Animal Models of Wilson Disease

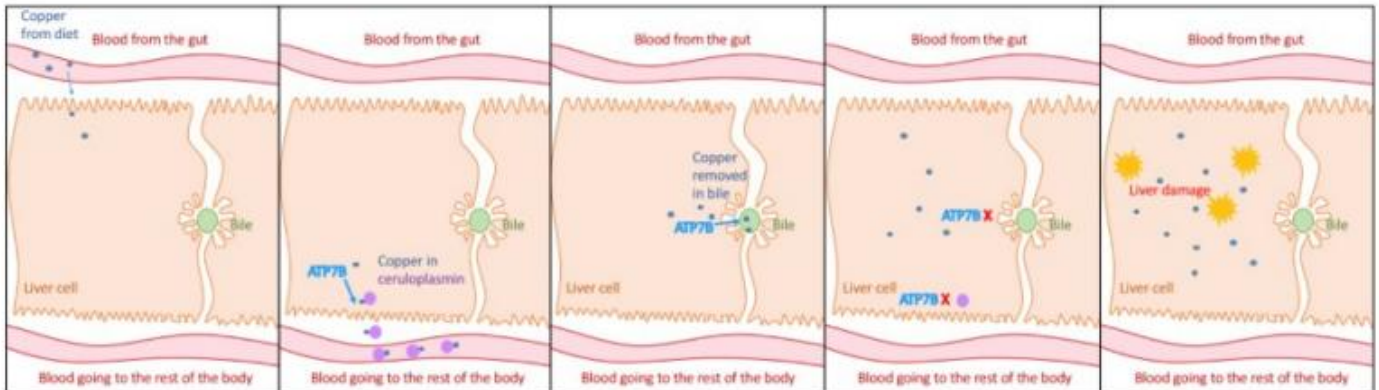
Why do we need animal models for Wilson disease?

Animal models are used by scientists to understand more about human diseases and to find new ways to test for them or treat them. Sometimes we need animal models instead of other models (such as cell samples taken from people) because we need to see how the disease progresses over time and spreads from one organ of the body to others. It is also often impossible to study how different types of cells contribute to the disease even if we just look at one organ, such as the brain. Most importantly, animal models are still very important to test new drug treatments.

Animal models and Wilson disease?

You might know that Wilson disease is caused by a defect in the gene ATP7B. The ATP7B gene is responsible for making ATP7B protein. Normal ATP7B protein mostly works in the liver, where it has two main roles. One is to package the copper that we absorb from our food into another protein, ceruloplasmin, which then circulates around the body in your blood. The other role is to remove copper from the body by adding it to bile in your liver. Bile then leaves the body through your gut as waste.

In Wilson disease, faulty ATP7B means that copper isn't added to ceruloplasmin, causing low levels of this protein in your blood (this is one of the tests we use to diagnose Wilson disease). It also means that copper isn't removed in bile. Because both these ways of removing copper from the liver are lost, copper builds up in the liver at toxic levels. Very often, the copper levels are also increased in other organs such as the brain.



Copper from your diet is transported by blood to the liver	**Normally…** ATP7B helps copper bind to ceruloplasmin which is transported around the body in blood	**OR** ATP7B removes copper from the liver by transporting it into bile	**In Wilson Disease…** Faulty ATP7B can't remove copper from the liver by moving it into bile or into ceruloplasmin.	Copper is trapped in the liver and builds up at toxic levels, causing liver damage. Copper spills out of damaged liver cells and builds up in other organs, such as the brain.

Diagram to show how ATP7B works in the liver

There are several different rodent (rat or mice) animal models of Wilson's disease, called the Long-Evans Cinnamon rat, the toxic milk mouse and the Atp7b knockout mouse. They all have copies of the Atp7b gene that don't work meaning that they also get toxic build-up of copper in their liver and other organs.

The Long-Evans Cinnamon (LEC) Rat

The LEC rat was discovered when scientists noted that a strain of rats with cinnamon-coloured coats developed liver disease at 3-4 months of age as a result of high levels of copper[1,2] . It was experiments in LEC rats that helped explain how ATP7B works and why the loss of ATP7B causes high copper[3,4]. LEC rats also gave us more information about how copper causes liver disease. The microscopic appearances of LEC rat livers are similar to human disease, with inflammatory, fatty and fibrous changes[1,5-8]. In LEC rat livers, it could be seen that copper causes oxidative stress (a process where charged oxygen particles damage parts of cells and cause inflammation) and damage to mitochondria (the cell batteries [9-16] .

New treatments targeting oxidative stress have been tested and found to be effective in treating liver disease in LEC rats. These include D-mannitol, N-acetylcysteine (NAC), D-galactosaminehydrochloride, proline solution, ascorbic acid, alpha-lipoic acid and thioredoxin[17-20]. A new chelation treatment (meaning it acts in a similar way to penicillamine or trientine), N-benzyl-D-glucamine dithiocarbamate (BGD) is also effective in treating liver disease in LEC rats[21] . Unfortunately, despite high copper levels in the brain, LEC rats do not develop symptoms of brain disease nor the brain changes seen in human disease[6,22,23]. This means we cannot use the LEC rat to study how the brain is affected by Wilson disease.

The toxic milk mouse

The toxic milk mouse has its name because the infant mice die early due to their mother's milk being too low in copper[24,25]. This can be prevented by feeding the pups from a healthy mother[26] . If normal milk is fed to these toxic milk mice, then they get a build-up of copper in the liver and develop liver disease[24,26,27] similar to the liver disease we see in patients with Wilson disease.

These mice also have high copper levels in their brain. They also have behavioural changes and changes in the way that they walk, which could be similar to a brain disorder caused by Wilson's disease. However, scientists think it is more likely these are symptoms of more general bad health. They also show some signs of having inflammation in their brains but, unlike in people who have a brain disorder because of Wilson's disease, there is no sign of brain cell loss[28] .

The Atp7b knockout mouse

The Atp7b knockout mouse is the only model of Wilson disease which was made intentionally by scientists [29] . Scientists have used this model to look very closely at liver disease in Wilson disease and by doing so, have divided it into 3 different stages. In stage 1 (early) disease, copper starts to build-up in the liver. Changes to the cell batteries (the mitochondria) and to the nucleus (the command centre of the cell) can be seen already but the rats are still healthy otherwise. In stage 2 disease, there is inflammation and cell death in the liver. By stage 3, the liver either starts to repair itself or fibrous changes and cancer can develop[30,31] .

Experiments in Atp7b knockout mice also revealed that the way the liver handles fat and cholesterol is changed in Wilson disease[31,32]. This is also seen in the brains of Atp7b knockout mice, which might help to explain how Wilson's disease causes brain damage[33]. Atp7b knockout mice also have oxidative stress and damage to mitochondria in their brains but, like the other models, they don't get loss of brain cells[33] .

Scientists have treated Atp7b knockout mice using "gene therapy". To do this, healthy Atp7b DNA was introduced into Atp7b knockout mice using a virus. This successfully improved liver function in the treated mice[34] .

Atp7b knockout mice have also been used to develop new ways to diagnose Wilson's disease using PET scanning. Scientists gave the mice radioactive copper before scanning. The radioactive copper caused the livers of the Atp7b knockout mice to light up on the PET scan more than the livers of healthy mice[35] .

What are the next steps?

Obviously, there is still a lot that we do not yet understand about Wilson disease and we are yet to find a cure for this disease! The three animal models of Wilson disease described in this article have given us lots of new information about Wilson disease and these could lead to new tests and treatments being developed in the future. Unfortunately, none of the currently available animal models for Wilson disease gets a brain disorder in the same way patients with Wilson disease often do. As it is often the brain disorder that is hardest to treat in Wilson's disease, we badly need to develop new models for Wilson disease. It would be particularly important for this new model to be suitable for drug screens so that we can find a cure.

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