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Value of molecular analysis of Wilson's disease in the absence of tissue copper deposits: a novel *ATP7B* mutation in an adult patient

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ABSTRACT

Wilson's disease (WD) is a disorder of copper metabolism leading to copper accumulation in the liver and in extrahepatic organs, such as brain and cornea. We present a patient with liver disease who did not fulfil the biochemical criteria for WD. Mutational analysis was necessary to make the diagnosis and show a new mutation. Our case supports the use of mutation analysis in cases with unclear liver disease and suggests that the spectrum of WD is broader than currently assumed.

KEYWORDS

Copper, hepatitis, mutation analysis, Wilson's disease

INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism. The *ATP7B* protein is an important transporter of copper and is responsible for WD; dysfunction can lead to copper accumulation in the liver and in extrahepatic organs, such as brain and cornea. This can lead to liver disease but also to (neuro)psychiatric disorders and Kayser-Fleischer (KF) rings around the iris. The diagnosis of WD is based on the results of several clinical and biochemical tests (decreased serum ceruloplasmin concentration, elevated 24-hour urinary copper excretion or elevated liver copper concentration). A liver biopsy with copper deposits is considered to be 'the gold standard' for the diagnosis.

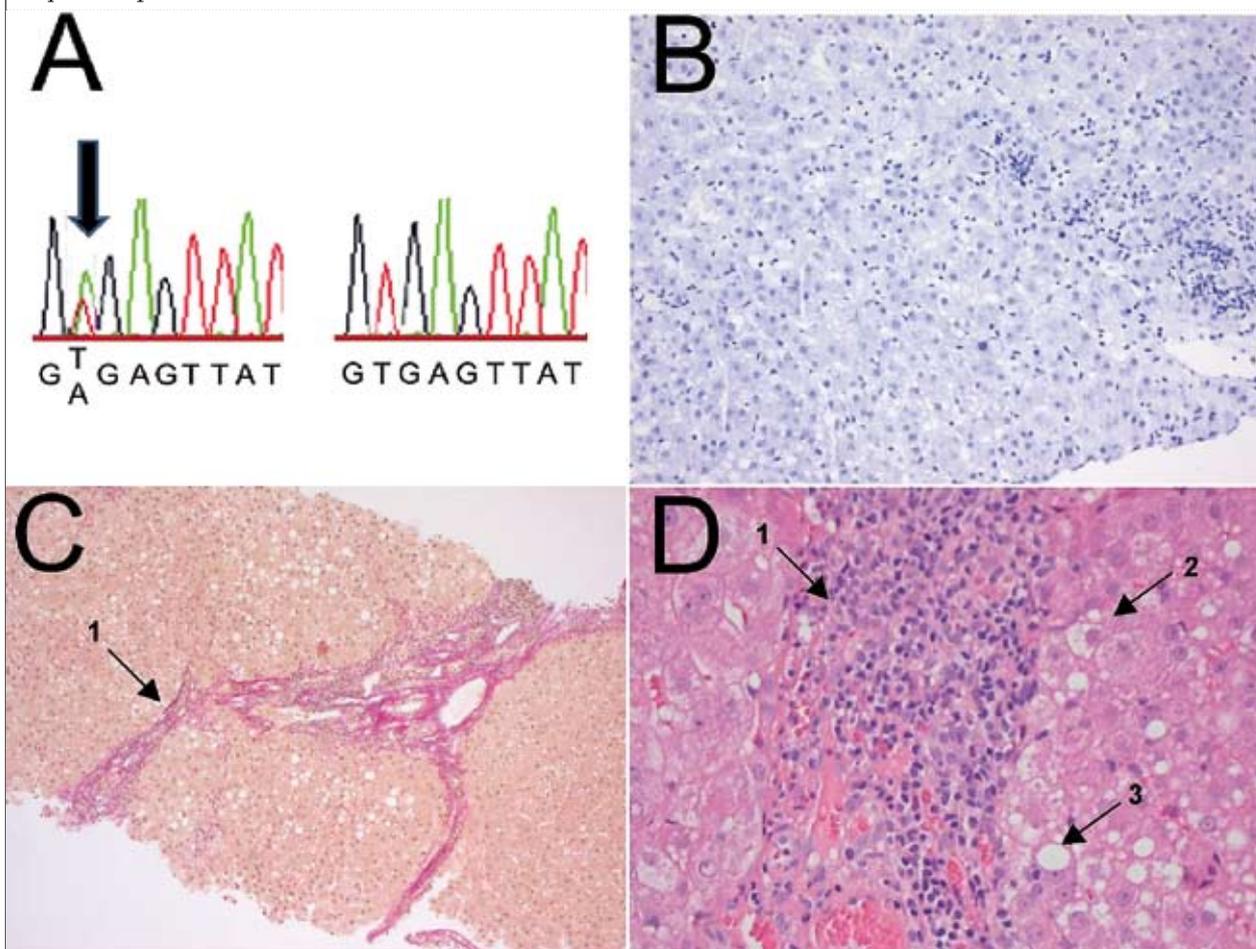
WD is usually diagnosed in childhood and most physicians are aware of the association of the above-mentioned clinical findings with WD. However, in the absence of 'typical'

clinical findings, WD is a diagnosis that can be hard to make. We would like to present a patient in whom we were only able to make the diagnosis of WD at adult age aided by molecular testing of the WD gene, *ATP7B*.^{1,2}

CASE REPORT

A 28-year-old woman visited our outpatient's clinic because of cryptogenic liver disease. At the age of 10 years she presented with disturbed liver enzymes without signs of viral hepatitis, autoimmune hepatitis, toxic hepatitis or metabolic liver diseases such as alpha-1 antitrypsin deficiency or hereditary haemochromatosis. Biochemistry demonstrated a decreased ceruloplasmin (0.13 g/l, normal 0.2 to 0.5 g/l), and mildly increased urinary copper excretion (2.31 $\mu\text{mol}/24$ h, normal <1.5 $\mu\text{mol}/24$ h). Repeated ophthalmological examination never showed Kayser-Fleischer rings. A liver biopsy at the age of 13 years showed steatosis and fibrosis. Copper staining was negative, reason to reject a diagnosis of WD at that time. At reassessment 15 years later, WD was reconsidered. The transaminases were still elevated (alanine aminotransferase 138 U/l; aspartate aminotransferase 63 (normal <45 U/l)). Serum copper was normal, most probably because the major copper binding protein in the plasma, ceruloplasmin, was only mildly decreased (0.17 g/l). In the absence of another explanation for the liver disease and in view of the decreased ceruloplasmin with increased urinary copper excretion of 2.9 $\mu\text{mol}/24$ h we proceeded to perform mutation analysis of the *ATP7B* gene. Upon sequencing we detected compound heterozygosity for c.3207C>A (p.H1069Q) and c.2447+2T>A (figure 1, panel A). The p.H1069Q mutation is the most commonly detected

Figure 1. A) The sequence analysis of *ATP7B* gene on an electropherogram, B-D) Liver biopsy specimen of a 28-year-old patient with Wilson's disease



A) The left insert shows the mutant sequence in the patient. There is a c.2447+2T>A mutation at the splice donor site of intron 9 that affects splicing pre-messenger-RNA from *ATP7B*.
 B) Copper staining is absent (rhodanine staining) (10x).
 C) Elastic-van Gieson's staining on a representative liver section compatible with bridging fibrosis (5x).¹
 D) Haematoxylin eosin staining, original magnification (20x): there is 1) periportal hepatitis with 2) ballooning of hepatocytes and 3) moderate steatosis.

mutation in WD; the second mutation c.2447+2T>A is a novel frame-shift mutation. We analysed the c.2447+2T>A variant for potential effects on the splice site using the splice site prediction by neural network (SSPNN; http://www.fruitfly.org/seq_tools/splice.html). The programme predicts the theoretical strength of donor and acceptor splice sites. The original splice site of intervening sequence 9 (IVS9) reaches the maximum probability score of 1.00 indicating that when the c.2447T>A variant is inserted in the sequence, the splice site is abolished.

We proceeded to perform a new liver biopsy which was consistent with periportal hepatitis, ballooning of hepatocytes, steatosis and bridging fibrosis (Metavir 2); again copper staining was negative (figure 1, panel B-D) The Metavir score assesses liver fibrosis on a five-point scale (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = numerous septa without cirrhosis, 4 =

cirrhosis). Given the positive molecular diagnosis of WD, we started her on chelating therapy with zinc sulphate (200 mg three times/day).

DISCUSSION

WD is characterised by a decreased biliary copper excretion and a defective incorporation of copper into ceruloplasmin, leading to copper accumulation in liver and brain. The prevalence is about 1 in 30,000. The range of clinical symptoms in Wilson's disease is wide; hepatic disease can lead to elevated transaminases, chronic hepatitis, cirrhosis and fulminant liver failure. Brain involvement is associated with neurological symptoms such as tremor, chorea, parkinsonism, pseudobulbar symptoms, dystonia and seizures and even psychiatric disease. The archetypical

ocular symptoms are KF rings and cataract. In rare cases other organs such as heart (cardiomyopathy) can be involved.

There is not a single specific test for the diagnosis of WD; symptoms are often nonspecific. KF rings are often present, especially in patients with predominantly neurological disease (85 to 98%), in contrast to patients with predominantly liver disease where KF were detected in only 52%.^{1,2} Quantitative copper concentration measurement remains the best biochemical evidence for WD, but due to a considerable sampling error, normal hepatic copper content does not exclude WD.³ Histochemical staining by rhodanine is not a substitute; less than 10% of confirmed cases had proteinaceous copper deposits in hepatocytes.⁴ To simplify the difficult diagnosis a diagnostic scoring system for WD has been proposed recently. This score includes data on the presence of KF rings, neuropsychiatric symptoms, haemolytic anaemia, urine copper, quantitative liver copper, rhodanine staining, serum ceruloplasmin and mutation analysis.⁵ Prior to mutational analysis our patient scored 2 points (decreased ceruloplasmin and increased urine copper excretion); after introduction of mutational analysis in the algorithm 4 additional points were gained: this made the diagnosis of WD 'highly likely'. Although it aids in making the diagnosis, we need to emphasise that this scoring system has not been assessed prospectively. The gene that is defective in WD is *ATP7B* which encodes a transmembrane protein ATPase which functions as a copper-dependent P-type ATPase. The *ATP7B* transporter has synthetic and excretory roles, facilitating transport of copper into the trans-Golgi compartment, for incorporation into ceruloplasmin and into bile. When copper excess is present, the transporter adopts an excretory role by increasing biliary copper excretion. The *ATP7B* gene was cloned in 1993; since then more than 380 mutations have been reported in patients with WD from many different populations (<http://www.medgen.med.ualberta.ca/database.html>). The p.H1069Q mutation is the most commonly detected mutation in WD, and has an allele frequency of up to 72% among WD patients. This mutation is seen in patients with late manifestations of WD and is associated with only a mildly disturbed copper metabolism. In compound heterozygotes, the phenotype of WD to a small extent depends on the type of mutation coinherited with p.H1069Q. Homozygosity for frameshift mutations in *ATP7B* is associated with severe disturbances of copper metabolism and presentation at young age. The effect of compound heterozygosity of a mild (p.H1069Q) and severe

(c.2447+2T>A) mutation is less well established although these patients develop WD at a later stage than carriers of two severe mutations.⁶

Our case history highlights several important aspects of WD. First, this case expands the clinical phenotype of Wilson's disease, showing that advanced WD can be present without readily detectable tissue copper staining. This apparently contradicts the concept that readily detectable liver copper accumulation should be present to diagnose WD. Negative copper staining results may be explained by heterogeneous liver copper distribution (sampling error) and differences in sensitivity of rhodanine for free copper and copper binding proteins, but might also suggest that other, hitherto unknown, cofactors might be present in order to cause liver fibrosis. It also shows the importance of molecular testing in cases with equivocal biochemical results as this showed the presence of two pathogenic *ATP7B* mutations consistent with WD. Lastly, the novel mutation adds to the mutational spectrum of WD.

Our data support the use of mutation analysis in cases with unclear liver disease with negative copper staining results and suggest that the spectrum of WD is broader than currently assumed.

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