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Clinical report

New mutations and polymorphisms of the *ATP7B* gene in sporadic Wilson disease

Cong-Xia Lu [a,1], Qing Lin [a,b,1], Wen-Qing Huang [b,1], Chi-Meng Tzeng [b,*]

[a] Department of Neurology, The First Affiliated Hospital of Xiamen University, Xiamen, Fujian 361003, China
[b] Translational Medicine Research Center, School of Pharmaceutical Sciences & Institute for Biomedical Research, Xiamen University, Xiamen, Fujian 361102, China

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ABSTRACT

Wilson's disease (WD) is a rare autosomal recessive genetic disorder of copper metabolism resulting in brain damage, liver failure, and neurological impairment and psychiatric disturbances, as a result of excessive copper accumulation in the brain, liver, kidneys and eyes. *ATP7B*, encoding a copper transporter P-ATPase was identified as the causative gene of WD. Mutations in the *ATP7B* gene lead to the defection of the transmembrane transporter so that it can not metabolize copper effectively. We reported the clinical and molecular features of three unrelated and non-consanguineous WD patients. We performed molecular genetic analysis of the *ATP7B* gene in all cases by DNA sequencing, and revealed 7 novel single nucleotide polymorphisms (SNPs) and 8 well known mutations. Among them, that novel SNP (c. -520 C>T) and two well known mutations (c. 2310 C>G/p. Leu700Leu, c. 2333 G>T/A/p. Arg778Leu/Gln) coexisted in all patients and they were heterozygous and homozygous in the youngest case, respectively, indicating that they may be correlated to the pathogenesis and potentially used as a genetic biomarker for early WD diagnosis.

1. Background

Wilson's disease (Hepatolenticular Degeneration, WD) is a rare autosomal recessive genetic disorder of copper metabolism that is fatal unless detected and treated with lifelong use of D-penicillamine or Zinc acetate before serious illness development from copper poisoning. WD affects between one in 30,000 and one in 100,000 individuals worldwide [Ala et al., 2007; Gupta et al., 2005; Ye et al., 2007], but in our observation, WD is the most common inherited liver disease with a higher incidence in Han population [Mak et al., 2008; Wu et al., 2006; Ye et al., 2007]. Clinical symptoms usually appear in the teenage years and twenties, but can also begin as late as age 40 or 50 [Ferenci et al., 2007; Schilsky, 2009], and the most characteristic signs include the Kayser–Fleischer (K–F) ring-a rusty brown ring around the cornea of the eye that can be seen only through an eye exam, and decreased ceruloplasmin concentration [European Association for Study of Liver, 2012; Mak and Lam, 2008]. In addition, 40–50% affected patients present some neurological or neuropsychiatric signs [Lorincz, 2010; Walshe, 1962]. The most common neurological symptoms are parkinsonism, ataxia or rigid dystonia, followed by cognitive impairment and seizures [Ala et al., 2007; Lorincz, 2010; Walshe, 1962]. Psychiatric symptoms include attention deficit, memory loss, disturbance of intelligence and slow in reaction, often accompanied by behavioral abnormalities and personality changes [Ala et al., 2007; Lorincz, 2010; Walshe, 1962]. The genetic defect causes decreasing biliary copper excretion with excessive hepatic copper accumulation, further leading to damage in the liver, kidneys, eyes and brain. If there was not promising treatment, WD can cause severe brain damage, liver failure, and neurological impairment or psychiatric disturbances, even death.

The gene, responsible for WD, named as *ATP7B* with 21 exons (about 80 kb) is a copper transporter P-ATPase on chromosome 13q14.3 [Tanzi et al., 1993]. *ATP7B* plays a key role in incorporating copper into ceruloplasmin and moving excess copper out of the liver. Mutations in the gene lead to an abnormal copper transporter so that it can not move copper effectively at all.

Here, we examined the mutations of *ATP7B* in three WD patients from three unrelated families in the three southern cities of China by using genomic DNA-targeted sequencing to identify 15 single nucleotide polymorphisms (SNPs). Among them, 7 SNPs are novel

* Corresponding author.
E-mail address: cmtzeng@xmu.edu.cn (C.-M. Tzeng).
[1] Contributed equally to this work.

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and 8 are agreed to be reported mutations, including the putative methylation modification-associated SNP (c. -520 C>T) and the most common missense mutation (c. 2333G>T/A/p. Arg778Leu/Gln) in Asian populations. This observation widens the clinical spectrum in deciphering the polymorphisms of *ATP7B* in WD.

2. Case presentation

Three Chinese patients with Wilson's disease from the First Affiliated Hospital of Xiamen University were included in the study (1 male and 2 female). They were denoted as WD001, WD002 and WD003, respectively. All patients are unrelated and non-consanguineous individuals with negative family history, and they come from three different cities in the southern of China. They all met the diagnostic criterion with Wilson's disease in EASL Clinical Practice Guidelines [European Association for Study of Liver, 2012]. The mean age of the patients was 25 years (age range, 18–33 years). Kayser–Fleischer (K–F) rings were present in all the patients. Fig. 1A shows the K–F rings, rusty brown rings around the cornea of the eyes in WD001 patient. All the patients displayed neurological and psychiatric symptoms to various certain extents, including ataxia, tremor, dystonia, slurred speech and poor mental state (Table 1). One of the patients presented with liver cirrhosis and hypersplenism. Table 1 shows the clinical presentation and laboratory results of the three patients in details. Patient 1(WD001) received MR imaging and showed abnormalities in the tectum of midbrain, tegmentum of pons, thalamus and lentiform nuclei (Fig. 1B).

3. Molecular genetic testing

Molecular genetic analyses of the *ATP7B* gene were performed with informed consent in all WD patients, and healthy parents and brother of WD001. Firstly, Genomic DNA was extracted from whole blood of the subjects using MagCore® Genomic DNA Whole Blood Kit. The 21 exons and the promoter regions spanning 2 kb upstream of transcription start site were amplified by PCR. Then, direct sequencing of PCR products were performed by 3730 automatic sequencer (Applied Biosystems, USA). Fifteen SNPs in *ATP7B* gene were identified in subjects (Table 2). Seven of these, including 3 substitutions (c. -676 A>G, c. -520 C>T, c. -210 A>T) in promoter, 2 substitutions (c. -218 A>C, c. -75 C>A) and 2 insertions (c.-128~-127insGCCGC, c.-119~-118insCGCCG) in 5′UTR were found to be novel. Among these, the substitution (c. -520 C>T) in promoter region had the highest frequency on the chromosomes of 6 subjects, and all of 3 WD patients were heterozygous for this SNP. In addition, eight reported mutations distributed the coding region of *ATP7B*, were also detected in our study, including the silent mutation (c. 2310 C>G, p.Leu700Leu) and the most frequent mutation (c. 2333G>T/A, p. Arg778Leu/Gln) in Asian populations, especially Chinese. Total of three Chinese patients have c. 2310 C>G (p.Leu700Leu) and c. 2333 G>T/A (p. Arg778Leu/Gln) mutations. They were heterozygous in WD001 and WD002, but homozygous in WD003 patient (Fig. 2).

4. Discussion

Among human rare diseases, Wilson's disease is a potentially life threatening, but rarely curable genetic disorder of copper metabolism, caused by the mutations in crucial gene *ATP7B*, which result in the impaired biliary excretion, deficient incorporation of copper into ceruloplasmin, and consequent toxic accumulation of copper in different tissues, especially in the liver, eyes and brain. It invariably results in severe disability, neurological or psychiatric symptoms, and even death if untreated promptly. Psychiatric manifestations, including the most common features, such as incongruous behaviors, irritability, depression and personality change, are shown particularly significant in Wilson's disease, and many of psychopathologic features seem to have an organic basis [Lorincz, 2010]. Therefore, early diagnose and lifelong drug treatment, together with diet control before serious illness development from copper poisoning are essential in decreasing morbidity and mortality of WD patients. In addition to neurological examination, biochemical markers (low ceruloplasmin), and K–F rings, and genetic biomarker (*ATP7B*) are common ways for WD diagnosis [Ala et al., 2007; European Association for Study of Liver, 2012; Mak and Lam, 2008].

More than 500 mutations of the *ATB7B* have been identified worldwide with different ethnic and geographical variation thus far, and most patients are compound heterozygotes [Ala et al., 2007; Kucinskas et al., 2008]. Sequencing analysis of *ATP7B* revealed 7 novel SNPs in our cases, which were not previously reported in the Wilson Disease Mutation Database of University of Alberta (accession to: December, 2012) (http://www.

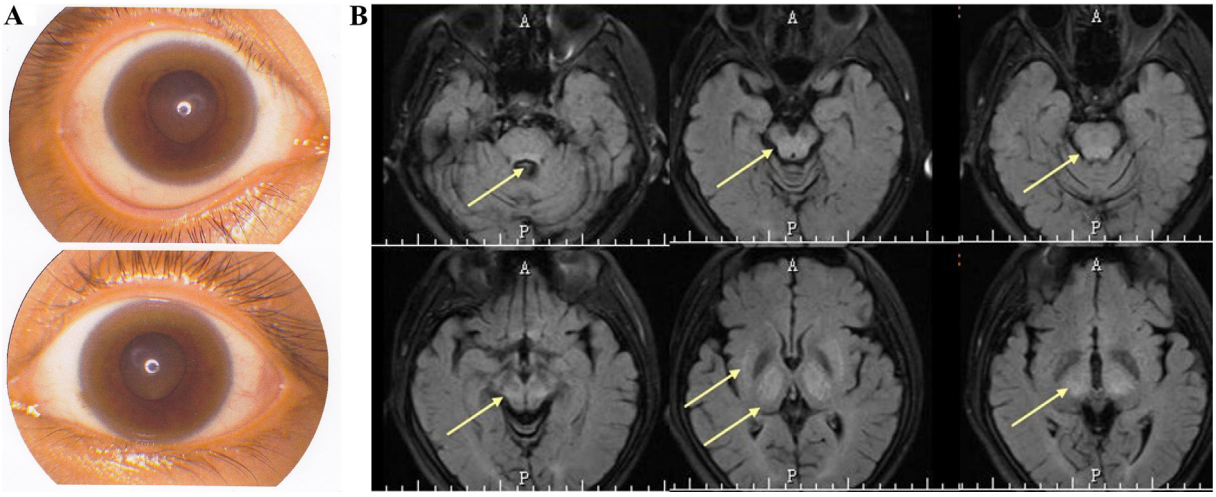

Fig. 1. K–F rings and abnormal MRI of the Wilson's disease patient (WD001). A. The results of slit lamp eye examination revealed the Kayser–Fleischer (K–F) rings, rusty brown ring around the cornea of the eyes, because of the deposition of copper in descemet's membrane in the patient (WD001). B. Brain Magnetic Resonance axial FLAIR image displaying symmetrical high signal intensity changes in the tectum of midbrain, tegmentum of pons, thalamus and lentiform nuclei (arrow head) in the Wilson's disease patient (WD001).