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Mini-review

Wilson disease: Current status and the future☆

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ABSTRACT

The focus of this minireview is on the current status and new advances in diagnosis and treatment of Wilson disease, an autosomal recessive disorder of copper metabolism. Molecular diagnostics have improved and complements current biochemical and clinical methods for screening for Wilson disease. Screening for Wilson disease in newborns is feasible and has been tested in limited populations, but is not yet widely performed. Identification of patients with Wilson disease as the cause of acute liver failure is possible using standard biochemical tests. Treatments for Wilson disease include chelating agents and zinc salts and liver transplantation. Future therapies may include hepatocyte transplantation and gene therapy, both of which have been tested and shown to work in animal models of Wilson disease. Future human studies await advances in these areas.

New advances in the diagnosis and treatment of Wilson disease, an autosomal recessive disorder of copper metabolism are the subjects of this review. Reduced biliary excretion that is now recognized as resulting from a dysfunctional copper transporting ATPase, ATP7B, leads to copper accumulation in the liver and other sites in the body, most notably the central nervous system. Copper incorporation into the protein ceruloplasmin is also impaired by loss of ATP7B function, leading to the reduced circulating levels of the holoprotein. This disorder, present in ~1 in 30,000 individuals, presents mainly as hepatic disease in younger patients in their first and second decades of life, and with neurologic or psychiatric symptoms in their late second or third decades (for review, see [1]). Rare patients present later in life [2]. Treatments available for this disorder can arrest and prevent disease progression in symptomatic patients, and prevent development of symptoms if begun while the patient is asymptomatic.

Current clinical and biochemical testing for Wilson disease successfully diagnoses most patients with this disorder, however the wide range of phenotype with respect to liver and neuropsychiatric disease at times poses challenges to clinicians. To aid clinicians in determining the degree to which a diagnosis of Wilson disease should be considered, a scoring system that uses the clinical, biochemical, pathological findings as well as considering results of the molecular testing was previously proposed [3]. This scoring system since has been validated by other authors [4,5]. Recently, an updated guidelines for the diagnosis and treatment of Wilson disease has been released by the American Association for the Study of Liver Disease, and this includes mention of the scoring system as well as suggestions as how to incorporate molecular testing into current clinical practice (Table 1) [6].

There is a diverse clinical and biochemical phenotype for Wilson disease, and establishing the diagnosis may be difficult in some patients. The use of molecular testing that is now available commercially and in dedicated research laboratories can be critical for providing definitive evidence for the presence or absence of mutations of *ATP7B* permitting a diagnosis of Wilson disease. Molecular testing for *ATP7B* mutations has therefore become an important advance in the diagnostic armamentarium of clinicians, especially when routine testing is equivocal for Wilson disease. The limitation to molecular testing (aside from availability and cost) has been the ability to identify all the affected alleles in suspect individuals, and in many prior studies only about 65% of all affected alleles were identified in patients with an appropriate phenotype [7]. Data presented by Prof. Deschampes at this International Congress on Metals and Genetics (2008) suggests that it may now be possible to detect greater than 95% of affected alleles [personal communication]. Continued compilation of results of the mutation analysis will allow better understanding which mutations of *ATP7B* represent function altering mutations or polymorphisms that may not alter protein function. A current database is being kept by Dr. Diane Cox from the University of Alberta (www.medicalgenetics.med.ualberta.ca/wilson/index.php), and future structure and function studies as well as testing of mutants for their ability to support copper transport in vitro models will yield additional useful information regarding the presence or absence of functional alteration for particular mutations (Table 2).

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Table 1
Summary of new developments for Wilson disease.

	References
Screening for Wilson disease	
Wilson disease scoring system	[3]
Identification of Wilson disease in patients with acute liver failure	[13]
Molecular screening	[7]
Newborn screening	[8–11]
Treatment of Wilson disease	
AASLD guidelines update	[6]
Tetrathiomolybdate	[12]
Experimental models for future treatment	
Hepatocyte cell transplant	[19–23]
Gene therapy	[24–27]

We currently search for the diagnosis of Wilson disease in family members of affected individuals and in those with unexplained liver or neuropsychiatric disease. However there are patients that develop advanced disease with neurological disabilities or chronic liver injury because the disease was not recognized early on, and others that present with acute liver failure. The ability to diagnose Wilson disease at birth is now possible by molecular genetic techniques; however this is not cost effective or well accepted as yet for population screening. Screening tests for disorders need to be cost effective and should be done for diseases like Wilson disease where establishing the diagnosis and starting therapy before the development of symptoms prevents disease progression. In an effort to preemptively diagnose Wilson disease, screening methods that may be applied for population screening have been devised that allow high throughput and low cost. One of these involves using an immunoassay searching for urinary ceruloplasmin and the other detection of ceruloplasmin in protein eluted from stored blood spots obtained from infants. In Japan in Hokkaido Prefecture, there is a mandatory examination and blood testing at age 3 years old. Taking advantage of the ability to obtain samples from these children at an age which is typically younger than that which patients with Wilson disease would develop clinical signs or symptoms, Nakayama et al. screened urines from 11,362 individuals for holoceruloplasmin [8,9]. Two individuals were identified as patients by this method, and physical examinations of these individuals confirmed their presymptomatic state while genetic testing for *ATP7B* mutations confirmed the diagnosis in both. In Korea, investigators screened 3667 patients where dried blood spots were available from newborns and identified one patient at the presymptomatic state [10]. This same method is being tested in the United States for screening by the group at the Mayo Clinic [11]. Whether either of these methods is adopted for newborn screening, the use of newborn screening for Wilson disease has clearly come of age. Identification of patients at this young age raises the issue of the best therapy and timing of therapy for these individuals. It must also be recognized that population screening will not identify all affected individuals, and therefore our current criteria for disease screening must continue.

Table 2
Comparison of current and future therapies for Wilson disease.

Mode of therapy	Specific treatment	Comments
Medical therapy	Chelating agents: trientine and D-penicillamine zinc salts	Requires lifelong administration; reversal of significant copper induced injury can occur over time
Liver transplantation	Whole liver, living donor, auxiliary or heterotopic liver transplant	Normal phenotype with respect to copper metabolism; requires lifelong immunosuppression and potential complications related to immunosuppressive use; may use liver from heterozygote for Wilson disease
Cell transplant	Hepatocytes from unaffected hosts	Requires lifelong immunosuppression, may require cells from more than one donor; may need to be repeated if cell survival not adequate or if population does not expand; need safe techniques for selective expansion of the population of donor cells
Gene therapy	Transfection of hepatocytes cells with *ATP7B* gene	Transfect of all hepatocytes should not be necessary, but a high transfection rate is desirable; safety concerns about integration of the virus into genome; theoretical potential to develop antibody to viral or transfected protein; transfection will need to be repeated if cell number transfected inadequate or if expression is transient

Once the diagnosis of Wilson disease is established, lifelong therapy with a copper chelator or zinc salts is necessary. The chelating agents trientine and penicillamine remain mainstays of initial and maintenance therapy, while zinc salts are more often used alone for maintenance therapy [6]. Tetrathiomolybdate, another avid copper chelator, is still investigational in the United States, and it may have a role in the future treatment of patients presenting with neurological Wilson disease [12]. This study by Brewer et al. that reviewed the use of tetrathiomolybdate did reveal an important finding that the period over which neurological improvement may occur with treatment extended over the four year period of time the study was conducted, contrary to earlier thinking that any clinical improvement would be completed during the first year of treatment.

Recognizing the presence of Wilson disease in the setting of acute liver failure is important in order to prepare the patient for transplant as early as possible and to initiate family screening, but also because specific treatment for lowering copper can be initiated. While many tests for copper used to help diagnose Wilson disease are not usually available on site, standard biochemical parameters can be used to help establish the diagnosis in a timely fashion. Wilson disease should be suspected when patients with acute liver failure have an alkaline phosphatase to bilirubin ratio of <4 (sensitivity 94%, specificity 96%) and an aspartate aminotransferase to alanine aminotransferase ratio of greater than 2.2 (sensitivity 94%, specificity 86%) [13]. Combining these two biochemical ratios, the diagnosis can be established with near certainty (sensitivity and specificity of 100%). Evidence of hemolytic anemia and a serum copper >200 mcg/dl (~ 31 micromoles/L) also suggests Wilson disease as the diagnosis.

Transplantation remains the treatment of choice for patients with acute liver failure due to Wilson disease as well as for treatment of patients with chronic liver failure unresponsive to medical therapy [2]. For those with acute liver failure, treatments such as plasmapheresis and hemofiltration or with other devices such as MARS or SEPAD that provides albumin dialysis exchange may help bridge the patients to transplantation [14,15]. These treatments rapidly lower the circulating copper that is present in marked excess during the acute liver injury. This may help reduce hemolysis and other second organ damage due to copper and copper complexes in the kidneys. With respect to other indications for liver transplant for Wilson disease, care must be taken when transplantation is used for treatment of patients with neurological disease as the primary indication since there may be less certain outcomes with respect to neurological recovery, and possibly less overall survival of the patient and graft [16]. Furthermore many of these patients may be managed medically with chelation therapy, raising the issue of appropriate organ allocation.

Given the shortage of organs for transplantation as well as cultural differences in organ donation, living donor liver transplant

represents a life saving treatment. The use of donor organs from parents of patients that are obligate heterozygotes for Wilson disease has proven safe and effective in maintaining normal copper balance in the recipient [17]. This is important as in some populations where cadaveric transplantation is not possible, and for pediatric patients where parents of affected individuals remain the most likely donors. With respect to transplantation for neurological Wilson disease, the ability to perform living donor liver transplant instead of utilizing a cadaveric organ from the donor pool permits more individualized decisions about whether or not to proceed for these patients. However not all patients have suitable donors, and performing a donor operation when medical alternatives are available raises other ethical concerns.

Whole organ liver transplantation has been shown to be effective in curing Wilson disease and in effect provides a very gross form of genetic therapy in changing the recipient phenotype [18]. However whether isolated hepatocyte transplantation can be applied in order to treat Wilson disease in man remains to be proved. The possibility of transplanting normal donor cells into a patients liver where they will integrate into the hepatic sinusoid and excrete circulating copper into bile more effectively than native cells has been demonstrated in animal models of Wilson disease, the LEC rat and toxic milk mice with *ATP7B* mutations [19–23]. These proof of principle experiments have also exposed the current Achilles heel of this therapeutic modality in its present form, namely the need to provide a stimulus for newly engrafted cells to repopulate the liver and toxic treatment to reduce the regrowth of the native cells. The current stimulus most frequently used in animal models is partial hepatectomy, and many of these studies have used alkaloid compounds to achieve suppression of regrowth of native cells in response to this stimulus. In an attempt to avoid this problem, LEC rats were transplanted at a very young age [21]. While encouraging in that some achieved growth of the transplanted cells to a level effective in ameliorating copper toxicity, engraftment and expansion to effective levels were not universally achieved in all study animals. Importantly, translating this to human studies, we know that most patients are not identified at a stage where this treatment would be effective. Another important drawback is that while these animal studies utilized congeneic cells, the use of allogeneic hepatocytes as would be necessary for adult human studies would necessitate lifelong immunosuppression to prevent rejection of the donor cells. Another critical limitation has been a steady and reliable source of human hepatocytes. Current research focuses on the potential of fetal cells and other in vitro methods to expand liver cell populations in hopes of alleviating this problem.

Proof of principal experiments for gene therapy for Wilson disease have utilized these same animal models, the LEC rat and the toxic milk mouse. These animal models are excellent for identifying the reversal of liver disease, but do not represent useful models for studying neurological disease due to differences in copper transport by the blood brain barrier in rodents and man. Testing of *ATP7B* gene delivery to the hepatocytes was performed by adenoviral and by lentiviral vectors [24–27]. The difficulties encountered in these studies were the transient expression of the transgene (measured in days) and the low transfection frequency. From the above experiments in cell transplantation we learned that not all hepatocytes need to have metabolic correction, and by extrapolation only 30–50% would need to be transfected with *ATP7B* to achieve adequate levels of biliary copper excretion. We can speculate that a smaller percent of cells may need to be transfected if a higher copy number of the expressed gene were achieved in the cells and this translated into increased capacity for biliary copper excretion. However whether or not there any ill effects of over expression of ATP7B protein on cell function is unknown. These studies were successful in demonstrating that the transfected genes were translated into full length ATP7B protein that was functional. Demonstration of functionality was accomplished by looking for production of the copper containing form of ceruloplasmin, holoceruloplasmin, in the circulation of treated animals. This was accomplished by enzymatic assay as well as electrophoretic separation of the holo and apo forms of ceruloplasmin in serum samples. Furthermore, the appearance of an increased amount of copper in the bile of treated animals also showed that the critical part of the pathway for copper excretion was augmented by the cells expressing the normal protein. Future experiments will aim to increase the number of cells that are transfected as well increasing the time of expression of the transfected gene. Gene therapy for Wilson disease should someday achieve the goal of metabolic correction and elimination of the need for lifelong medical therapy for this disorder.

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References

[1] A. Ala, A.P. Walker, K. Ashkan, J.S. Dooley, M.L. Schilsky, Wilson's disease, Lancet 369 (2007) 397–408.
[2] A. Ala, J. Borjigin, A. Rochwarger, M. Schilsky, Wilson disease in septuagenarian siblings: raising the bar for diagnosis, Hepatology 41 (2005) 668–670.
[3] P. Ferenci, K. Caca, G. Loudianos, G. Mieli-Vergani, S. Tanner, I. Sternlieb, M. Schilsky, D. Cox, F. Berr, Diagnosis and phenotypic classification of Wilson disease, Liver Int. 23 (2003) 139–142.
[4] T. Müller, S. Koppikar, R.M. Taylor, F. Carragher, B. Schlenck, P. Heinz-Erian, F. Kronenberg, P. Ferenci, S. Tanner, U. Siebert, R. Staudinger, G. Mieli-Vergani, A. Dhawan, Re-evaluation of the penicillamine challenge test in the diagnosis of Wilson's disease in children, J. Hepatol. 47 (2007) 270–276.
[5] A. Xuan, I. Bookman, D.W. Cox, J. Heathcote, Three atypical cases of Wilson disease: assessment of the Leipzig scoring system in making a diagnosis, J. Hepatol. 47 (2007) 428–433.
[6] E.A. Roberts, M.L. Schilsky, Diagnosis and treatment of Wilson disease: an update, Hepatology 47 (2008) 2089–2111.
[7] L.P. Davies, G. Macintyre, D.W. Cox, New mutations in the Wilson disease gene, *ATP7B*: implications for molecular testing, Genet. Test. 12 (2008) 139–145.
[8] M. Owada, K. Suzuki, M. Fukushi, K. Yamauchi, T. Kitagawa, Mass screening for Wilson's disease by measuring urinary holoceruloplasmin, J. Pediatr. 140 (2002) 614–646.
[9] K. Nakayama, M. Kubota, Y. Katoh, Y. Sawada, A. Saito, K. Nishimura, E. Katsura, N. Ichihara, T. Suzuki, H. Kouguchi, M. Tamura, H. Honma, S. Kanzaki, H. Itami, A. Ohtake, K. Kobayashi, T. Ariga, K. Fujieda, N. Shimizu, T. Aoki, Early and presymptomatic detection of Wilson's disease at the mandatory 3-year-old medical health care examination in Hokkaido Prefecture with the use of a novel automated urinary ceruloplasmin assay, Mol. Genet. Metab. 94 (2008) 363–367.
[10] S.H. Hahn, S.Y. Lee, Y.J. Jang, S.N. Kim, H.C. Shin, S.Y. Park, H.S. Han, E.S. Yu, H.W. Yoo, J.S. Lee, C.S. Chung, S.Y. Lee, D.H. Lee, Pilot study of mass screening for Wilson's disease in Korea, Mol. Genet. Metab. 76 (2002) 133–136.
[11] C.A. Kroll, M.J. Ferber, B.D. Dawson, R.M. Jacobson, K.A. Mensink, F. Lorey, J. Sherwin, G. Cunningham, P. Rinaldo, D. Matern, S.H. Hahn, Retrospective determination of ceruloplasmin in newborn screening blood spots of patients with Wilson disease, Mol. Genet. Metab. 89 (2006) 134–138.
[12] G.J. Brewer, F. Askari, M.T. Lorincz, M. Carlson, M. Schilsky, K.J. Kluin, P. Hedera, P. Moretti, J.K. Fink, R. Tankanow, R.B. Dick, J. Sitterly, Treatment of Wilson disease with ammonium tetrathiomolybdate: IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease, Arch. Neurol. 63 (2006) 521–527.
[13] J.D. Korman, I. Volenberg, J. Balko, J. Webster, F.V. Schiodt, R.H. Squires Jr., R.J. Fontana, W.M. Lee, M.L. Schilsky, Pediatric and Adult Acute Liver Failure Study Groups, Screening for Wilson disease in acute liver failure: a comparison of currently available diagnostic tests, Hepatology 48 (2008) 1167–1174.
[14] S. Sen, M. Felldin, C. Steiner, B. Larsson, G.T. Gillett, M. Olausson, R. Williams, R. Jalan, Albumin dialysis and molecular adsorbents recirculating system (MARS) for acute Wilson's disease, Liver Transplant. 8 (2002) 962–967.
[15] K.L. Collins, E.A. Roberts, K. Adeli, D. Bohn, E.A. Harvey, Single pass albumin dialysis (SPAD) in fulminant Wilsonian liver failure: a case report, Pediatr. Nephrol. 23 (2008) 1013–1016.
[16] V. Medici, V.G. Mirante, L.R. Fassati, M. Pompili, D. Forti, M. Del Gaudio, C.P. Trevisan, U. Cillo, G.C. Sturniolo, S. Fagiuoli, Liver transplantation for Wilson's disease: the burden of neurological and psychiatric disorders, Liver Transplant. 11 (2005) 1056–1063.

[17] S. Tamura, Y. Sugawara, Y. Kishi, N. Akamatsu, J. Kaneko, M. Makuuchi, Living-related liver transplantation for Wilson's disease, Clin. Transplant. 19 (2005) 483–486.
[18] C.G. Groth, R.S. Dubois, J. Corman, A. Gustafsson, S. Iwatsuki, D.O. Rodgerson, C.G. Halgrimson, T.E. Starzl, Metabolic effects of hepatic replacement in Wilson's disease, Transplant. Proc. 5 (1973) 829–833.
[19] Y. Yoshida, Y. Tokusashi, G.H. Lee, K. Ogawa, Intrahepatic transplantation of normal hepatocytes prevents Wilson's disease in Long–Evans cinnamon rats, Gastroenterology 111 (1996) 1654–1660.
[20] A.N. Irani, H. Malhi, S. Slehria, G.R. Gorla, I. Volenberg, M.L. Schilsky, S. Gupta, Correction of liver disease following transplantation of normal rat hepatocytes into Long–Evans Cinnamon rats modeling Wilson's disease, Mol. Ther. 3 (2001) 302–309.
[21] H. Malhi, A.N. Irani, I. Volenberg, M.L. Schilsky, S. Gupta, Early cell transplantation in LEC rats modeling Wilson's disease eliminates hepatic copper with reversal of liver disease, Gastroenterology 122 (2002) 438–447.
[22] S.M. Park, K. Vo, M. Lallier, A.S. Cloutier, P. Brochu, F. Alvarez, S.R. Martin, Hepatocyte transplantation in the Long Evans Cinnamon rat model of Wilson's disease, Cell Transplant. 15 (2006) 13–22.
[23] H. Malhi, B. Joseph, M.L. Schilsky, S. Gupta, Development of cell therapy strategies to overcome copper toxicity in the LEC rat model of Wilson disease, Regen. Med. 3 (2008) 165–173.
[24] Y. Meng, I. Miyoshi, M. Hirabayashi, M. Su, Y. Mototani, T. Okamura, K. Terada, M. Ueda, K. Enomoto, T. Sugiyama, N. Kasai, Restoration of copper metabolism and rescue of hepatic abnormalities in LEC rats, an animal model of Wilson disease, by expression of human *ATP7B* gene, Biochim. Biophys. Acta 1690 (2004) 208–219.
[25] D. Ha-Hao, U. Merle, C. Hofmann, H. Wesch, J. Doll, G. Auburger, S. Tuma, M. Strauss, W. Stremmel, Chances and shortcomings of adenovirus-mediated *ATP7B* gene transfer in Wilson disease: proof of principle demonstrated in a pilot study with LEC rats, Z. Gastroenterol. 40 (2002) 209–216.
[26] U. Merle, J. Encke, S. Tuma, M. Volkmann, L. Naldini, W. Stremmel, Lentiviral gene transfer ameliorates disease progression in Long–Evans cinnamon rats: an animal model for Wilson disease, Scand. J. Gastroenterol. 41 (2006) 974–982.
[27] U. Merle, W. Stremmel, J. Encke, Perspectives for gene therapy of Wilson disease, Curr. Gene Ther. 7 (2007) 217–220.