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16. WILSON'S DISEASE

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Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism characterized by excessive accumulations of copper in the liver, central nervous system, kidneys, eyes and other organs. WD is characterized by reduced incorporation of copper into ceruloplasmin and a decreased biliary copper excretion. The disease is progressive and ultimately fatal if untreated. The worldwide prevalence of WD is estimated to be approximately one case per 30000, whereby at least a half of WD patients remain undiagnosed and die of untreated disease. The age at onset and clinical presentation greatly vary among WD patients.

The disease most commonly occurs in people under 40; in children the onset of symptoms may occur around age 4 - but it is most common during teenage years. Liver failure and damage to the central nervous system (CNS) are the predominant and most severe WD sequels. Approximately 40% of all patients are first seen because they experience symptoms of liver disease. Blood tests show an elevation in liver enzymes, and the symptoms of acute hepatitis, fulminant hepatitis; chronic hepatitis or cirrhosis, with all complications, may be present. If the liver injury is acute, copper may be released into the blood and cause haemolytic anemia. If the liver injury is chronic, copper may accumulate in the brain and cause neuropsychiatric symptoms.

In general, the younger the age at symptom onset, the greater the degree of liver involvement. In patients with WD, neurological symptoms seem to be predominant after the age of 20. These neurological symptoms may include tremor of the head, arms, or legs; generalized, impaired muscle tone and sustained muscle contractions that produce abnormal postures, dystonia, bradykinesia, ataxia, loss of intellectual functions, loss of memory, depression, suicidal impulses, confusion and dementia if untreated. Sometimes the initial symptoms may manifest as abruptly inappropriate behavior and inexplicable deterioration of school work, neurosis or psychosis. The disease may mimic Parkinson's disease and schizophrenia. In the eyes, the characteristic feature is the Kayser-Fleischer ring, a pigmented ring at the outer margin of the cornea.

Other findings that may be associated with WD include impaired kidney function, development of unusually dark skin patches (hypermelanotic pigmentation), thrombocytopenia, softening and thinning of the bones, and problems with major joints.

Many cases of WD occur due to spontaneous gene mutations. Differences in the phenotypic disease presentation can be partially explained by various mutations. Mutations that completely prevent the function of the gene produce a more severe phenotype than certain types of mis-sense mutation. In general, the most severe mutations result in the onset of symptoms before 12 years of age, frequently with liver manifestations.

The WD gene, designated ATP7B, has been mapped to chromosome 13q14.3 and encodes the copper transporting P-type ATPase expressed predominantly in the liver. The gene has approximately 80 kb and contains 21 exons that encode an approximately 7.5 kb transcript. ATP7B is a copper transporter involved in the intracellular transport of copper in hepatocytes. The protein possesses a metal-binding domain, a cation channel and a phosphorylation region, and a transduction domain responsible for the conversion of the energy of ATP hydrolysis to cation transport. It is an integral membrane protein, predominantly found in the trans-Golgi network (TGN). The copper-binding motifs closed to the transmembrane channel of ATP7B are probably directly involved in copper transport, transferring copper to residues within the channel for subsequent translocation across the membrane. The remaining N-terminal motifs may not be directly involved in copper transport. Instead, they may act cooperatively to induce conformational changes in the domain, sensing cytosolic concentracions, thereby inducing redistribution of ATP7B within the cell. ATP7B is not redistributed to the plasma membrane in response to elevated copper levels.

There are two isoforms of ATP7B: short and long isoform. The short isoform is expressed in the brain, and not in the liver.

Copper is absolutely required for aerobic life, and yet, paradoxically, is highly toxic. Within the living cell, it coexists with high concentrations of electron-rich molecules such as thiols or ascorbate that are essential to life. Cu, like other redox-active metals, is sequestered in non-reactive forms as it is transported into cells and moves through cellular compartments. Copper chaperones are required for proper intracellular delivery of Cu so that Cu is incorporated into specific targets within different cellular compartments. Moreover, because it is believed that the cell possesses a high copper-chelating capacity, the level of intracellular free Cu is kept extraordinarily low, and the toxic effects of intracellular Cu are minimized. Copper chaperones function to sequester and deliver Cu to their respective protein targets. Three different chaperones have been identified that transport Cu to different cellular locations: to the mitochondria for insertion into cytochrome C oxidase (CCO), the terminal oxidase of the respiratory chain; to Cu-ZnSOD, a primary antioxidant enzyme in the cytosol; and to a post-Golgi compartment by way of P-type

adenosine triphosphatase transmembrane Cu transporter, for final insertion into ceruloplasmin.

To date, more than 200 ATP7B mutations have been reported in patients with WD (http://www.uofa-medical-genetics-org/wilson/index.html). These mutations are distributed over the entire gene, suggesting that the physiological function of ATP7B can be disrupted in a number of ways. The mutations may alter protein folding, the ability to transport copper, the stability of protein in a cell, subcellular localization, and trafficking of ATP7B. The specific effects of most mutations are yet unknown. Several studies used in vivo and in vitro systems to investigate how several ATP7B mutations affect the intracellular localizations of this protein. In the hepatocytes from a WD individual, homozygous H1069Q, the most common mutation in the populations of European origin, mutant protein was localized in rough ER, where it appeared to be situated on the cytoplasmic side of the ER. This mutation occurs at a frequency of 26%-70% in various populations, and is associated with neurologic or hepatic disease and a mean onset at age 20. The mutation was present in only 13% of non-Sardinian Mediterranean patients and was absent in those from Sardinia, India and Asia. Various ethnic groups carry a different range of specific mutations. The most common mutation in Oriental populations is an amino acid substitution, arginine778leucine, R778L found in 57% of patients younger than 18. A number of short tandem repeats (STR), which closely flank the gene (e.g., D13S314, D13S301, D13S316/D13S129) are inherited in specific combinations or hAPLotypes, with each mutation. The identification of the specific allele, or type of marker at each, can sometimes be helpful in pinpointing which mutation is present.

The diagnosis of WD may involve one or more of the following laboratory tests, findings, or procedures:

· Low ceruloplasmin (Cp) levels (<200 mg/L). Decreased levels are also found in 20% of heterozygous Cp deficiency, in conditions associated with renal Cp losses, in the presence of massive burns as well as in liver disease due to defective synthesis. Low normal levels are found in 5% of WD during the chronic, clinically inapparent stage and in 15% of those with hepatic damage. In fulminant liver failure, Cp concentration can be higher than during the preceding asymptomatic stage. However, ceruloplasmin may be normal in up to 10% of WD individuals.

· 24-hour urinary copper excretion test (>1.6 µmol/24 h) is done in all patients with clinically symptomatic WD. The degree of cupriuria is markedly elevated after a penicillamine challenge. Slightly increased urinary copper can also occur in cholestatic liver disease.

· Serum copper level is usually <11.8 µmol/L. However, in case of an acute hepatic type of manifestations, the levels are higher and may be high normal.

· Free Cu (fCu) in serum is increased. In healthy people, it ranges from 0.9 to 3.0 µmol/L, and in WD exceeds 3.2 µmol/L. It is calculated by use of the equation:

fCu (µmol/L) = Cu (µmol/L) - [Cp (mg/L) x 0.0535]

· Kayser-Fleischer rings are most reliably observed in the cornea by slit lamp examination. They are found in 50%-85% of individuals presenting with liver disease and 90% of individuals presenting with either neurologic findings or psychiatric disturbance.

Diagnosis may be difficult to reach in the absence of typical symptoms and in asymptomatic siblings because all biochemical markers of impaired copper metabolism can be normal.

Incorporation of radiolabeled 64Cu may be helpful. Radioactive labeled 64Cu is administered orally and serum levels of radioactivity are measured at 1 to 2 hours and at 48 hours. In WD patients there is no secondary rise in plasma radioactivity due to impaired incorporation of radio copper into Cp. Heterozygotes have a pattern of incorporation that is intermediate between that of WD patients and healthy individuals. If Kayser-Fleischer rings or neurologic abnormalities are absent, or serum Cp level is normal in the presence of Kayser-Fleischer rings, a liver biopsy for quantitative copper determination is the gold standard to establish the diagnosis of WD. The liver Cu content is normally <50 µg/g, whereas in WD it is >250 µg/g of liver dry weight.

Groups of authors from Japan suggest that Cp level in dried blood samples from children aged 1 to 6 years appears to be a reliable marker for the early detection of WD. A screening test of this sort should be used once in infancy in every child. During the neonatal period, ceruloplasmin levels are not always decreased in patients with WD, and are not reliable for detecting the disease.

The diagnostic approach to WD should be tailored to clinical presentation. It should be emphasized that, in the absence of definitive DNA analysis, the diagnosis of WD should not be based on the results of an individual laboratory test, and can be only established in a setting of confirmatory clinical and biochemical data. Patients with neurological or psychiatric manifestations should undergo slit-lamp examination of the eyes and determination of serum ceruloplasmin. The documentation on Kayser-Fleischer rings and low Cp concentration is sufficient to make the diagnosis, which can be confirmed by the presence of an increased 24-h urinary copper excretion. A liver biopsy with quantification of hepatic copper content is essential if either Kayser-Fleischer rings are absent (in order to exclude the possibility that the patient is heterozygous for the gene) or the Cp levels are normal (as occurs in up to 15% of cases).

In patients who present primarily with hepatic dysfunction, the diagnosis may be difficult, since Cp levels may be falsely elevated and ophthalmologic findings absent. It is imperative that biochemical screening for WD be performed in all patients under 40 years of age who have clinical or histologic findings compatible with chronic active hepatitis, and in whom autoimmune and viral hepatitis have been excluded.

Mutation analysis of the ATP7B gene for diagnostic purposes is available for a limited number of mutations. Linkage analysis of the ATP7B gene is clinically available primarily for the early diagnosis of siblings at risk. People with only one abnormal gene are called carriers (heterozygotes). They do not become ill and should not be treated. People with two different mutant alleles are compound heterozygotes and they develop symptoms in their mid-teens. Possessing one mutant allele at the ATP7B locus could potentially render a person more susceptible to liver, brain, or heart damage from other causes.

Without proper treatment, WD is generally fatal. If the treatment is introduced on time, symptomatic recovery is usually complete, and life of normal length and quality can be expected. Treatment must be life-long. Prophylactic therapy in the affected but presymptomatic patients can prevent the onset of symptoms. Prompt and accurate presymptomatic diagnosis is critically important. Treatment of WD consists of anti-copper agents to remove excess copper from the body and to prevent it from reaccumulating. Most cases are treated with the drugs penicillamine, trientine or zinc acetate, sulfate, glucuronate. Penicillamine and trientine increase urinary excretion of copper,

however, they both cause side effects. Zinc blocks the absorption of copper and increases copper excretion in the stool. Pyridoxine (vitamin B6) is used to counteract nervous tissue damage. A low copper diet is recommended, including avoiding shellfish, nuts, chocolate, dried fruit, liver, mushrooms.

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