CLINICAL GENOMICS

Wilson's Disease

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Wilson's disease (WD) is an autosomal recessive inherited disorder leading to impaired intrahepatic trafficking and biliary excretion of copper, resulting in the accumulation of copper in various organs including the liver, cornea, and brain. The WD gene (OMIM 277900) codes for a copper transporting P-type ATPase (ATP7B). Although the finding of the gene resulted in a major breakthrough for understanding the pathophysiology of WD, the role of genetic testing in the clinical management of WD patients is not yet established. There is no gold standard for diagnosis of WD. Diagnosis requires a combination of clinical and biochemical tests. None of these parameters alone allows a certain diagnosis of WD. To facilitate diagnosis, a scoring system was developed at the 8th International Meeting on Wilson Disease in Leipzig, Germany in 2001. For clinical purposes, the use of mutation analysis is limited by the occurrence of many mutations (more than 200) causing WD. In contrast to direct DNA sequencing, direct mutation detection by using allele-specific probes is rapid and clinically very helpful, if a mutation occurs with a reasonable frequency in the population (ie, H1069Q in European WD patients or R778L in WD patients from the Far East). To date, mutation analysis is the only reliable tool for screening the family of an index case with known causative mutation. Alternatively, haplotype analysis can be used to address diagnostic dilemmas in differentiating heterozygote gene carriers and affected asymptomatic siblings.

Wilson's disease (WD) is an autosomal recessive inherited disorder of copper metabolism. The hallmarks of the disorder are the presence of liver disease, neurologic symptoms, and Kayser-Fleischer corneal rings. The disease is common in younger subjects[1] but might be present even as late as the eighth decade of life.[2] The basic defect in WD is the impaired biliary excretion of copper, resulting in the accumulation of copper in various organs including the liver, cornea, and brain. Copper is an essential nutrient needed for such diverse processes as mitochondrial respiration (cytochrome C), melanin biosynthesis (tyrosinase), dopamine metabolism (DOPA-β-monooxygenase), iron homeostasis (ceruloplasmin), antioxidant defense (superoxide dismutase), connective tissue formation (lysyl oxidase), and peptide amidation. The consequence of copper accumulation is the development of severe hepatic and neurologic disease. Copper's unique electron structure allows these "cuproenzymes" to catalyze redox reactions, but it causes ionic copper to be very toxic, readily participating in reactions that promote the synthesis of damaging reactive oxygen species. Copper overload particularly affects mitochondrial respiration. Damage to mitochondria is an early pathologic effect in the liver. Damage to the liver has been shown to result in increased lipid peroxidation and abnormal mitochondrial respiration both in copper-loaded dogs and in patients with WD.

Wilson's Disease Gene

The WD gene (OMIM 277900) was identified by 3 independent groups in 1993[3,4] and codes for a copper transporting P-type ATPase (ATP7B).[5] ATP7B contains several functional domains: 6 copper binding domains, a transduction domain (amino acid residues 837–864, containing a Thr-Gly-Glu motif) involved in the transduction of the energy of ATP hydrolysis to cation transport, a cation channel and phosphorylation domain (amino acid residues 971–1035, containing the highly conserved Asp-Lys-Thr-Gly-Thr motif), an ATP-binding domain (amino acid residues 1240–1291), and 8 hydrophobic transmembrane sequences (1–8), in one of which (region 6) is the Cys-Pro-Cys sequence found in all P-type ATPases. The 6 N-terminal metal-binding sites (MBS) are required for trafficking and are essential for the copper transport function. Structural analysis of the N-terminus has revealed that both secondary and tertiary structural changes take place after the binding of copper.[6] Furthermore, it was demonstrated that copper co-

Abbreviations used in this paper: ATP7B, copper transporting P-type ATPase; MBS, metal-binding sites; N-domain, nucleotide-binding domain; WD, Wilson's disease.

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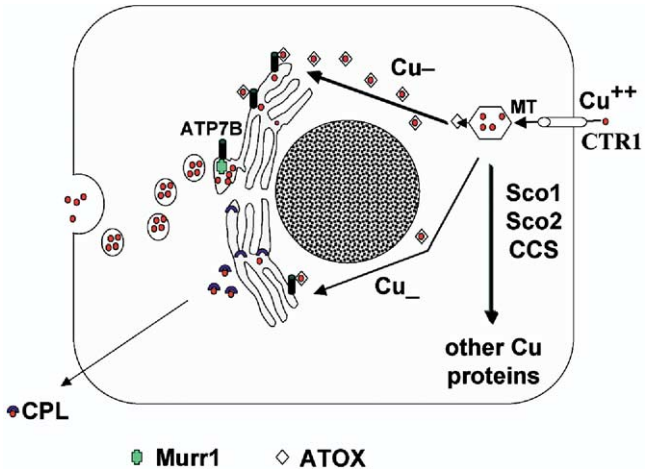

Figure 1. Model of hepatobiliary copper transport. For details see text. *CTR1*, copper transporter 1; *MT*, metallothionein; *CPL*, ceruloplasmin; *ATOX, Sco1, Sco2, CCS*, copper chaperones; *Murr 1*, protein that binds to ATP7B and is involved in the transfer of copper into vesicles.

ordination induces the phosphorylation of ATP7B, which coincides with the trafficking of the protein to vesicular compartments.[7] Either MBS 5 or MBS 6 alone is sufficient to support the redistribution of ATP7B to vesicular compartments. The first 3 N-terminal motifs were not required for copper-dependent intracellular trafficking and could not functionally replace sites 4–6 when placed in the same sequence position.[8]

Hepatic Copper Metabolism and the Role of ATP7B

Dietary copper intake (approximately 1–2 mg/day) far exceeds the trace amounts required. Approximately 10% of dietary copper is absorbed in the upper intestine and is taken up by the liver. The hepatic uptake of diet-derived copper is a carrier-mediated, energy-independent mechanism. The copper transporter 1 transports copper with high affinity in a metal-specific, saturable fashion at the hepatocyte plasma membrane.[9,10] Metallothioneins, a group of cysteine-rich intracellular proteins capable of binding metal ions, including copper, cadmium, and zinc, have a critical role to protect intracellular proteins from copper toxicity.[11] The copper stored in metallothionein can be donated to other proteins, either after degradation in lysosomes or by exchange via gluthathione-complexation. Biliary excretion is the only mechanism for copper elimination, and the amount of copper excreted in the bile is directly proportional to the size of the hepatic copper pool. Specific pathways allow the intracellular trafficking and compartmentalization of copper, ensuring adequate cuproprotein synthesis while avoiding cellular toxicity (Figure 1).[12] Metallochaperones transfer copper to the site of synthesis of copper-containing proteins.[13–15]

The cytoplasmic copper chaperone ATOX1 is required for copper delivery to ATP7B in the hepatocyte by direct protein-protein interaction. ATP7B delivers copper to apo-ceruloplasmin and mediates the excretion of excess copper into bile.[16] These distinct functions require the protein to localize at 2 different subcellular compartments. At the *trans*-Golgi network, ATP7B transports copper for incorporation into apo-ceruloplasmin to form ceruloplasmin. In the copper-limiting environment of the cell, the delivery of copper by ATOX1 is responsible for initiating the catalytic activity and the intracellular trafficking of ATP7B.[17] Other chaperones (Sco1, Sco2, Cox17, Lys7) carry copper for synthesis of the other cuproenzymes and do not require an interaction with ATP7B.[18] Ceruloplasmin contains 6 tightly bound copper atoms. Its main function is to carry copper to various tissues. Another important physiologic role of ceruloplasmin is to act as ferrooxidase, converting Fe^{++} to Fe^{+++}. When intracellular copper levels are increased, ATP7B traffics to *post*-Golgi vesicles in close proximity to the canalicular membrane to facilitate biliary copper excretion. For a comprehensive review of intrahepatocellular copper trafficking, see the excellent review article by Gitlin.[19]

ATP7B Mutations in Wilson's Disease

Molecular genetic analysis of patients with WD has revealed more than 200 distinct mutations (database maintained at the University of Alberta: http://www.medgen.med.ualberta.ca). The molecular defects include missense and nonsense mutations, deletions, and insertions. Some mutations are associated with a severe impairment of copper transport, resulting in severe liver disease very early in life. Other mutations appear to be less severe, with disease appearance in mid adulthood. Several WD mutations are clustered within the WD-protein nucleotide-binding domain (N-domain), where they are predicted to disrupt ATP binding. The mechanism by which the N-domain coordinates ATP is presently unknown (Figure 2). Mutations of the invariant WD-protein nucleotide residues E1064A and H1069Q drastically reduce nucleotide affinities, pointing to the likely role of these residues in nucleotide coordination. In contrast, the R1151H mutant exhibits only a 1.3-fold reduction in affinity for ATP. The C1104F mutation significantly alters protein folding, whereas C1104A does not affect the structure or function of the N-domain.[20] These results directly demonstrate the pheno-