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Wilson's Disease:
First Report of Two Combined Mutational Variants in a Portuguese Patient

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ABSTRACT
Wilson's disease is a rare autosomal recessive condition. A defect on the copper carrier protein ATP7B prevents the excretion of copper, which then accumulates in several organs. The prognosis of Wilson's disease is favourable if the diagnosis is made early. The Leipzig criteria standardized phenotypic classification and diagnostic criteria, thus simplifying the diagnostic approach. A search for ATP7B mutations is not necessary for diagnostic purposes and studies of genotype–phenotype correlation have not produced any conclusive evidence so far. More information is needed to reliably assess the prognosis for each patient. Here we describe a young patient with a combination of two mutational variants: c.3402del and c.3061-12T>A. To our knowledge, this is the first report of this compound heterozygote genotype.

LEARNING POINTS
- Wilson's disease should be suspected in a young patient with subacute liver failure.
- The diagnostic approach to Wilson's disease can be difficult as there are a great variety of clinical scenarios.
- Further studies on matching genotypic variations with clinical phenotypes could improve the diagnosis and treatment of these patients.

KEYWORDS
Wilson's disease, liver failure, copper, genetic testing

BACKGROUND
Wilson's disease (WD) is a rare autosomal recessive disease with a prevalence of around 1 in 30,000 individuals [1]. A defect on the copper carrier protein ATP7B is responsible for the disease. The ATP7B gene encodes a transmembrane copper-transporting ATPase that mediates the excretion of copper into bile and delivers copper for the functional synthesis of ceruloplasmin. Pathogenic mutations on the ATP7B gene prevent copper excretion leading to copper overload in hepatocytes, release of excess copper into the circulation and secondary pathological accumulation in tissues, particularly in the liver and central nervous system [2]. If left undiagnosed, both liver and neurological dysfunction is irreversible. However, the prognosis is favourable if the diagnosis is made early [1].
WD can present clinically at any age but most cases present symptomatically before the age of 40 with liver and/or neuropsychiatric disease. Liver disease is non-specific and can manifest as acute, subacute or chronic hepatitis and cirrhosis. Neurological disease can express itself as a wide range of syndromes from a subtle tremor to full-blown parkinsonism, severe ataxia or a dystonic syndrome. Behavioural symptoms can be present as manifestations of neuropsychiatric illness. Another frequent clinical manifestation can be Coombs-negative haemolytic anaemia in 12% of patients [3].
The Leipzig diagnostic criteria for WD were developed in 2001, establishing a phenotypic classification, and standardized the diagnostic approach (*Table 1*) [3].

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Signs, symptoms and typical findings			Complementary examinations		
Kayser-Fleischer rings	Present	2	Hepatic copper content	>4 µmol/g	2
	Absent	0		0.8–4 µmol/g	1
Neurological symptoms	Severe	2		Normal (<0.8 µmol/g)	–1
	Mild	1		Rhodamine-positive granules	1
	Absent	0	Urinary copper	>5×ULN after DPA	2
Serum ceruloplasmin	0.1 g/l	2		>2×ULN	2
	0.1–0.2 g/l	1		1–2 ULN	1
	>0.2 g/l	0		Normal	0
Coombs-negative haemolytic anaemia	Present	1	Mutation analysis	Both chromosomes	4
	Absent	0		One chromosome	1
				No mutation detected	0

Table 1. Leipzig score, adapted from the 8th International Meeting on Wilson's disease, Leipzig 2001.

Score ≥4 indicates diagnosis is established, score =3 indicates diagnosis is possible, but more tests are needed; and score ≤2 indicates diagnosis is very unlikely. DPA: D-penicillamine; ULN: upper limit of normal.

A diagnosis of WD is highly likely if the Leipzig score (LS) is >3, and highly unlikely if it is <2. Between those values, WD is considered probable, and additional tests are needed such as determination of the ATP7B mutation.

Around 850 mutations of the ATP7B gene have been described [4], the most common being H1069Q in exon 14, which is frequently found in European patients. In other geographical areas, different mutations predominate such as R778L in Southeastern Asia [2]. WD can develop in a homozygotic or a compound heterozygote fashion when two disease-causing mutations coexist in the same patient.

Genetic testing is not needed for diagnosis if the patient has typical clinical signs and laboratory features with a high LS. The absence of a known mutation does not exclude WD. However, the presence of known disease-causing mutations in both chromosomes makes the diagnosis certain, even in the absence of clinical symptoms [3]. This is not true in a heterozygote condition when only one chromosome has a known mutation with the patient being then considered a carrier.

There is no straightforward correlation between genotype and clinical phenotype in WD [5]. Some clinical studies have been published, but the evidence is inconclusive. Late diagnosis, poor reporting, and difficulty in applying objective measures to various degrees of neuropsychiatric symptoms further hampered these studies. Therefore, more information is needed as reporting of the clinical phenotype is important in genetic diseases, particularly when uncommon mutations are discovered.

CASE DESCRIPTION

A 24-year-old Portuguese Caucasian woman with no previous medical history, relevant family history (both parents were Portuguese) or medication was admitted with a 1-month history of bilateral, symmetric palpebral and lower limb oedema and choluria. No other signs or symptoms, specifically neurological or psychiatric manifestations, were noted. The blood work-up revealed hyperbilirubinemia (2.7 mg/dl), hypoalbuminemia (2.1 g/dl) and severe coagulopathy (prothrombin time 39.2 seconds). No anaemia or renal dysfunction was present. Abdominal ultrasonography showed hepatomegaly with no nodules and a permeable portal vein. The abdominal CT showed a homogeneous hepatomegaly with umbilical vein permeabilization. Serology was negative for viral hepatitis (A, B and C) and autoimmune hepatobiliary

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diseases. The patient reported no new medications and no alcohol or recreational drug consumption. Serum ceruloplasmin was low (0.04 g/l) and she was started on copper chelating treatment with trientine.

Subsequent investigation revealed low total serum copper (36 µg/dl), increased free serum copper (20.3 µg/dl), and markedly elevated urinary copper excretion (3840 µg/24 h). Factor V was 20.5%. There were no Kaiser-Fleischer rings. The liver biopsy showed acute hepatocellular injury with confluent necrosis and without evidence of copper pigment deposition (*Fig. 1*). Hepatic copper content was not measured. A brain MRI showed a bilateral high T2 signal in the red nucleus and substantia nigra (*Fig. 2*). Genetic analysis revealed pathogenic mutations in the ATP7B gene with compound heterozygosity: c.3402del and c.3061-12T>A. The LS was 7 and the diagnosis of WD was made.

Follow-up was uneventful. The patient was started on spironolactone in addition to trientine with a progressive improvement in liver function (*Table 2*). A mild tremor was reported did not interfere with daily activities and did not require specific medical treatment. Three years after the diagnosis the patient is well with no clinical symptoms and complete recovery of liver function tests, and maintains copper chelating therapy.

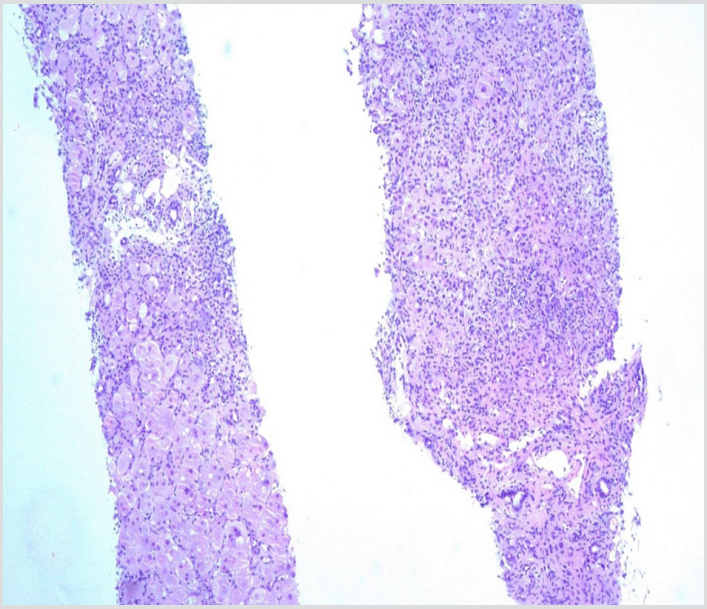

Figure 1. Acute hepatocellular injury with confluent necrosis, portal fibrosis, extensive lymphocytic inflammatory infiltrate and no evidence of copper pigment deposition (haematoxylin-eosin ×50)

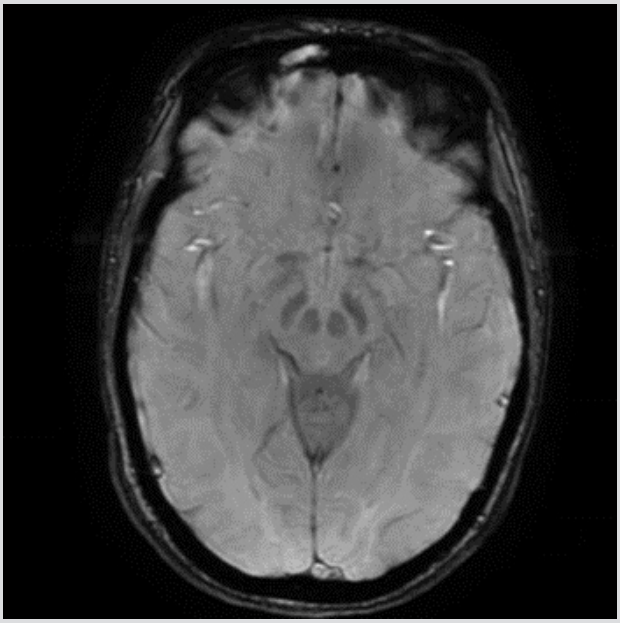

Figure 2. Bilateral high T2 signal in the red nucleus and substantia nigra consistent with the panda sign of the midbrain, classically seen in Wilson disease

	Mar/18	Jun/18	Sep/18	Apr/19	Nov/19	Jul/20
Prothrombin time	30.8	26.6	–	12.8	12.9	12.5
International Normalized Ratio	2.9	2.3	1.6	1.2	1.1	1.1
Factor V (%)	20.5	15	–	70.5	–	–
Bilirubin (mg/dl)	3.4	3.1	2.1	0.4	0.5	1.1
Albumin (mg/dl)	2.1	2.6	–	4	4.5	–
Urinary copper (µg/24 h)	3840	556	173	149	123	–

Table 2. Evolution of liver function on follow-up

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DISCUSSION

WD is rare and the diagnostic suspicion should be high in young patients with subacute or acute on chronic liver disease. A diagnosis made in time can avoid liver transplantation. There are no established criteria for liver transplantation in WD [6] but the most commonly used were taken into consideration when treating our patient: factor V reached a minimum of 15% partially fulfilling Clichy criteria but no King's College criteria were met [7]. Fortunately, our patient improved with medical therapy and intervention was not necessary.

However, the diagnostic work-up was not straightforward: suspicion for WD was high after a compatible clinical presentation and a very low ceruloplasmin result, but slit-lamp examination was negative for Kayser-Fleischer rings (as expected for a predominantly hepatic disease), haemolytic anaemia was not present, and there were no neurological symptoms at the time. The LS was 2 and it was decided to start copper chelating therapy while complementary investigations were carried out to confirm the diagnosis.

While there is plenty of evidence concerning the value the D-penicillamine (DPA) test as part of WD work-up [10–12], there is no evidence for other tests with other chelating agents such as trientine. However, there are studies on copper chelating therapy in heterozygotes and control subjects [12, 13] that show an increase in urinary copper excretion in both, with control subjects having a mean excretion of 24±16 µg/24 h before and 640±230 µg/24 h after DPA (maximum reported of 1160 µg/24 h) [14]. Our patient had a urinary copper excretion of 3840 µg/24 h and although she was already on trientine, we considered this value fulfilled the Leipzig criteria. Third, the liver biopsy was negative for rhodamine-positive granules, and the hepatic copper content was not measured.

The above work-up had an LS of 3 making WD a possible diagnosis. Therefore, we conducted genetic testing for confirmation because of the therapeutic and family implications. The molecular study reported pathogenic mutations in the ATP7B gene with compound heterozygosity of two mutational variants: c.3402del and c.3061-12T>A. The LS was then 7 and a WD diagnosis was finally made.

The first mutation c.3402del is a frameshift mutation first described in 1993 and classified as a pathological variant in the ClinVar database[15]. It is the most common mutation in WD patients in Brazil, with homozygote and heterozygote carriers of this variant commonly reporting ancestors of Portuguese origin [16-17]. It is also the most common variant in Venezuela, with some patients reporting European ancestry, possibly from Portugal [18]. The second mutation c.3061-12T>A is less well described: it could lead to a splicing error on exon 14 and is currently described as likely pathogenic [19]. The first report of this mutation was in 2002 in a compound heterozygotic patient [20] with significant neurological disease. It was also described in two consecutive generations of a French family with Portuguese ancestors [21] as well as in two other reports: an analysis of WD mutations in a French population [22] and a Portuguese clinical report of a homozygous child, again with predominantly neurological manifestations[23].

Our patient had subacute liver failure (with complete recovery under copper chelating therapy) and a mild neurological disease with 2 years of follow-up.

The c.3402del mutation has been described as liver predominant in heterozygous and neurologically predominant in homozygous patients[17]. The c.3061-12T>A mutation is described as neurologically predominant. To our knowledge, this is the first report of this compound heterozygote genotype. The evolution of our patient remains to be seen in the long-term follow-up. Cohort studies are inconclusive for the most common variants [1, 5] and the variety of heterozygotic expressions increases complexity. Some reports also seem to indicate that even homozygotic patients with environmentally similar background can have vastly different outcomes [24]. Regardless, the authors believe continued reporting to provide more information on WD is necessary so the molecular implications of this disease can be fully understood in the future.

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