7b−/− mice following the same pattern as the aminotransferase in the same period. However, acidophilic bodies, such it is those observed in viral hepatitis, were mild compared with the global liver injuries observed and especially compared with the cellular atypia. This could suggest that cellular atypia, such as ballooned cells in Nonalcoholic Fatty Liver Disease (NAFLD),[24] could reflect the activity of hepatitis in WD. Since the cellular atypia was correlated to liver function tests and expressed apoptotic markers, we suggest that activity in WD should be evaluated not only based on acidophil bodies but associated with the evaluation of cellular atypia and inflammation.

The fibrosis features described in our study include a progressive perisinusoidal fibrosis that begins between 6 and 20 weeks and progresses to diffuse fibrosis from 39 to 50 weeks without reaching a completed cirrhotic stage. More extended experimentation could be necessary to reach

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the cirrhosis that might occur after 50 weeks. The fibrosis development along sinusoids suggests an evolution close to the metabolic hepatitis models (such as NAFLD) rather than viral hepatitis. No fibrosis score has been validated in WD. This model suggests that the evaluation of fibrosis in WD should preferably use the fibrosis score of the scoring system, such as NAFLD Activity Score, rather than METAVIR.

Interestingly, in treated mice with D-penicillamine, we observed at 50 weeks a decrease in both inflammation and fibrosis in comparison to untreated mice. In humans, the treatment allows the normalisation of transaminases and an improvement of fibroscan values reflecting a decrease in inflammation and fibrosis. Histological effects of chelator treatment using D-penicillamine between 39 and 50 weeks in KO mice were significant; however, a longer course of treatment may be necessary to amplify the improvement of liver injuries, especially concerning inflammation and fibrosis but not the regression of the nodules, an earlier start to prevent the development of more severe liver damage.

Although Atp7b−/− mouse is a good model for apprehending liver injuries related to copper overload, this model presents some notable differences compared with WD in humans and/or other animal models. First, in the Atp7b−/− mice cerebellum, ATP7A compensates for the absence of ATP7B, preventing neurological damage. So far, no animal models mimic both liver and neurological involvement. Second, Atp7b−/− mice only present with chronic hepatitis and is not a model to study fulminant hepatitis, consistent with previously published data.[16] Third, no significant steatosis was detected in our model, unlike what is usually described not only in WD patients' livers but also in LEC rats.[25 26] In Atp7b−/− mice, it has been shown that copper overload affects lipid metabolism, particularly the cholesterol biosynthesis pathway, which is downregulated. Microvesicular or macrovesicular hepatic steatosis is an early histological lesion in patients with WD but was not described in Atp7b−/− mice by Lustenko.[16] It is unclear if the liver biopsy at 6 weeks is too early and 20 weeks too late. In LEC rats, steatosis was seen at 12 weeks of age. Last, no malignancy or dysplastic features were observed in our model despite what has been reported by Huster *et al* in the Atp7b−/− mice.[22] According to the literature, the rate of hepatobiliary malignancies in WD is considerably low, even in cirrhotic patients.[27] This observation is consistent with our personal experience based on examining the patient's samples from our centre.

In conclusion, we validate a valuable model of fibrotic hepatitis in WD that will be useful for future experiments, such as testing new drug candidates. The Atp7b−/− mice represent a valuable model for analysis of the consequences of copper accumulation in the liver. Several important phenotypic characteristics of the Atp7b−/− mice look like the WD phenotype, encouraging the use of these animals for dissecting the WD pathology. We report the different stages of liver involvement induced by copper overload that will facilitate a deeper understanding of the progression of WD.

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Contributors P-ML: performed histological analysis and wrote the manuscript. OG: statistical analysis and manuscript approval. JD: project conception and manuscript approval. EM: animal care, biochemical analysis and manuscript approval. VB: animal care, biochemical analysis and manuscript approval. SH: project conception and manuscript approval. ECB: manuscript writing and approval and is responsible for the overall content as guarantor. AL: project conception and manuscript approval. MB: biochemical analysis and manuscript approval. VH: project conception, histological interpretation and manuscript approval.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Breeding, genotyping, housing and all experimental procedures were performed according to a protocol approved by the ethics committee (C2EA—12 Comité d'éthique ComEth Grenoble), the veterinary authorities and the French Ministry for Research (reference C38-18510001).

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement Data are available upon reasonable request.

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REFERENCES

1 Peña MM, Lee J, Thiele DJ. A delicate balance: homeostatic control of copper uptake and distribution. *J Nutr* 1999;129:1251–60.
2 Petrukhin K, Fischer SG, Pirastu M, *et al*. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nat Genet* 1993;5:338–43.
3 Bull PC, Thomas GR, Rommens JM, *et al*. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* 1993;5:327–37.
4 Tanzi RE, Petrukhin K, Chernov I, *et al*. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993;5:344–50.
5 Schaefer M, Roelofsen H, Wolters H, *et al*. Localization of the Wilson's disease protein in human liver. *Gastroenterology* 1999;117:1380–5.
6 Bartee MY, Lutsenko S. Hepatic copper-transporting ATPase Atp7B: function and inactivation at the molecular and cellular level. *Biometals* 2007;20:627–37.
7 Harris ZL, Gitlin JD. Genetic and molecular basis for copper toxicity. *Am J Clin Nutr* 1996;63:836S–41S.
8 Ferenci P, Caca K, Loudianos G, *et al*. Diagnosis and phenotypic classification of Wilson disease . *Liver International* 2003;23:139–42.
9 Loudianos G, Gitlin JD. Wilson's disease. *Semin Liver Dis* 2000;20:353–64.
10 Riordan SM, Williams R. The Wilson's disease gene and phenotypic diversity. *J Hepatol* 2001;34:165–71.
11 Giacchino R, Marazzi MG, Barabino A, *et al*. Syndromic variability of Wilson's disease in children. Clinical study of 44 cases. *Ital J Gastroenterol Hepatol* 1997;29:155–61.
12 Beinhardt S, Leiss W, Stättermayer AF, *et al*. Long-term outcomes of patients with Wilson disease in a large Austrian cohort. *Clin Gastroenterol Hepatol* 2014;12:683–9.
13 European Association for Study of Liver. EASL clinical practice guidelines: Wilson's disease. *J Hepatol* 2012;56:671–85.
14 Roberts EA, Schilsky ML, American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. *Hepatology* 2008;47:2089–111.
15 Huster D. Structural and metabolic changes in Atp7B-/- mouse liver and potential for new interventions in Wilson's disease. *Ann N Y Acad Sci* 2014;1315:37–44.
16 Lutsenko S. Atp7B-/- mice as a model for studies of Wilson's disease. *Biochem Soc Trans* 2008;36:1233–8.
17 Heissat S, Harel A, Um K, *et al*. Evaluation of the accuracy of exchangeable copper and relative exchangeable copper (REC) in a mouse model of Wilson's disease. *J Trace Elem Med Biol* 2018;50:652–7.
18 Buiakova OI, Xu J, Lutsenko S, *et al*. Null mutation of the murine Atp7B (Wilson disease) gene results in intracellular copper accumulation and late-onset hepatic nodular transformation. *Hum Mol Genet* 1999;8:1665–71.
19 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* 1996;24:289–93.
20 Burkhead JL, Gray LW, Lutsenko S. Systems biology approach to Wilson's disease. *Biometals* 2011;24:455–66.
21 Fuentealba IC, Aburto EM. Animal models of copper-associated liver disease. *Comp Hepatol* 2003;2:5.

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22 Huster D, Finegold MJ, Morgan CT, *et al*. Consequences of copper accumulation in the livers of the Atp7B−/− (Wilson disease gene) knockout mice. *Am J Pathol* 2006;168:423–34.
23 Huster D, Purnat TD, Burkhead JL, *et al*. High copper selectively alters lipid metabolism and cell cycle machinery in the Mouse model of Wilson disease. *J Biol Chem* 2007;282:8343–55.
24 Brunt EM, Tiniakos DG. Histopathology of nonalcoholic fatty liver disease. *World J Gastroenterol* 2010;16:5286–96.
25 Stromeyer FW, Ishak KG. Histology of the liver in Wilson's disease: a study of 34 cases. *Am J Clin Pathol* 1980;73:12–24.
26 Levy E, Brunet S, Alvarez F, *et al*. Abnormal hepatobiliary and circulating lipid metabolism in the long-Evans cinnamon rat model of Wilson's disease. *Life Sci* 2007;80:1472–83.
27 Pfeiffenberger J, Mogler C, Gotthardt DN, *et al*. Hepatobiliary malignancies in Wilson disease. *Liver Int* 2015;35:1615–22.

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