

Wilson disease Database: an Aid to DNA Diagnosis

Cox DW*, Bugbee DA, Wilson AME, Deeb TMM, Macintyre G, Kenney SM, Davies LM.
Depts. of Medical Genetics, Medicine and Pediatrics, University of Alberta, Edmonton,
Canada T6G 2H7

The copper transporting ATPase, ATP7B, is defective in Wilson disease (WND), in which copper is retained in, and damages, liver, kidney and brain. Variability in clinical and biochemical features is extensive (onset from 3 to 70 years; hepatic and neurological features), so diagnosis for this treatable disorder is easily missed. Identification of WND mutations serves two important purposes: identification of specific amino acid residues important for normal function, and reliable molecular diagnosis of WND.

We have documented all reported *ATP7B* mutations in the Wilson Disease Database: (<http://www.wilsondisease.med.ualberta.ca/database.asp>). This includes more than 500 probable disease-causing variants and over 50 probable normal variants from populations worldwide. Variants, assembled from our data, literature searches, and direct submission, have been entered into a searchable, web-based database using Microsoft Access and Adobe Dreamweaver.

An important goal for our database is to ensure that variants are correctly classified as disease vs non-disease causing. We are systematically testing function of these variants, and also applying and testing prediction programs. Functional testing of variants is important to ensure reliable application of mutation analysis for DNA diagnosis.

ATP7B has two major functions: 1) intracellular transport of copper to the Golgi for incorporation into proteins, including ceruloplasmin, which requires copper for normal function 2) elimination of copper from the liver. This function requires the trafficking of *ATP7Bp* in copper-loaded vesicles to the plasma membrane of hepatocytes, to allow export of excess copper into bile.

To test for functional defects in potential *ATP7B* variants, we use a highly conserved copper transport pathway in yeast. Human *ATP7B* can replace the yeast homologue, *Ccc2p*, and allows for normal copper transport in *ccc2⁻* yeast strains. Copper is then available for incorporation into Fet3p (ceruloplasmin homologue) that imports iron into yeast. Normal transport function is measured by growth on low iron medium. Variants that do not support normal copper transport function do not grow on low iron media, and are considered to be *ATP7B* mutations. Variants that display a normal copper transport function could have a specific trafficking defect, and are tested in mammalian copper viability and trafficking assays, using CHO cells stably transfected with normal or variant *ATP7B*. We have also developed an assay to test variants that could influence normal splicing.

Other tools we use help to identify disease-causing variants are:

- Prediction programs SIFT (Sorting Intolerant From Tolerant), PolyPhen, Align-GVGD. Missense variants with functional data were tested in these prediction programs. For the 29 tested, SIFT (Sorting Intolerant From Tolerant) and PolyPhen agreed more frequently with functional data than did Align-GVGD.
- For splice site prediction, Splice Site Prediction by Neural Network (SSPNN) and RESCU-ESE were useful to establish priorities for testing.
- 3-D protein modeling aids in assessment of the potential impact of amino acid changes on *ATP7B* protein structure and function.

Our approaches aid in the elucidation of the mechanism of action of *ATP7B*, and fulfill a valuable role in molecular diagnosis of this highly variable disease.