

Wilson Disease in Septuagenarian Siblings: Raising the Bar for Diagnosis

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Wilson Disease (WD) usually presents in the first decades of life, although rare patients have a later presentation. We report the clinical features, diagnostic evaluation, and outcome with treatment of two septuagenarian siblings evaluated as part of a research trial for treatment of neurological WD. The index case was a 72-year-old woman who suffered progressive neurological disability, then developed sub-fulminant liver failure. Her sibling was a 70-year-old man with minimal neurological symptoms and a mild depressive disorder. His liver biopsy revealed only steatosis and minimal fibrosis and an elevated hepatic copper content (671 $\mu\text{g/g}$ dry weight liver). Molecular studies demonstrated compound heterozygosity for disease specific *ATP7B* mutations E1064A and H1069Q in both patients. Both individuals were treated with trientine and Zn followed by Zn maintenance therapy. Over the last 5 years, the clinical course stabilized and improved, although the index case recently died from bronchopneumonia. In conclusion, advanced age and different clinical presentations of these two subjects with identical *ATP7B* mutations raises the question of the degree of penetrance for these and other *ATP7B* mutations. Environmental and extragenic factors are pivotal determinants of disease phenotype. We suggest that WD must be considered at all ages in patients with hepatic disease, neurological disease, or psychiatric symptoms. (HEPATOLOGY 2005;41: 668-670.)

Wilson Disease (WD) usually presents in the first decades of life, although rare patients were diagnosed with this disorder in their fifth and sixth decades after late onset of disease presentation.^{1,2} Its clinical expression varies, and disease penetrance and modifying factors are not known. We report on the presenting features, diagnostic evaluation, and outcome with treatment in two septuagenarian siblings who were initially diagnosed in their eighth decade of life.

Patient 1, a 72-year-old woman, suffered progressive neurological disability for 5 years when she developed pneumonia and decompensated clinically with the development of ascites and encephalopathy. Further evaluation by radiological imaging suggested cirrhosis. Screening for WD showed a normal serum ceruloplasmin (Cp), 37 mg/dL, and a nondiagnostic 24-hour urine Cu, 39 $\mu\text{g}/24$ hours. Repeat 24-hour urinalyses for Cu were elevated above 100 $\mu\text{g}/24$ hours (143 and 147 μg), and after a penicillamine challenge, increased to 9,390 $\mu\text{g}/24$ hours. Kayser-Fleisher rings were identified by slit-lamp examination. Patient 2, a 70-year-old male sibling of Patient 1, reported a mild hand tremor beginning at approximately age 45 years that was previously treated with a beta blocker. He also had a history of mild clinical depression. At age 69 years, he developed mild gait dyscoordination. Screening for WD was performed after his sibling was diagnosed with WD. His clinical examination showed no stigmata of chronic liver disease. Studies showed Cp 20 mg/dL, urine Cu 218 $\mu\text{g}/24$ hours, and Kayser-Fleisher rings were identified by slit-lamp examination. Liver biopsy showed steatosis and minimal fibrosis, and staining with rhodanine was positive (Fig. 1). Hepatic Cu was 671 $\mu\text{g/g}$ dry weight.

For molecular diagnostic studies, DNA was isolated from peripheral blood using QIAamp DNA Blood kit

Abbreviation: WD, Wilson disease.

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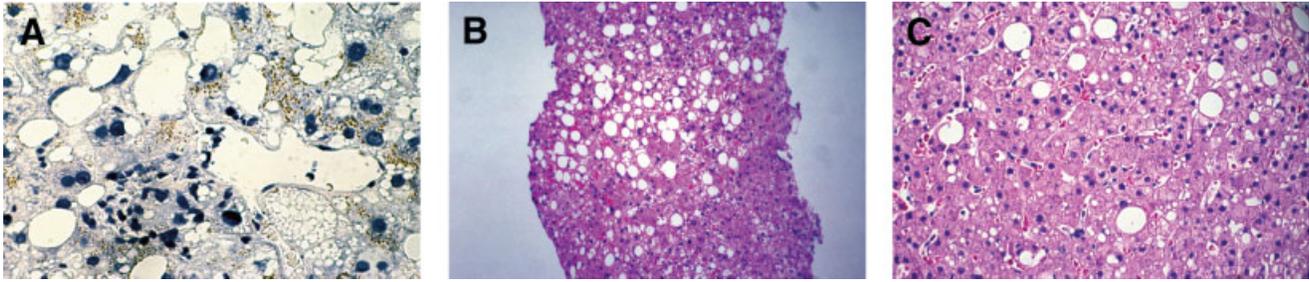


Fig. 1. (A) Rhodanine stain of the liver biopsy specimen from patient 2 showing positive granules in hepatocytes, consistent with an elevated hepatic copper content (671 $\mu\text{g/g}$ dry weight). (B-C) Low- and high-power (B and C, respectively) magnification of the liver biopsy (hematoxylin-eosin staining) from patient 2 showing microvesicular steatosis, nuclear vacuolation, and lack of advanced fibrosis (confirmed on trichrome stain) and Mallory bodies.

(Qiagen, Valencia, CA). *ATP7B* exons 1 through 21 were amplified with polymerase chain reaction (PCR), purified with QIAquick PCR purification kit (Qiagen, Valencia, CA), and sequenced directly using BigDye Terminator sequencing kit with an ABI Prism 377 DNA sequencer (both from PE Applied Biosystems, Foster City, CA). The primers used for *ATP7B* amplification were based on Petrukhin et al.³ To verify that the mutations were located on two separate chromosomes, the exons containing two different alleles were subcloned using the TA cloning kit (Clontech, Palo Alto, CA) after the PCR amplification. Plasmid DNA from individual subclones was sequenced as above. Results indicated compound heterozygosity for disease specific *ATP7B* mutations E1064A and H1069Q in both patients.

Patients were treated with trientine and zinc for the first 8 weeks, and then with zinc thereafter for maintenance therapy as part of a clinical research protocol. This protocol randomized initial therapy with trientine and zinc or tetrathiomolybdate and zinc, followed by zinc maintenance therapy. Both patients' conditions stabilized and improved these last 5 years. Patient 1 died earlier this year of complications of bronchopneumonia. Patient 2 is alive and well, now 6 years after first presentation.

The confirmation of WD in these two siblings makes them the oldest patients at the time of diagnosis to date. The advanced age and different clinical presentations of these two subjects with identical *ATP7B* mutations raises the bar as to the age for which WD must still be considered. Furthermore, it begs the question of the degree of penetrance for these and other *ATP7B* mutations. This is a critical issue when molecular genetic testing is used for disease diagnosis rather than biochemical and clinical evaluations that rely on phenotypical expression of the disease.

The clinical variability in the phenotypical presentation of many WD patients has led to the quest for understanding whether specific genotypes are responsible for a specific phenotypical presentation. An important con-

founding factor is the limited number of individuals with homozygous *ATP7B* mutations. In one of the few larger populations with homozygosity (H1069Q) in Europe, there is a predominance of neurological presentation of the disease and a slight increase in the average age of presentation compared with other patients.⁴ The presence of apolipoprotein E genotype 3/3 amongst these homozygous patients with H1069Q mutations may be one of the extragenetic factors that ameliorates the disease and delays the onset of disease presentation.

Although the clinical phenotypes of WD and age of onset are often similar among sibling patients with the same genotype,⁴ rarely do younger patients show neurological symptoms earlier than their older siblings, and there are reports of markedly different clinical phenotypes appearing in siblings.^{5,6} Some phenotypical variability in patients with WD is likely attributable to other extragenetic and environmental factors, as demonstrated by differences in mode of presentation, hepatic copper content, ceruloplasmin levels, and disease course in patients with the same *ATP7B* mutations, even in identical twins and siblings such as those described in this report. Multiple variables such as dietary copper and zinc intake, upregulation of intestinal metallothioneins, and capacity for countering copper-induced oxidative stress at the cellular level via glutathione, superoxide dismutase, catalase, and heat shock protein pathways may all be important in modulating the phenotypic expression of disease. Other genetic variations that modulate the expression or function of factors necessary for the intracellular sensing and trafficking functions of the *ATP7B* protein, including the HAH1 chaperone protein essential for delivery of cytosolic copper to the *ATP7B* protein, also may contribute to the phenotypical variation in disease expression of WD.⁷

The normal ceruloplasmin present in our index patient may have contributed to the delay in the initial diagnosis of WD. The level of serum ceruloplasmin is reduced in

most patients with WD to a level below 20 mg/dL, although at least 5% of patients have levels above this value.⁸ Interestingly, when patient 1 was treated for her WD, the level of serum ceruloplasmin became less than 20 mg/dL, indicating that the initial normal level was a response to acute inflammation that subsequently resolved with treatment.

In summary, this report on 2 individuals with WD identified in their eighth decade of life highlights the larger range of phenotypical expression for WD than previously recognized. We suggest the clinical need for excluding WD in patients of all ages with evidence of liver disease, neurological disease, or psychiatric symptoms.

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