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LETTER TO THE EDITORS

Next-generation sequencing: a decisive diagnostic aid for atypical Wilson's disease

Amory Jardel[1] · Céline Bonnet[2,3] · Solène Frismand-Kryloff[1] · Jean Marie Ravel[2,3] · Emmanuelle Schmitt[4] · Mickael Alexandre Obadia[5] · Sébastien Delassaux[6] · Myriam Bronner[2] · Aurelia Poujois[5,7] · Mathilde Renaud[1,3,8]

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Introduction

Wilson's disease (WD) is an autosomal recessive copper metabolic disease, first described by Kinnier Wilson in 1912. Its clinical prevalence is low: 1 in 30,000 to 100,000 births, but its genetic prevalence is higher [1].

Kayser–Fleischer ring is present in almost all Wilson's disease patients at disease diagnosis when neurologic symptoms are observed [2].

Regarding neurological symptoms, they are reported in approximately 18–68% of patients [3] and are mostly due to basal ganglia or brainstem lesions.

Finally, hepatic manifestations of Wilson's disease at presentation can be extremely variable [4].

Typically, diagnosis is based on the association of compatible clinical signs and classical biological triad: low ceruloplasmin (Cp), low serum copper and elevated 24-h urinary copper excretion.

Disease confirmation comes from the molecular analysis of *ATP7B* gene revealing one mutation in each allele.

Case history

The present report concerns the case of a woman with a neurological complaint starting in 2016 at the age of 42. The symptomatology is marked by a progressive onset of head tremor with kinetic and postural tremor of the hands.

No family history of tremor was reported. The pedigree revealed the notion of amyotrophic lateral sclerosis (ALS) affecting one of her deceased brothers as well as sudden cardiac deaths in both her parents at the age of 45 and 60 Fig. 1.

The patient is under oestro-progestative contraceptives.

In 2017, brain MRI was prescribed due to the rapid worsening of the tremor and revealed a diffuse cortical–subcortical atrophy and a bilateral mesencephalon T2 hyper-signal.

Diagnosis of essential tremor was then retained, and treatments with propranolol first and then primidone were tried without any efficacy.

In 2018, the postural and kinetic tremor of the upper limbs continued to worsen and dysarthria appeared.

At the same time, an alteration of the general state was noticed with anorexia, anxiety and weight loss without swallowing problems.

A metabolism assessment was then performed showing ceruloplasmin and serum copper in the normal range, but 24-h urinary copper excretion was not measured. Hepatic biological assessment was normal (transaminases, Bilirubin, PT, and hemogram).

A new assessment was carried out in 2020 because of the clinical worsening: Video 1.

A complete biological check-up was requested again including a new copper metabolism assessment: total

Aurelia Poujois and Mathilde Renaud contributed equally to this work.

✉ Aurelia Poujois
apoujois@for.paris

1 Service de Neurologie, CHRU Nancy, Nancy, France

2 Laboratoire de Génétique Médicale, CHRU Nancy, Nancy, France

3 Université de Lorraine, Inserm U1256, NGERE, 54000 Nancy, France

4 Service de Neuroradiologie, CHRU Nancy, Nancy, France

5 Service de Neurologie, Hôpital Fondation Adolphe de Rothschild, Paris, France

6 Centre Hospitalier Emile Durkheim d'Epinal, Epinal, France

7 Centre de Référence de la Maladie de Wilson et Autres Maladies Rares Liées au Cuivre, Service de Neurologie, Hôpital Fondation Adolphe de Rothschild, 29 rue Manin, 75019 Paris, France

8 Service de Génétique Médicale, CHRU de Nancy, Nancy, France

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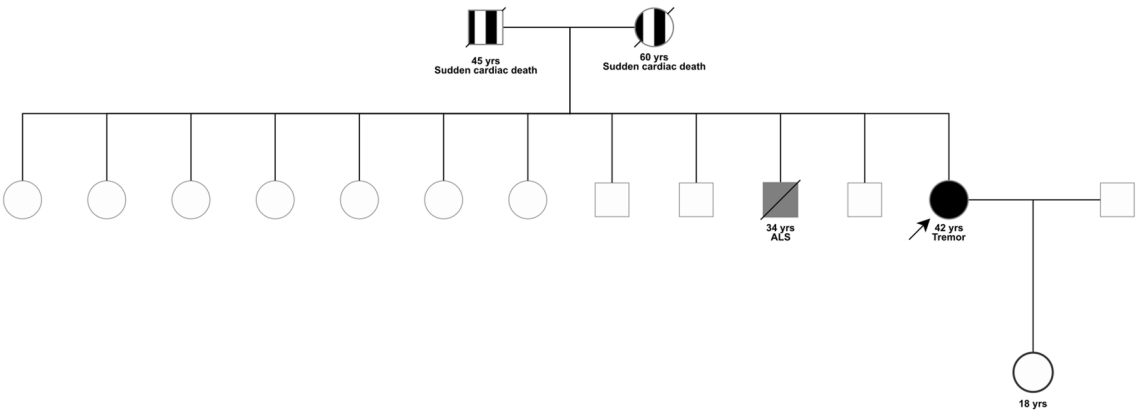

Fig. 1 Pedigree of WD patient

serum copper (17.7 µmol/l), ceruloplasmin (30 g/l) and REC (13.2%) remained normal, but exchangeable copper and 24-h urinary copper excretion were raised 2.32 µmol/l ($N < 1.05$) and 1.28 µmol/24 h ($N < 0.40$). Once again, there was no disturbance in the hepatic biological tests.

A specific assay of copper in the cerebrospinal fluid (CSF) was performed and was in favor of an abnormally high level.

Brain MRI showed the same isolated abnormalities previously described in the brain stem.

A complementary hepatic MRI and liver elastometry confirmed liver cirrhosis and slit lamp examination did not reveal any Kayser–Fleischer ring.

Next-generation sequencing (NGS) analysis was performed on genomic DNA.

Clinical exome sequencing identified compound heterozygous variants in the ATP7B gene: a splice-site variant NM_000053.3(ATP7B):c.51 + 4A > T and a missense variant,

NM_000053.3 (ATP7B):c.2128G > A,p.(Gly710Ser). According to ClinVar, these variants are classified as follows: pathogenic rated 2 stars, with 6 submissions, 13 publications and no conflicts for c.51 + 4A > T and 10 submissions, 19 publications and no conflicts for c.2128G > A.

Overall assessment entrusted to the National Center for Wilson's Disease (Rotschild Foundation Hospital) concluded that Wilson's disease was highly probable despite several atypical features.

Treatment with Trientine salt was introduced gradually associated with an active search of the disease in the history.

After 3 months of well-conducted treatment, the patient started to improve slowly on the UWDRS-specific clinical score.

Discussion

This patient presented both a classic neurological picture of Wilson's disease and a copper balance sheet, not in favor of it, along with the damage to other target organs.

Arguments that lead us to refute too quickly WD hypothesis were:

- normal serum copper and ceruloplasmin levels
- very limited MRI brain lesions
- the absence of Kayser–Fleischer ring while there is significant clinical neurological damage
- the late onset of the disease

We retain five main messages from this clinical case:

Ceruloplasmin may be falsely normal or elevated in a patient under oestro-progestative contraceptives [5].

Twenty four hour urinary copper excretion must be performed along with the serum copper and ceruloplasmin.

The direct dosage of the free copper, so-called exchangeable copper, is elevated in extra-hepatic form of the disease, but the REC (ratio exchangeable copper/total serum copper) may be falsely decreased in case of normal serum copper induced by oestro-progestative contraceptive treatment [6, 7].

It seems that Wilson's disease clinical expression varies with age, with a majority of initial neurological findings in patients over forty, especially in women like our patient [8].

Other message: in neurological form of the disease, liver disease may be limited to thrombocytopenia. Absence of Kayser–Fletcher ring and very focal brain MRI abnormalities in neurological form of WD are possible [9].

Finally, we used CSF copper assay to provide arguments about copper imputability in the patient's symptomatology [10].

We conclude from this clinical case that a standard but incomplete serum copper test with no abnormality cannot refute Wilson's disease hypothesis when the clinical suspicion is strong. NGS is then of great diagnostic utility, allowing patients to receive an effective specific treatment earlier and to improve the clinical outcome of these patients.

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Author contributions All authors listed have made substantial, direct and intellectual contribution to the work and approved it for publication.

Data availability statement Raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Conflicts of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical statement Ethical approval was not provided for this study on human participants because this research was performed as part of a routine molecular diagnostic of patients. Written informed consent to participate in this study was provided by the participant.

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