

Copper: two sides of the same coin

R.H.J. Houwen

Department of Pediatric Gastroenterology (KE.01.144.3), University Medical Center Utrecht,
PO Box 85090, 3508 AB Utrecht, tel.: +31 (0)88-755 40 01, fax: +31 (0)88-755 53 42,
e-mail: r.h.j.houwen@umcutrecht.nl

Copper is a highly toxic trace element, due to its oxidising potential, yet it is essential for proper growth and development. Both sides of this coin are shown in two inborn errors of copper metabolism: Menkes disease and Wilson's disease. In Menkes disease, due to mutations in the *ATP7A* gene, copper cannot leave the small intestinal epithelial cells after its absorption from the gut, resulting in copper deficiency throughout the body. This will primarily cause neurological dysfunction and connective tissue abnormalities, due to insufficient function of enzyme systems in which copper is an essential co-factor, such as dopamine- β -hydroxylase and lysyloxidase.¹ In Wilson's disease, due to mutations in the closely related *ATP7B* gene, copper cannot be excreted by the liver, giving a slow accumulation of copper in the liver and secondarily in other organ systems, such as the brain.^{2,3} This copper overload can become symptomatic as early as at 4 years, and as late as 70 years of age. As is to be expected, patients presenting early more often have hepatic symptoms, while patients presenting later in life generally have neurological symptoms.⁴

Diagnosis of Wilson's disease can be difficult, as is described in the case report by Kok *et al.*⁵ The classical biochemical abnormalities, such as a low serum ceruloplasmin and serum copper, elevated urinary copper excretion and abnormal liver copper can be partially absent. Conversely, some of these abnormalities can also be found when Wilson's disease is definitely not present, such as the low serum ceruloplasmin and serum copper frequently encountered in healthy carriers of an *ATP7B* mutation, and the increased liver copper and urinary copper excretion found in cholestatic liver disease. Therefore, a combination of several diagnostic parameters should be used in the diagnosis of Wilson's disease, for example by applying the scoring system developed by Ferenci *et al.*⁶ In this scoring system some weight is given to the presence or absence of Kayser-Fleisher rings. Indeed, the presence of these pathognomonic rings, a deposit of a greenish brown copper pigment in Descemet's membrane in the cornea, is almost

diagnostic for Wilson's disease. However, these rings are absent in up to 50% of the patients presenting with hepatic symptoms, as was the case in the patient described by Kok *et al.*⁵ This is not surprising, as copper starts to accumulate in the liver and dissemination to other organ systems, especially the brain, of which the eyes are an extension, only develops later in the course of the disease.

Molecular analysis of the *ATP7B* gene, as done in the patients described by Kok *et al.* and Balkema *et al.*, can either confirm a diagnosis already established, or be an essential part of the diagnostic process.^{5,7} However, in up to 20% of patients with an unequivocal diagnosis of Wilson's disease either one or both causative mutations can not be found. These patients probably have (a) mutation(s) in the promotor region, which is not analysed during routine analysis of the gene. So a negative genetic analysis does not exclude Wilson's disease.

In Wilson's disease the intracellular levels of copper within the liver will exceed buffering capacity after years to decades of copper accumulation. Then mitochondrial membranes will become oxidised, activating the Fas pathway, and causing apoptosis of liver cells.⁸ In this process unbound copper will be released, challenging the remaining liver, and causing even more cells to go down the apoptosis route. This process can result in a rapid reduction in functioning liver mass, causing liver insufficiency and yet is characterised by a relatively modest increase in the levels of transaminases. This not very well-known characteristic of Wilson's disease may cause diagnostic delay, thereby postponing treatment. As chelating therapy is especially effective in the early stages of liver disease, any loss of time can make the difference between recovery of the patient's own liver, or a liver transplant. Whether it is indeed possible for a patient to recover with chelators only, or that an emergency liver transplant is warranted, can be reliably predicted by using the revised King's score.⁹ With a score of 11, the patient described by Balkema *et al.*, came indeed close to having to be listed for a liver

transplant.⁷ The phase with rapid decay of liver cells is also characterised by the release of significant amounts of unbound copper into the circulation, which can induce oxidative damage in circulating red blood cells, and may result in severe haemolysis in some patients with Wilson's disease. It can even be the presenting symptom, as in the patient described by Balkema *et al.*⁷

Interestingly, it might be possible to inhibit the most devastating consequences of oxidative cellular damage, i.e. the apoptosis displayed by liver cells, using amitriptyline. This effectively reduces apoptosis of liver cells *in vitro* by inhibiting acid sphingomyelinase, which is an essential part of the relevant signalling pathway. When applying this principle *in vivo*, by using LEC rats, an animal model of Wilson's disease, apoptosis could also be effectively blocked, thereby significantly increasing survival.¹⁰ It seems logical to extend these findings to humans, as amitriptyline prescribed for other reasons has proven to be safe in a very large number of patients and over a wide dosage range.

Current treatment of Wilson's disease is aimed at reducing copper overload. With the availability of at least four treatment modalities to achieve this (zinc, penicillamine, trientine and tetrathiomolybdate), the best therapeutic choice continues to be a matter of debate. Unfortunately, in a recent literature search that we performed, identifying almost 1000 articles devoted to this topic, we could identify less than 20 retrospective patient series in which one of these medications was evaluated, and only one prospective trial, albeit non-randomised and non-blinded. This number was far exceeded by the number of statements and editorials claiming the superiority of one treatment over the other. Clearly, proper randomised trials with a sufficient number

of patients should answer the question which therapy is the best in a specific situation, e.g. neurological presentation, mild hepatic symptoms, etc. Within EuroWilson, an EU sponsored multinational collaboration, such trials are now being developed. In the coming years the results of these trials should aid in the therapeutic choice for patients with Wilson's disease.

REFERENCES

1. Kaler SG. Diagnosis and therapy of Menkes syndrome, a genetic form of copper deficiency. *Am J Clin Nutr* 1998;67:S1029-34.
2. Brewer GK, Yuzbasiyan-Gurkan V. Wilson disease. *Medicine* 1992;71:139-64.
3. Houwen RHJ, van Hattum J, Hoogenraad TU. Wilson disease. *Neth J Med* 1993;43:26-37.
4. Stapelbroek JM, Bollen CW, Ploos van Amstel JK, et al. The H1060Q mutation in ATP7B is associated with late and neurologic presentation in Wilson disease: results of a meta-analysis. *J Hepatol* 2004;41:758-63.
5. Kok KF, Hoevenaars B, Waanders E, Drenth JPH. Value of molecular analysis of Wilson's disease in absence of tissue copper deposits: a novel ATP7B mutation in an adult patient. *Neth J Med* 2008;66(8):348-50.
6. Ferenci P, Caca K, Loudianos G, et al. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003;23:139-42.
7. Balkema S, Hamaker ME, Visser HPJ, Heine GDN, Beuers U. Haemolytic anaemia as a first sign of Wilson's disease. *Neth J Med* 2008;66(8):344-7.
8. Strand S, Hofmann WJ, Grambihler A, et al. Hepatic failure and liver cell damage in acute Wilson's disease involve CD95 (APO-1/Fas) mediated apoptosis. *Nature Med* 1998;4:588-93.
9. Dhawan A, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37 years experience and revised King's score for liver transplantation. *Liver Transplant* 2005;11:441-8.
10. Lang PA, Schenk M, Nicolay JP, et al. Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. *Nature Med* 2007;13:164-70.