Review on Wilson's Disease

KM CHEUNG, YK CHAN

Abstract
Wilson's disease is an autosomal recessive disorder of copper metabolism. A case was presented and the historical background, genetics, pathogenesis, clinical manifestations, diagnosis and treatment were reviewed.

Key words
Children; Wilson's disease

Case Summary
YML presented with one-year history of clumsiness in hand-writing and difficulty in walking as well as jaundice at age 13. Physical examination revealed complete Kayser-Fleischer ring. Choreiform movement, involuntary hand tremor and slightly unsteady gait were noted. Wilson's disease was diagnosed with liver biopsy in Taiwan. D-penicillamine and benzhexol was started. She tolerated the medications with no allergic side effects and her symptoms gradually improved. Her parents were of consanguineous marriage. She was later found to be homozygous for Arg1319X exon CGA->TGA. Her parents, her elder brother and her two young children were heterozygous carriers (Figure 1). On subsequent follow up in our hospital over twenty years, she remained well despite having mild residual tremor and dysarthria. Kayser-Fleischer rings had resolved. Twenty-four hours urine for creatinine clearance, protein and copper excretion was checked every 6 months. Her creatinine clearance was normal (85-106 mL/min). Twenty-four hour urine protein ranged from 0.04 to 0.1 gm/day. The urine copper ranged from <2 umol/day to 16 umol/day (normal references <1 umol/day). Complete blood picture and liver function tests were monitored every 6 months and the results were all normal (Latest blood test results were: haemoglobin = 13 g/dL, white cell count=8.3 x 10^9/L, platelet count=154 x 10^9/L, total bilirubin=11 mmol/L, alkaline phosphatase=74 iu/L, aspartate aminotransferase=18 iu/L, alanine aminotransferase=13 iu/L, albumin=39 g/L, globulin=32 g/L). Ultrasonogram of liver showed coarse echogenicity consistent with parenchymal disease. No gallstone was noted. CT brain showed symmetrical hypodensities without contrast enhancement in bilateral globus pallidus and atrophy of caudate nuclei with adjacent enlarged frontal horn of lateral ventricles. Throughout these years, she tolerated penicillamine well except mild epigastric pain, which ameliorated after her maintenance dosage was decreased from 1000 mg/day to 500 mg/day in the recent five years. She was also maintained on benzhexol 2 mg and vitamin B complex 1 tablet thrice per day. Fertility was not affected as she had two normal spontaneous deliveries. The penicillamine dosage was cut down to 250 mg bd during her pregnancy. Both children were normal with no dysmorphic features. They were heterozygous for Arg1319X mutation. The thyroid function tests and calcium levels were normal. The patient had not developed renal tubular function impairment. No haemolytic episode was found through out these years. No orthopaedic complication of Wilson's disease was noted.
Historical Background

Wilson's disease (WD) is an autosomal recessive condition with a prevalence of one in 30,000 in most populations, and a corresponding carrier rate of approximately 1 in 90. WD is a disorder of biliary copper excretion and is clinically characterised by Kayser-Fleischer rings, and by hepatic and neurological manifestations related to the accumulation of copper in the liver and the lenticular nuclei. Wilson first described WD as a distinct clinical entity in his M.D. dissertation in 1912. He reported four patients who presented with progressive lenticular degeneration and liver cirrhosis at autopsy. He proposed that they were suffering from the same disease as eight similar cases he had found in the literature. He hypothesised that it was a familial disease of which the abnormalities in the brain were caused by a "toxin" generated within the cirrhotic liver. Kayser described pigmented rings in the cornea in 1902 and Fleischer reported similar observation in 1909. Cummings identified that copper was the "toxin" affecting the brain and the liver in 1945. In 1952, Scheinberg and Gitlin reported deficiency of ceruloplasmin in the serum of affected individuals. Comprehensive family studies by Bearn demonstrated the autosomal recessive nature of this disease. In 1985, Frydman et al established linkage of the WD gene to the esterase D locus on Chromosome 13. The WD gene was cloned in 1993 and shown to encode a novel member of the cation transporting P-type ATPase family. More than 200 mutations have been described in the ATP7B gene on chromosome 13 in patients with WD. The spectrum of mutations and their clinical consequences have been investigated in patients with WD in different ethnic populations. The most common mutation in people of Northern European origin is His1069Gln (40%), whereas Arg778Leu is more common in Oriental patients (30%).

Pathogenesis of Wilson's Disease

The liver is the central organ of copper homeostasis. It has a huge capacity for both storage and excretion of this metal. The control of copper balance is determined by biliary copper excretion. The amount of copper appearing in the bile is directly proportional to the size of the hepatic copper pool. Hepatocytes are the primary site of copper uptake and accumulation in the liver. They sense the copper status in the cytoplasm and regulate copper excretion into the bile depending on the intracellular concentration of this metal. This regulation is accomplished by the protein...
product of the ATP7B gene. It is a putative copper transporting P type ATPase that is presumed to transport copper across cell membranes. It is abundantly expressed in hepatocytes and localized to the trans-Golgi network.\textsuperscript{10-12} When the copper content of the hepatocyte increases, this ATPase moves from the trans-Golgi network to a cytoplasmic vesicular compartment near the canalicular membrane. After accumulation of copper within this vesicular compartment, the accompanying decrease in cytoplasmic copper results in redistribution of the ATPase back to the trans-Golgi network and copper excretion into the bile. This mechanism provides a rapid and responsive system to maintain intracellular copper balance and ensures that excess cytosolic copper is rapidly excreted. Immunofluorescent studies in human liver localize the ATPase near but not on the canalicular membrane, suggesting that additional mechanistic steps are involved in this final stage of copper excretion from the hepatocyte.\textsuperscript{11-12} ATP7B also contains a mitochondrial target sequence, once cleaved, a processed WD protein is localized to mitochondria. Whether it serves any similar function in controlling copper transport in mitochondria is not known.

**Copper is an important component of cytochrome oxidase (complex IV) of the mitochondrial respiratory chain. It may substitute for iron in redox reactions generating free radicals. Copper accumulation might be associated with increased oxidative stress and damage within target tissues where copper concentrations are high. High intra-mitochondrial copper concentrations might increase free-radical generation. Mitochondrial changes including abnormal morphology have been identified in liver from patients with WD.\textsuperscript{13} In addition, there is evidence of a 33-fold increase of mitochondrial copper and of oxidative damage in the liver in these patients.\textsuperscript{14} Gu et al had shown significant decrease in mitochondrial enzyme activities in livers of patients with WD as compared with healthy control.\textsuperscript{15} On the other hand, the mitochondrial enzyme activities of patients with cholestatic liver diseases with high copper accumulation were similar to that of the healthy control. This implies that oxidative damage and mitochondrial dysfunction are important in the pathogenesis of WD.

**Clinical Manifestations**

The presenting features of WD are related to deposition of copper in specific organs, most commonly the liver and central nervous system.

**Hepatic Manifestations**

All patients with WD have hepatic involvement. Most patients present at teenage or young adulthood but the disease can occur as early as three years of age and as late as 50 year of age. Patients who are homozygous for ATP7B gene usually present earlier while patients who are compound heterozygous may present in middle age. Most children had no or nonspecific symptoms. On the other hands, children with WD can also present with acute hepatitis, chronic active hepatitis, cirrhosis, and fulminant hepatic failure. Hepatocellular carcinoma is a rare consequence of WD. In recent series reported by Australian and American hepatologists,\textsuperscript{16,17} about one fourth patients had fulminant hepatitis. It is a devastation presentation. Adolescents and occasionally adults develop icteric hepatitis, which rapidly evolves over few days to weeks to hepatic insufficiency with extreme jaundice (because of accompanying haemolysis), hepatic coma and death if liver transplantation is not performed. Even when correctly and quickly diagnosed these patients virtually never recover without liver transplantation. Therefore, prompt referral of these patients to liver transplantation center is necessary to save the patient's life. In the absence of encephalopathy, WD patients presented with severe hepatic insufficiency and coagulopathy might survive without liver transplantation if D-penicillamine was started early.\textsuperscript{18}

**CNS Manifestation**

Neurological or psychiatric disease typically occurs in the second to third decades of life but they may present as early as six year of age. Motor system involvement predominates, with the sensory system virtually spared. Dystonia, incoordination, tremor, and difficulty in fine task are common symptoms, while rigidity, drooling, dysarthria, mask-like faces and gait disturbances are late features. Behaviour problems include compulsivity, aggressiveness, impulsiveness, depression, phobias and declining academic performance. Intellect is unchanged, however. Neurological dysfunction is slow in progression but may be irreversible even with copper chelation therapy.\textsuperscript{17} Neurological features mirror the underlying pathologic changes of cavitary degeneration in the basal ganglia with extensive gliosis and neuronal loss in association with a marked increase of the copper content in this region of the brain. These underlying structural changes and the copper deposition can be detected by magnetic resonance imaging at an early stage in symptomatic patients and may be observed to decrease with chelation therapy.\textsuperscript{19}
Ophthalmological Manifestation

Kayser-Fleischer rings are present in 98% of WD patients with neurological or psychiatric signs and symptoms but in only 50% in those presented with hepatic disease. These rings may rarely be seen in long-standing cholestatic disorders also. Kayser-Fleischer rings consist of copper granules, deposited primarily in the stromal layer, with color change visible only in Descemet’s membrane. Although visualisation with the naked eye is possible, slit-lamp evaluation is mandatory. With appropriate treatment, these rings should fade and disappear. Another less common ophthalmologic finding is sunflower cataract.

Miscellaneous

Accumulation of copper in kidney results in renal tubular defects. Skeletal abnormalities include osteoporosis, osteomalacia, rickets, spontaneous fracture, osteoarthritis, and osteochondritis dissecans have been described though rarely occurred in children. Haemolysis is induced by release of copper from the necrotic hepatocytes and Coomb’s negative haemolytic anaemia may be the initial presentation of WD in children.\textsuperscript{20,21} Cholelithiasis can occur either from haemolysis and/or cirrhosis. Endocrinopathy such as hypothyroidism, hypoparathyroidism and secondary amenorrhoea can also occur.\textsuperscript{22}

Diagnosis

Diagnosis is usually based on the presence of major clinical and laboratory features including typical hepatic and/or neurological symptoms and signs. Kayser-Fleischer rings, low serum ceruloplasmin level and increased urinary copper excretion. A ceruloplasmin level less than 20 mg/dL or 24-hour urine copper excretion greater than 100 ug/day is suggestive of WD. Liver biopsy remains the gold standard, patients with WD typically have values of hepatic copper above 250 ug dry weight. Elevated hepatic copper concentrations as a diagnostic clue is not infallible, however.\textsuperscript{18} Sampling error may occur because of the heterogeneous distribution of copper within the liver,\textsuperscript{23} which could be up to 500-fold difference.\textsuperscript{24} Furthermore, hepatic copper concentrations may be normal in WD patients who present with fulminant hepatic failure because of massive release of copper from necrotic hepatocytes. Therefore, none of the above test is confirmatory because these can occur in other conditions and their absence cannot rule out WD. For example, ceruloplasmin is normal in 10% of WD patients with neurological presentation and 20% patients with hepatic presentation. It is an acute phase protein and its level is elevated during active liver inflammation. The serum level of ceruloplasmin is low in any situation with protein deficiency, e.g. nephrotic syndrome, protein losing enteropathy. Infants less than 6 months of age usually have low serum ceruloplasmin. Twenty percent of people who are heterozygous for WD also had low ceruloplasmin. In patients with aceruloplasminaemia (congenital absence of ceruloplasmin), the copper excretion is normal and they do not have WD. Patients with fulminant hepatic failure of causes other than WD can also have low ceruloplasmin and high urine copper excretion. Moreover, a liver biopsy may not be obtainable because of impaired coagulation.

Gow et al stated that diagnosis of WD will be assisted by the findings of a Coomb’s negative haemolytic anaemia, a high non-ceruloplasmin copper concentration (325-1743 ug/L), and greatly increased urine copper excretion (844-9375 ug/24h).\textsuperscript{17} A mildly elevated alanine transaminase and alkaline phosphatase which contrast sharply with markedly raised transaminases usually found in fulminant hepatic failure due to virus, drugs or toxins also point to the diagnosis of WD.\textsuperscript{17}

Patients present with liver disease alone are another diagnostic challenge because features of Kayser-Fleischer rings and low ceruloplasmin are often absent.\textsuperscript{17,21} This situation is even more common in paediatric group of patients as majority of them have hepatic presentation.\textsuperscript{20} Steindl et al proposed an algorithm for diagnosis of WD with hepatic involvement (Figure 2).\textsuperscript{21} In the absence of typical clinical findings and after exclusion of other liver diseases, measurement of hepatic copper is mandatory in every patient with unexplained elevations of liver enzymes and any abnormal parameters of copper metabolism.\textsuperscript{21}

Figure 2  Family pedigree of the index patient. (Arrow: index patient; double line: cousin marriage; half-shaded: carrier; slanted: death due to unknown; dotted: unknown status).
Furthermore, in patients with ceruloplasmin levels in the normal ranges a radioactive copper test may be useful.\textsuperscript{17,21} All WD homozygote patients will fail to incorporate copper into ceruloplasmin with the hepatocyte.\textsuperscript{25} Steindl suggested that selected patients with borderline and confusing results or in whom a liver biopsy cannot be performed because of impaired coagulation, a test treatment with decoppering agents can be initiated for a period of 6-12 months.\textsuperscript{21} A favourable clinical response and high urinary copper excretion following penicillamine treatment was considered diagnostic of WD.\textsuperscript{21} On the other hand, other authors believed that liver biopsy remains the "gold standard" and a trial of penicillamine will usually not resolve a diagnostic dilemma.\textsuperscript{17,26} For example, in the absence of advanced disease, penicillamine may not induce cupriuria.\textsuperscript{27} In general, no single test is diagnostic of WD. However, the diagnosis can usually be established (or ruled out) with a constellation of appropriate tests, provided that the clinicians maintain a high index of suspicion.

**Treatment**

D-penicillamine is the mainstay of treatment of WD.\textsuperscript{28} It is a sulfur containing metabolite of penicillin. It acts as a copper chelating agent. It should be started as small dose because large amount of released copper from liver will deposit in CNS and may worsen the neurological symptoms. The dosage should be gradually increased to 20 mg/kg body weight per day and should not exceed 1 gm/day. It should be taken 1 hour before meal or 2 hours after because it is better absorbed in the absence of food. Hypersensitivity reaction such as fever, rash, leukopenia, thrombocytopenia and lymphadenopathy may occur within weeks of starting therapy in as many as 20% of patients. Later, nephrotoxicity such as proteinuria, lupus-like syndrome, Goodpasture's syndrome may occur even years later. Dermatologic side effects include pemphigoid of mouth and aphthous stomatitis. Any reaction to penicillamine should prompt discontinuation of the drug and a change to an alternative treatment because no role now exists for desensitisation of this drug. Clinicians must beware that abrupt cessation of therapy can lead to massive copper release and subsequent deposition. Mortality due to sudden discontinuation had been reported. Whether this drug is tetratogenic is controversial. Some reports have indicated that penicillamine may induce birth defects such as cutis laxa.\textsuperscript{29,30} However continuation of penicillamine outweighs the risk as maternal exacerbation of liver disease and fetal liver damage had been reported in untreated patient.\textsuperscript{31} A reduced dosage of 500 mg/day of penicillamine had been reported to be safe and effective during pregnancy.\textsuperscript{32} Penicillamine may affect wound healing and the use of it should be cautious in pregnant ladies who may need Caesarean section. Dosage between 300-600 mg was shown not to affect the wound healing.\textsuperscript{33}

Trientine and zinc can be used as alternative therapy.\textsuperscript{28,34} Trientine is a copper chelator and acts by increasing urinary excretion of copper. It is less potent than penicillamine but had been proven to be an effective substitute in WD when patients had penicillamine intolerance. It has a better safety profile, side effects include bone marrow suppression and sideroblastic anaemia.

Zinc is an antagonist of copper absorption. It induces intestinal cell metallothionein which has a high affinity for copper and prevents the serosal transfer of copper into circulation. The safety and long term efficacy of zinc as maintenance therapy has been well shown in both adults and children.\textsuperscript{35,36} The main drawback of zinc therapy is the slow rate at which copper is depleted. Clinical efficacy of these drugs is summarised in Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>In pregnancy</th>
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<tr>
<td>Penicillamine</td>
<td>Small initial dose and gradually increases to 20 mg/kg/day (&lt;1 g/day)</td>
<td>Fast acting</td>
<td>50% risk of neurological worsening. 25% of patients never recover. High incidence of toxicity.</td>
<td>100% efficacy. Possibly tetratogenic causing complex mesenchymal abnormalities.</td>
</tr>
<tr>
<td>Zinc</td>
<td>25 mg tds 1 hour after meal</td>
<td>Low toxicity</td>
<td>Slow acting. Permit disease progression.</td>
<td>100% efficacy. Low toxicity. Non-tetratogenic.</td>
</tr>
<tr>
<td>Trientine</td>
<td>20 mg/kg/day in divided doses 1 hour before meals</td>
<td>Fast acting</td>
<td>High incidence of adverse effects. Moderate incidence of toxicity.</td>
<td>100% efficacy. Tetratogenic in animals.</td>
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Dietary copper sources should also be restricted. Examples of copper rich food are animal liver, kidney, shellfish, chocolate, broccoli, mushrooms, dried beans, peas and unprocessed wheat. These kinds of food should be excluded from diet.

Orthotopic liver transplant is now an accepted form of therapy with specific indications: fulminant hepatic failure, decompensated cirrhosis with end-stage liver failure, and worsening disease despite intensive therapy, although not indicated for neurological symptom alone. Living related liver transplant, with grafts chosen from heterozygote carrier do not appear to confer any risk of recurrence in the recipients. The survival rate is nearly 100% in developed country. Improvement in neurological symptoms is remarkable and quality of life is good after liver transplantation.

Genetic Counselling

Each full sibling of the index patient with WD is at 25% risk of having the disease. Other relatives have an increased risk up to 200 times that of the general population. Screening of all first degree relative is mandatory. Physical examination including slit lamp examination for Kayser-Fleischer rings, blood for ceruloplasmin, and 24-hour urine for copper excretion should be performed. Any abnormalities from the above investigations should prompt a liver biopsy to establish a diagnosis. In the absence of laboratory abnormalities, haplotype analysis, which is commercially available, is helpful in identifying affected family members of an index patient with WD. Mutation analysis is also recently being used to identify very young patients and carrier of WD. Shimizu et al had reported an eight months old asymptomatic infant with low ceruloplasmin, diagnosis WD was established by demonstration of a mutated ATP7B gene. Since WD gene is considered fully penetrant, once a sibling is diagnosed to have WD, treatment should be commenced as soon as possible after a liver biopsy was obtained. Brewer et al recommended the use of zinc acetate in asymptomatic WD patient as this agent has relatively less side effects than penicillamine.

Parents of the patient should also be screened to identify their carrier status. Prenatal diagnosis is feasible with the combination of linkage analysis and mutational analysis. Close monitor of the presymptomatic baby is indicated to allow early institution of anticopper treatment. A Checklist outlining the management and monitoring of WD is summarised in Table 2.

### Table 2 Checklist for the management of patient with Wilson's disease

<table>
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<th>A. Avoid copper rich food:</th>
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<tr>
<td>- Animal organs: liver, kidney, etc.</td>
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<td>- Seafood: shellfish, shrimp, crab</td>
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<tr>
<td>- Vegetables: broccoli, mushrooms, dried beans, peas.</td>
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<td>- Others: chocolate, unprocessed wheat.</td>
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<th>B. Family screening.</th>
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<th>C. Choices of anticopper drugs:</th>
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<tbody>
<tr>
<td>- Penicillamine.</td>
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<tr>
<td>- Zinc.</td>
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<tr>
<td>- Trientine.</td>
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<th>D. Initial therapy (first 6 months):</th>
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<tr>
<td>- Watch out for the hypersensitivity reaction of penicillamine such as rash, fever, leukopenia, thrombocytopenia, lymphadenopathy, or proteinuria.</td>
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<tr>
<td>- Monitor CBC, R/LFT, AST, urine for multistix, urinalysis, renal tubular function (e.g. urine for reducing substance and amino acids), 24-hour urinary copper excretion every 2-4 weeks.</td>
</tr>
<tr>
<td>- Physical examination every 2-4 weeks for the signs of liver disease, anaemia, renal tubular function, proteinuria, reversal of neurological signs and the disappearance of Kayser-Fleischer rings.</td>
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<th>E. Maintenance therapy (6 months onwards):</th>
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<tr>
<td>- Monitor the compliance and the long term adverse effects of the anticopper treatment, e.g. examine oral mucosa for any ulceration.</td>
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<tr>
<td>- Check 24-hour copper every 2-4 months.</td>
</tr>
<tr>
<td>- Check CBC, R/LFT, AST, 24-hour urine for protein every 2-4 months.</td>
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<th>F. Pregnancy – for prenatal counselling +/- prenatal diagnosis:</th>
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<td>- Inform your physician if pregnancy is planned or as soon as you get pregnant.</td>
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<tr>
<td>- Reduce penicillamine to 500 mg/day.</td>
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<tr>
<td>- If zinc is used as maintenance therapy, no dosage adjustment of zinc is required.</td>
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Conclusion

WD is a rare but treatable autosomal recessive hereditary disorder of copper accumulation. The genetic defect is at the ATP7B gene on chromosome 13 which encoded a cation transporting P-type ATPase. Diagnosis is easily made in patient presenting with the classical features of liver impairment, Kayser-Fleischer rings, low ceruloplasmin and high urinary copper but it may be difficult when patients present with fulminant hepatitis and or present with liver disease alone. In general, no single test is diagnostic. Careful history taking, physical examination and appropriate tests are the prerequisite to reach a diagnosis. Life long anticopper treatment is required. Patients on any kind of anticopper treatment should be monitored regularly for the possible side effects. Compliance is particularly important as abrupt discontinuation of the treatment can lead to mortality. In the future, direct molecular diagnosis may become possible.

References

32. Berghella V, Steele D, Spector T, Cambi F, Johnson A. Successful


