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MINI REVIEW

Wilson disease: What is still unclear in pediatric patients?

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Summary Since Wilson disease (WD) may not be present with evident clinical symptoms of liver injury and neurological presentation is rare in children, establishing a diagnosis is often challenging, especially in childhood. Increased transaminases can be the only abnormality found in early course of WD. In clinical practice, high suspicion is crucial for early diagnosis and timely treatment to ensure better outcomes. Conventional diagnostic criteria established for adults are commonly agreed for children but may not always be appropriate in very young age. Currently, the best therapeutic approach for each specific presentation of the disease remains controversial and there are no clear indications about how to treat pediatric WD patients with a mild liver disease.

Introduction

Wilson's disease (WD) is an autosomal-recessive human copper (Cu) storage disorder caused by mutations in the *ATP7B* gene [1]. Clinical presentation can vary widely, but the key features of WD are liver disease and neuropsychiatric disturbances [2,3]. Diagnosis of WD remains a challenging patchwork involving clinical, laboratory, histological and molecular tools [4]. The therapeutic success using oral copper chelating agents and zinc therapy makes WD one of the few treatable metabolic liver diseases.

Genetics and pathogenesis

The *ATP7B* gene is large and encodes copper-translocating ATPase expressed primarily in the liver. ATP7B resides in the trans-Golgi membrane compartment and mainly loads Cu on newly synthesized apoceruloplasmin [5]. The exact mechanism of copper hepatotoxicity and brain injury remains unclear. It is suggested that copper metabolism disturbance

Abbreviations: WD, Wilson disease; Cu, copper; KF, Kayser-Fleischer; ALF, acute liver failure; CuB, basal urinary copper; PCT, urine copper after penicillamine challange (CuPCT); AP, alkaline phosphatase; TB, total bilirubin; AST, aspartate transaminase; ALT, alanine transaminase; REC, relative exchangeable copper.

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in WD is associated with significant changes in systemic antioxidant capacity parameters in a direction favoring enhanced oxidative stress [6].

To date, over 520 mutations in *ATP7B* gene have been reported in the Human Genome Mutation Database [7].

The sensitivity of molecular genetic testing for WD was initially reported as 80% [8], but subsequent studies using more sensitive DNA sequencing methods have raised the sensitivity to greater than 98% [9—11]. Furthermore, cases with a confirmed clinical and biochemical diagnosis of WD in whom two ATP7B mutations could not be identified have been reported [9]. The possibility of a second WD gene has been discussed, but causative mutations in other genes involved in copper homeostasis have not yet been identified [3].

The real prevalence of WD is still debated. The widely cited prevalence figure of 1:30,000 with a carrier frequency of 1:90 pre-dates the discovery of *ATP7B* as the disease-causing gene defect and has been questioned. Mass screening for WD in East Asian populations, based on ceruloplasmin, suggested a significantly higher frequency, ranging from 1:500 to 1:3000 [11]. Regional clustering of mutations has been very well established. The highest incidence of WD reported within a single population is in a mountainous area of Crete, in which six out of 90 births were diagnosed as WD patients [12]. Even if updated Italian epidemiological data are missing, WD incidence in the Sardinian population (1:2707 live births) remains one of the highest in the world and six mutations account for 85% of all WD cases [12].

Genotype-phenotype correlation in WD remains a hotly debated topic [9]. Patients with common H1069Q mutation tend to present WD later and with neurological disease [13]; and the fulminant hepatic failure is more likely in patients with truncating mutations [14]. Still, it is clear that not only the type of mutation determines the phenotype. Indeed an additional role of unknown modifier genes and environmental factors has been hypothesized. These include polymorphisms in genes: apolipoprotein E, prion protein, methylenetetrahydrofolate reductase, copper metabolism gene *Murr1*, antioxidant 1, inhibitor of apoptosis linked to chromosome X, as well as those related to iron metabolism, inflammatory processes, oxidative stress, and even gender.

Clinical features

Although the failure to excrete biliary copper is present from birth, WD symptoms generally do not develop until about three years of age, and rarely become evident before age of five. Unfortunately, symptoms at any age are frequently non-specific. Most of the pediatric WD patients present with liver disease, whereas neuropsychiatric symptoms are more common after the age of 18 years [15].

The hepatic clinical presentation ranges widely from asymptomatic hypertransaminasemia and/or fatty liver at ultrasound until cirrhosis or less commonly acute liver failure (ALF) [1,2]. In childhood, the percentages of WD patients with hepatic, neurologic or neuropsychiatric presentation can vary widely according to expertise of care units and health policy. For example, the percentage of WD children presenting with isolated elevated serum aminotransferases ranges from 14 to 88% [15,16]. In particular, in Italy where aminotransferases serum levels are evaluated in the context of check-up, it is more common that WD patients are identified during the first decade of life, when liver disease is mild [16,17]. There is evidence that copper silently accumulates in the liver during childhood, so that alterations in liver function tests may precede the onset of symptoms for a considerable time. The acute hepatic presentation is characterized by the presence of liver failure and Coombs negative hemolytic anemia. WD is the identified etiology in about 5% of ALF patients worldwide [18].

In childhood, presenting symptoms of WD can be sudden behavioral changes, worsening in school performances, inability to carry out activities that need good hand-eye coordination and modifications in handwriting as the micrographia. In approximately 10—25% of WD patients, a psychiatric disturbance is the initial clinical presentation, even before the appearance of any movement disorder [19]. Diagnosis of WD is rarely made during the period in which psychiatric symptoms predominate. Although neuropsychiatric symptoms are considered secondary to liver damage, neurologic and psychiatric manifestations without hepatic involvement have been described also in children [19].

Ophthalmic findings include Kayser-Fleischer (KF) rings and sunflower cataracts. Both findings are reversible with medical therapy or after liver transplantation. It should be pointed out that KF, although very specific for WD, is rarely described in early pediatric ages and in WD children presenting with clinically asymptomatic hypertransaminasemia [17], being more frequent (up to about 50% of cases) in adolescents and young adults with more severe liver disease and/or with neurologic symptoms [18].

Diagnosis

If WD is not recognized and adequately treated, the progression of hepatic and neurologic damage can be very rapid and ALF can occur. Therefore, the prompt detection of this condition is vital. Unfortunately, the diagnosis of WD is an especially challenging task [17].

In 2003, Ferenci et al [20] proposed a diagnostic score for WD, including clinical, biochemical, histologic and molecular findings. Table 1 shows scoring system clarifying for each item the diagnostic validated cutoff in pediatric population. Recently, it has been confirmed that WD scoring system may be a reliable tool also in children with a mild liver disease [17].

The first step in WD investigation is the measurement of ceruloplasmin serum level, which is reduced because of its impaired biosynthesis [5]. Ceruloplasmin is an acute-phase reactant, so in presence of histologically active hepatitis, it may therefore be falsely normal. Up to 20% of pediatric and adult WD patients show normal ceruloplasmin level [1,2]. On the contrary, low levels of ceruloplasmin are not always indicative of a copper storage disorder because both heterozygotes for WD and patients with other disorder may share this feature [9]. In particular, ceruloplasmin deficiency has been observed in decompensated liver failure and in other pathological conditions, including congenital disorder of glycosylation. It has been recently demonstrated that also in children the best WD diagnostic threshold of ceruloplasmin is 20 mg/dL [17].

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Table 1 Diagnostic score for Wilson disease.

Laboratory tests		Clinical symptoms and signs	
Serum ceruloplasmin		*Kayser-Fleisher rings*	
Normal (> 20 mg/dL)	0	Present	1
10—20 mg/dl	1	Absent	0
< 10 mg/dl	2		
Urinary copper (in absence of acute hepatitis)		*Neurologic involvement*	
Normal (< 40 μg/24 h)	0	Severe	2
1—2 × ULN* (40—80 μg/24 h)	1	Mild	1
>2 × ULN* (> 80 μg/24 h)	2	Absent	0
Normal, but > 5 × ULN*	2		
(> 200 μg/24 h after penicillamine challenge)			
Liver copper (in absence of cholestasis)		*Coombs negative hemolytic anemia*	
Normal (< 50 μg/g dry weight)	−1	Present	1
< 5 × ULN* (50—250 μg/g dry weight)	1	Absent	0
> 5 × ULN* (> 250 μg/g dry weight)	2		
Rhodanine stain		*Mutation analysis*	
Absent	0	2 chromosomes mutations	4
Present	1	1 chromosome mutation	1
		No mutations detected	0

Modified from Ferenci et al. [20].
Assessment of the WD-diagnostic score: 4 or more = affected by WD; 2—3 = WD likely, do more investigations; 0—1 = WD unlikely.
* ULN: upper limit of normal.

Serum non-ceruloplasmin bound copper (improperly named serum free copper), has been proposed as a diagnostic test [2] but false negatives values are often encountered. Recently, a more rapid and reliable biological method, known as relative exchangeable copper (REC), has been proposed. Newly diagnosed WD patients had REC levels significantly higher than controls (0.62—1.15 $\mu mol.L^{-1}$), giving 100% specificity and sensitivity in this small group [21]. Further investigations are needed to include this test into routine diagnostic approach.

Basal urinary copper (CuB) is another test useful for recognizing WD. The level taken as diagnostic of WD in symptomatic patients is commonly > 100 μg/24 hours [2]. Anyway, in several pediatric series up to 19% of WD patients showed urine copper values below the mentioned cutoff. CuB seems to be directly correlated with the age at diagnosis of WD, suggesting an accumulation of the metal over time [9]. It has been recently suggested that diagnosis of WD in children should be considered when this test produces the value >40 μg/24/h [17].

Urine copper excretion measurement after a penicillamine challange (CuPCT) is considered a diagnostic tool for WD, and it has been commonly considered diagnostic when >1600 μg/24 h [2]. As suggested by our recent study, CuPCT should not be performed in children without symptomatic liver disease, because only patients with severe liver damage due to WD had a positive CuPCT [17].

For diagnostic purposes, liver biopsy is only required if the clinical signs and non-invasive tests do not provide a final diagnosis or if there is suspect of additional liver pathologies [2,3]. Hepatic copper accumulation is the hallmark of WD. Qualitative measurements of hepatic copper content, such as stains for copper or copper-associated proteins (e.g. orcein, rhodanine, rubeanic acid stain), are useful but unreliable tools for diagnosis or exclusion of WD. Liver copper content greater than 250 μg/g dry weight is considered diagnostic for WD [2]. It is well known that in long standing cholestatic disorders, hepatic copper content may also be increased above this level. Values < 40—50 μg/g dry weight exclude diagnosis of WD [2]. Hepatic copper threshold value has been criticized as being too high. The problem to apply a lower threshold is linked mainly to the fact that the concentration of hepatic copper in heterozygotes is frequently higher than normal [2,17].

Establishing a diagnosis of fulminant WD can be difficult because KF rings may not be present and parameters of copper metabolism are neither specific nor diagnostic. Some series supported that values of less than 2.0 for the alkaline phosphatase (AP)-total bilirubin (TB) ratio and greater than 4.0 for the aspartate (AST)-alanine transaminase (ALT) ratio provide a good sensitivity and specificity in identifying fulminant hepatic failure caused by WD from other etiologies [22]. However, a pediatric study showed that differentiation from other causes of fulminant liver failure in children on the basis of these biochemical parameters was not sufficient [18]. In particular, in children, AP levels are higher as they originate also from the bone component.

From a genetic point of view, the diagnosis of WD is based on the identification of two disease causing mutations. It should be performed for individuals in whom the diagnosis is difficult to establish by clinical and biochemical testing or to screen asymptomatic sibling of patient with WD [2]. A further indication is in case of WD-related fulminant hepatitis, when the conventional diagnostic tools are unreliable. Detection of mutations on both chromosomes allows a definitive diagnosis of WD, whereas the diagnosis cannot be excluded because of their absence.

Sibling screening is mandatory when a new case of WD is diagnosed. This should include clinical examination, liver function tests, biochemical tests of copper metabolism and

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Table 2 Drugs available for treatment of children with Wilson disease ([0,1]2).

Drugs	Dosage	Adequacy of treatment parameters
Penicillamine	20 mg/kg/die divided in 2—3 divided dosages; in young adults 1000 mg/die (maximum 1500 mg/die) in 2—4 divided dosages Maintenance dose 10—20 mg/kg/die up to 750—1000 mg/day in 2 divided dosages 1 h before or 2—3 hours after meals Supplemental pyridoxine should be provided (25—50 mg/day) Reduce dose during pregnancy Reduce dose for surgery to promote wound-healing	Urinary copper: around 1000 μg (16 μmol) after starting treatment Urinary copper: 200—500 μg/24 h (3—8 μmol/24 h) on maintenance treatment
Trientine	20 mg/kg/die divided in 2—3 doses Maintenance dose 900—1500 mg/day 1 h before or 2—3 h after meals Reduce dose during pregnancy Reduce dose for surgery to promote wound-healing	Urinary copper: 200—500 μg/24 h (3—8 μmol/24 h) on maintenance treatment
Zinc salts	*Elemental zinc (acetate)* Age < 6 yrs: 25 mg twice daily 6—16 yrs with weight < 50 kg: 25 mg three times daily Age > 16 yrs or weight > 50 kg: 50 mg three times daily *Zinc sulphate* Age < 6 yrs: 100 mg twice daily 6—16 yrs with weight < 50 kg: 100 mg three times daily Age > 16 yrs or weight > 50 kg: 200 mg three times daily: 1 h before or 2—3 h after meals and no dosage reduction for surgery and pregnancy	Urinary copper: < 75 μg/24 h (1.2 μmol) on maintenance treatment Urinary zinc: > 2 mg/24 h on maintenance treatment Serum zinc: > 1250 μg/L on maintenance treatment

mutational analysis if the proband's mutations are known. If mutational analysis is not available, biochemical testing should be delayed until after six months of age, because ceruloplasmin levels are low in the newborn. In definitive, despite the availability of multiple tools, diagnosis of WD is very challenging in childhood. The first essential in making the diagnosis is to think of it and an high suspicion index is required.

Treatment

The overall therapeutic aim for WD is the generation of a negative copper balance. Today this can be achieved either by liver transplantation, which phenotypically corrects the gene defect in the liver, or by medical therapy. Obviously liver transplantation is a treatment option for patients with severe life-threatening conditions in whom the window for medical treatment is not wide enough. It cannot be proposed as a therapeutic strategy, given the high rate of complications and the need for immunosuppressive therapy for life. For all other WD patients, lifelong medical therapy is indicated. Conventional medical therapies for children comprise treatment with either copper chelators (penicillamine or trientine) and zinc (Table 2) [2,3]. Chelators mobilize intracellular copper into the circulation and enhance urinary excretion of copper, while zinc acts inducing copper-binding metallothione in both in enterocytes, reducing metal intestinal absorption into portal circulation, and in hepatocytes, reducing the damaging effects of free liver copper.

Today, it has been recently supported that, regardless of the drug used, appropriately treated patients with WD have an excellent long-term prognosis, with a survival probability not differing from the general age and sex-matched population [23]. It has been described that more than 30% of WD patients adequately treated since childhood with the available drugs do not exhibit complete normalization of liver enzymes but have a good quality of life and a favourable outcome [16]. On the other hand, regardless of type of treatment, it has been described that a poor compliance or discontinuation of medical therapy is associated with high risk of hepatic decompensation requiring even liver transplantation [2,3].

American and European guidelines [2,3] provide an useful therapeutic support even if many points remain unclarified for pediatric patients. There is a lack of high quality evidence to compare the relative treatment effects of the available drugs in children.

For pre-symptomatic patients, who include the subjects identified following family screening before the onset of symptoms, the recommended approach is therapy with zinc, considered its proven efficacy and safety profile [2].

Initial treatment for patients with significant chronic liver disease (portal hypertension, cirrhosis etc.) should include always a chelating agent. Penicillamine and trientine have many beneficial effects but also multiple potential toxicities, which may require discontinuation in up to 30% of cases. In particular in patients with neurological symptoms chelators should be introduced gradually over time to avoid rapid deterioration of neurological function. In contrast to

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copper chelators, zinc has a lower toxicity and rarely leads to worsening in neurological symptoms. Its efficacy is contested especially in adult patients with liver disease, when applied for very long periods [3]. Pediatric experience is totally different from that concerning adult patients. In particular, in patients with mild liver disease, diagnosed in childhood, zinc monotherapy seems to be effective in controlling WD related liver disease both as first-line and as maintenance treatment, with a low rate of adverse events. The following side effects of zinc therapy were reported: nausea, vomiting and epigastric pain, mild gastric irritation, decreased blood iron levels and anemia. Still, in our experience [24] zinc sulfate may also cause gastric/duodenal mucosal ulceration or erosion.

In patient presenting with neurologic or psychiatric signs, zinc may have a role as a first line therapy as it is not associated with neurological deterioration [2,3]. As maintenance therapy after the induction phase with chelators, the patients with a good control of the disease may be treated with lower doses of chelators or be shifted to zinc [2,3].

The adherence to a life-long therapeutic regimen may be poor, mainly in the teenage age. Therefore, the patients should be checked periodically to supervise the occurrence of side effects and to assess the adherence to the prescribed regimens.

In conclusion, the available medical treatment is effective in most cases to treat WD patients but not to cure WD gene defect. Therefore, there is an increasing demand for novel treatment strategies in addition to conventional therapy. A desirable goal might be the identification of new molecular targets suitable for correction of *ATP7B* mutants, towards WD cure. This goal becomes every day more and more feasible in the light of new discoveries in cell biology. In particular, novel strategies in WD directly aim at enhancing the protein expression of mutant *ATP7B* with residual copper export activity. It remains to be established to what extent *ATP7B* expression and function need to be restored to relieve toxic copper accumulation, and the experience with alpha1-antitrypsin deficiency suggests that effects seen in cell culture may not be mirrored in patients.

Disclosure of interest

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References

[1] Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. Lancet 2007;369:397—408.
[2] 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.
[3] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Wilson's disease. J Hepatol 2012;56:671—85.
[4] Vajro P, Nicastro E. Wilson's disease. In: Arrojo V, Jara P, editors. International hepatology updates. Liver disease in children. 1st ed. Barcelona: Permanyer Publications; 2009.
[5] de Bie P, Muller P, Wijmenga C, Klomp LW. Molecular pathogenesis of WD and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. J Med Genet 2007;44:673—88.
[6] Bruha R, Vitek L, Maracek Z, Pospisllova L, Nevsimalova S, Martasek P. Decreased serum antioxidant capacity in patients with Wilson disease is associated with neurological symptoms. J Inherit Metab Dis 2012;35:541—8.
[7] The Human Gene Mutation Database. http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ATP7B
[8] Schmidt HH. Role of genotyping in Wilson's disease. J Hepatol 2009;50:449—52.
[9] Nicastro E, Loudianos G, Zancan L, D'Antiga L, Maggiore G, Marcellini M, et al. Genotype-phenotype correlation in Italian children with Wilson's disease. J Hepatol 2009;50:555—61.
[10] Coffey AJ, Durkie M, Hague S, McLay K, Emmerson J, Lo C, et al. A genetic study of Wilson's disease in the United Kingdom. Brain 2013;136:1476—87.
[11] Schilsky ML, Ala A. genetic testing for Wilson disease: availability and utility. Curr Gastroenterol Rep 2010;12:57—61.
[12] Loudianos G, Gitlin JD. Wilson's disease. Semin Liver Dis 2000;20:353—64.
[13] Stapelbroek JM, Bollen CW, van Amstel JK, van Erpecum KJ, van Hattum J, van den Berg LH. The H1069Q mutation in ATP7B is associated with late and neurologic presentation in Wilson disease: results of a meta-analysis. J Hepatol 2004;41:758—63.
[14] Merle U, Weiss KH, Eisenbach C, Tuma S, Ferenci P, Stremmel W. Truncating mutations in the Wilson disease gene *ATP7B* are associated with very low serum ceruloplasmin oxidase activity and an early onset of Wilson disease. BMC Gastroenterol 2010;10:2—6.
[15] O'Connor JA, Sokol RJ. Copper metabolism and copper storage disorders. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. Liver diseases in children. New York, NY USA: Cambridge University Press; 2007. p. 626—59.
[16] Iorio R, D'Ambrosi M, Marcellini M, Barbera C, Maggiore G, Zancan L, et al. Serum transaminases in children with Wilson's disease. J Pediatr Gastroenterol Nutr 2004;39:331—6.
[17] Nicastro E, Ranucci G, Vajro P, Vegnente A, Iorio R. Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. Hepatology 2010;52:1948—56.
[18] Dhawan A, Taylor RM, Cheeseman P, De Silva P, Hatsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. Liver Transpl 2005;11(4):441—8.
[19] Zimbrean PC, Schilsky ML. Psychiatric aspects of Wilson disease: a review. Gen Hosp Psychiatry 2014;36:53—62.
[20] Ferenci P, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003;23:139—42.
[21] El Balkhi S, Trocello JM, Poupon J, Chappuis P, Massicot F, Girardot-Tinant N, et al. Relative exchangeable copper: a new highly sensitive and highly specific biomarker for Wilson's disease diagnosis. Clin Chim Acta 2011;412:2254—60.
[22] Korman JD, Volenberg I, Balko J, Webster J, Scholodt FV, Squires RH, et al. Screening for Wilson disease in acute liver failure: a comparison of currently available diagnostic tests. Hepatology 2008;48:1167—74.
[23] Schilsky ML. Long-term outcome for Wilson disease: 85% good. Clin Gastroenterol Hepatol 2013;16:S1542—3565.
[24] Wiernicka A, Jańczyk W, Dądalski M, Avsar Y, Schmidt H, Socha P. Gastrointestinal side effects in children with Wilson's disease treated with zinc sulphate. World J Gastroenterol 2013;19:4356—62.

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