Pitfalls in diagnosing Wilson's Disease by genetic testing alone: the case of a 47-year-old woman with two pathogenic variants of the *ATP7B* gene

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To the Editors:

Wilson's Disease (WD) is an inherited, multisystem disorder of copper metabolism in which pathological copper accumulation in different organs results in secondary damage of affected tissues (mainly the liver and brain) and symptoms related to affected systems (mainly hepatic and/or neuropsychiatric) [1–2]. Notably, WD is one of the few genetic neurodegenerative disorders that can be successfully treated with pharmacological agents. The most important determinants of outcome are early diagnosis and treatment [1–2].

The diagnosis of WD has been performed mostly by copper metabolism assessment and genetic tests. Nowadays, an additional algorithm including genetics, copper metabolism and clinical symptoms score (the Leipzig score) is used to improve WD diagnosis in doubtful cases (Tab. 1) [1]. However, despite the progress in WD diagnosis (genetic tests, algorithms), difficulties often occur, as highlighted in the following case.

We present the case of a 47-year-old female patient who had been suffering from idiopathic immunodeficiency syndrome for 19 years and was receiving monthly intravenous immunoglobulin administration. In addition, she was diagnosed, in gastroenterology departments, based on histological and serological examinations (according to international guidelines) [3, 4], with coeliac disease (treated with gluten-free diet) and ulcerative colitis (treated with mesalazine).

Three years ago, she began to experience hepatic symptoms including abdominal pain, increased weakness and weight loss. Liver enzymes were increased and she also had low serum albumin levels (2.03 g/dL; normal 3.5–5.0) and a high international normalised ratio (1.78; normal 0.8–1.2), indicating the impairment of liver synthetic function. Ultrasound examination of the liver documented cirrhosis (no liver biopsy was performed due to coagulopathy). Triggers of cirrhosis, namely autoimmunology illnesses, infectious diseases, and metabolic causes were excluded. The cause of liver injury remained unknown; however, WD was suspected and she was admitted to our department for further diagnosis. She was underweight (body mass index 13 kg/m^2), had enlarged abdominal circumference, ankle oedema and subcutaneous haemorrhage on the limbs.

She had no neurological signs characteristic of WD, with no tremor, ataxia, dystonia, or speech problems. Serum ceruloplasmin level was very low at 4.7 mg/dL (normal 25–45 mg/dL) and total serum copper level was also very low at 14.7 µg/dL (normal 70–140 µg/dL). Daily urinary copper excretion was normal at 9 µg/24 h (normal 0–50 µg/24 h). Her mother, aged 75, was alive and not suffering from liver, neurological or psychiatric symptoms. Her father was unknown and she had no siblings or children. Brain magnetic resonance imaging (MRI) showed only discreetly hyperintense changes in T1-weighted images in the globus pallidus, which are changes that are atypical for WD, but are often seen in the course of liver failure, probably due to brain manganese accumulation (Fig. 1). A slit-lamp examination excluded the presence of Kayser-Fleischer (K-F) rings.

DNA analyses with Sanger's sequencing method showed two variants classified as disease-causing variants in the Wilson Disease Mutation Database (http://www.wilsondisease.med.

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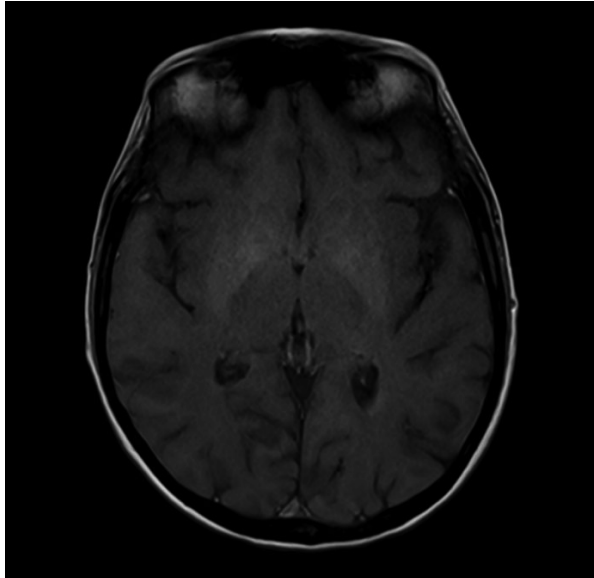
Figure 1. Brain magnetic resonance imaging (MRI). Hyperintense changes in T1-weighted images in the globus pallidus characteristic of hepatic encephalopathy

ualberta.ca): c.1924 G>G/C (p.D642H) in exon 6 (missense mutation) and c.3842 G>G/ (p.G1281D) in exon 18 (missense mutation) [5].

Due to the divergence between findings i.e. late occurring hepatic symptoms, absence of neurological signs, and no changes typical for WD despite age, lack of family history, normal daily urinary copper excretion, but low serum ceruloplasmin and the presence of two pathogenic variants of *ATP7B* gene, a ^{64}Cu radioactive copper incorporation test was performed.

This test measures the incorporation of radioactive intravenous copper into ceruloplasmin and involves the assessment of blood radioactivity after 2 (the starting value), 24, and 48 hours [6]. In healthy people, radioactive copper accumulates in the liver after a few hours, forms ceruloplasmin and is released into the blood, with almost all radioactive copper found in the blood after 24–48 hours. Calculated 24 hour/2 hour ^{64}Cu ratios and 48 hour/2 hour ^{64}Cu ratios are typically at or around 1 in healthy individuals. In WD cases, copper accumulates in the liver more slowly and only partially incorporates into ceruloplasmin; most radioactivity stays in liver cells and much less radioactivity can be measured in blood.

In WD patients, 24 hour/2 hour and 48 hour/2 hour ^{64}Cu ratios are generally ≤ 0.36 and ≤ 0.4, respectively [6]. This test reflects the functional activity of the copper transporter ATP7B and is characterised by very high sensitivity (48 hour/2hour ^{64}Cu ratio – 98.6%) and specificity (48 hour/2hour ^{64}Cu ratio – 100%). The test was performed on groups of patients who had genetically confirmed or excluded WD [6]. The limitations of this test are restrictions in copper isotope use in laboratories, so currently it is performed rarely for diagnostic needs. Radioactive copper test is regarded as a useful tool in experimental works where the aim is to restore function of mutated gene and potentially could be used in human gene therapy studies [7, 8].

Table 1. Scoring system (Leipzig score) for the diagnosis of Wilson's Disease developed at the 8th International Meeting on Wilson's Disease and Menkes Disease, Leipzig 2002 [1]

Clinical symptoms, sings and other tests	Score		
Kayser-Fleischer rings	Present (2 points)	Absent (0 points)	
Neuropsychiatric symptoms suggest WD (or typical brain MRI)	Yes (2 points)	No (0 points)	
Coombs negative haemolytic anaemia	Yes (1 point)	No (0 points)	
24-hour urinary copper excretion (in the absence of acute hepatitis)	> 2 x ULN or normal but > 5 x ULN after challenge with 2 x 0.5 g D-penicillamine (2 points)	1–2 x ULN (1 point)	Normal (0 points)
Quantitive liver copper assessment	> 5 x ULN (2 points)	< 5 x ULN (1 point)	Normal (-1 point)
Rhodanine-positive hepatocytes (if no quantitative liver copper assessment is available)	Present (1 point)	Absent (0 points)	
Serum ceruloplasmin (nephelometric assay, normal > 20 mg/dL)	< 10 mg/dL (2 points)	10–20 mg/dL (1 point)	Normal (0 points)
Mutation analysis	Disease causing mutations on both chromosomes (4 points)	Disease causing mutations on one chromosome (1 point)	No mutation detected (0 points)
Evaluation based on total WD diagnosis score:			
≥ 4 points: diagnosis of WD highly likely			
2–3 points: diagnosis of WD probable, more investigations needed			
0–1 points: diagnosis of WD unlikely			

MRI — magnetic resonance imaging; ULN — upper limit of normal; WD — Wilson's Disease

In our patient, the incorporation of radioactive copper was similar to that seen in healthy people (24 hour/2 hour: 1.61 and 48 hour/2 hour: 1.51), indicating that incorporation of ^{64}Cu into apoceruloplasmin was preserved and that a diagnosis of WD could be excluded.

However, it raised the question as to why, despite having two disease-causing variants of gene, was the patient not suffering from WD?

We suspect that two mutations confirmed as pathogenic, in our case, were present on one allele (uniparental isodisomy), which is rare but has been previously observed [9]. In our recent study of 248 patients with WD, we found three cases with three mutations [10]. As WD is an autosomal recessive disease, pathogenic mutations must be present on two alleles [2]. Unfortunately, we were unable to perform DNA analysis to check if one of this case's parents also had two mutations.

Additionally, the low level of ceruloplasmin observed in our patient is a frequent 'false positive' test for WD, especially in the case of malabsorption, and should be taken into account carefully in such cases [1]. We suspect that the low ceruloplasmin and copper serum levels were the result of malabsorption and general cachexia. The liver injury could be explained as a common extraintestinal manifestation of coeliac disease and ulcerative colitis and as the adverse effect of mesalazine treatment [11, 12]. She had normal daily urinary copper excretion, which is a very sensitive test for WD. Her clinical course was not typical of WD, with neither K-F rings nor neurological symptoms, despite her age.

The patient died two months after discharge from our department in another hospital. A post mortem was not performed, so the diagnosis of liver disease remains uncertain.

Using our case as an example, we would like to emphasise that the final diagnosis of WD cannot be guided solely by the results of genetic tests. Based on our experience as a reference WD centre, we strongly recommend that the diagnosis of WD must always be confirmed by clinical, laboratory and genetic compatibility.

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