Wilson Disease With Novel Compound Heterozygote Mutations in the *ATP7B* Gene Presenting With Severe Diabetes

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OBJECTIVE

To determine the relationship between *ATP7B* mutations and diabetes in Wilson disease (WD).

RESEARCH DESIGN AND METHODS

A total of 21 exons and exon-intron boundaries of *ATP7B* were identified by Sanger sequencing.

RESULTS

Two novel compound heterozygous mutations (c.525 dupA/ Val176Serfs*28 and c.2930 C>T/ p.Thr977Met) were detected in *ATP7B*. After D-penicillamine (D-PCA) therapy, serum aminotransferase and ceruloplasmin levels in this patient were normalized and levels of HbA_{1c} decreased. However, when the patient ceased to use D-PCA due to an itchy skin, serum levels of fasting blood glucose increased. Dimercaptosuccinic acid capsules were prescribed and memory recovered to some extent, which was accompanied by decreased insulin dosage for glucose control by 5 units.

CONCLUSIONS

This is the first report of diabetes caused by WD.

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism, which is characterized by hepatic and neurologic diseases and caused by mutations in the *ATP7B* gene (1). *ATP7B* codes for a copper-transporting P-type ATPase, which plays an important role in the transmembrane transport of copper (2,3).

WD is among a limited number of genetic diseases that can be partly treated or prevented by copper-chelating agents (penicillamine and dimercaptosuccinic acid). The absence or delay in treatment for WD may cause death or disorders of the liver, brain, and cornea (2,4). Therefore, early diagnosis and treatment are critical for better outcomes of WD.

RESEARCH DESIGN AND METHODS

Patient

This study was approved by the Ethics Committee of the Liyuan Hospital (Wuhan, China). Informed consent was obtained from the patient's family member.

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Information Collection

Genomic DNA was extracted from peripheral blood. Clinical information and biochemical characteristics were collected. Liver ultrasound examination, ophthalmologic examination, and brain nuclear MRI examination were performed.

Mutational Analysis by Sanger Sequencing

A total of 21 exons and exon-intron boundaries of the *ATP7B* gene were amplified by PCR. PCR products were determined by Sanger sequencing.

RESULTS

Current Medical History

The patient is a 47-year-old man who was admitted to the hospital for uncontrolled high blood glucose and abnormally increased hepatic transaminases. However, no obvious signs of dry mouth, polydipsia, polyuria, polyphagia, or weight loss were observed. The patient's memory had gradually declined since 2005. In 2009, the patient was diagnosed with diabetes after he had suffered an episode of diabetic ketoacidosis. He was prescribed metformin and subcutaneous injections of insulin; however, he failed to achieve ideal outcomes. In general, the HbA_{1c} level fell within the range of 10–15% (86–140 mmol/mol). The WD patient's mother, brother, and an older sister all died of unknown reasons at an age between 30 and 40.

Clinical Characteristics

There was trembling of the patient's hands. Blood pressure and serum lipids levels were in the normal range. Fasting plasma glucose (FPG) and HbA_{1c} levels were found to be extremely high, diabetic autoantibodies (GAD, islet cell antibody, and insulin autoantibody) were all negative, and fasting C-peptide decreased significantly. Together, the above results suggested that the function of islet cells might be severely impaired in this patient. The laboratory data of the patient demonstrated elevated liver enzymes, decreased ceruloplasmin, and slightly increased 24-h urinary copper excretion. Antibodies directed against hepatitis A, B, C, and E, and as well as autoimmune hepatitis antibodies were negative. The ophthalmologic examination showed negative Kayser-Fleischer rings. Ultrasound examination of the liver and pancreas demonstrated that liver and pancreas morphology was normal, and echography of the parenchyma was uniform, without obvious abnormal signs. T1-weighted image waves showed a symmetrically triangular higher density of the lentiform nuclei in the bilateral basal ganglia as shown by brain nuclear MRI.

Direct DNA Sequencing for Mutation Analysis of *ATP7B*

In this patient, a total of 21 exons and exon-intron boundaries of the *ATP7B* gene were sequenced, identifying one heterozygous mutation (c.525 dupA, Val176Serfs*28) in exon 2 and one heterozygous mutation (c.2930 C>T, p.Thr977Met) in exon 13, which were known to pathogenic mutations rs758115611 and rs72552255, respectively.

Follow-up of the Patient Treated With Copper-Chelating Agents

The patient was admitted to the Liyuan Hospital Endocrinology Department with diabetes. When combining the clinical characteristics, family disease history, and the sequencing results of *ATP7B*, we concluded that the patient suffered from hepatolenticular degeneration. Therefore, D-penicillamine (D-PCA) was recommended at 0.25 g, three times daily. After 3 weeks of treatment, the serum concentrations of ceruloplasmin increased from 0.11 to 0.17 g/L, serum concentrations of alanine aminotransferase and AST declined from 75.9 to 41.1 units/L and from 45.7 to 25.7 units/L, respectively, the serum concentrations of bilirubin-direct or γ-glutamyl transpeptidase declined from 8.0 to 3.9 μmol/L and from 70.9 to 30.6 units/L, respectively, and the patient's neurologic symptoms and the difficulty in speaking were alleviated.

When the patient visited the hospital to review his condition 3 months later, the HbA_{1c} level declined from 9.5% (80 mmol/mol) to 7.6% (60 mmol/mol), the serum concentration of ceruloplasmin declined from 0.17 to 0.12 g/L, and the serum concentration of fasting C-peptide and glucagon increased from 0.44 to 0.86 ng/mL and from 52.5 to 76.2 ng/L, respectively, which suggested that the patient's islet cell function had slightly recovered. The patient refused to use D-PCA because of an itchy skin (allergic to D-PCA). We therefore recommended that the patient use another copper chelator, dimercaptosuccinic acid capsules (0.5 g/8 h, use 3 days a week, then stop for 4 days). After 3 months of treatment, the HbA_{1c} level of the patient approached normal levels, memory recovered significantly, and insulin use decreased by 5 units.

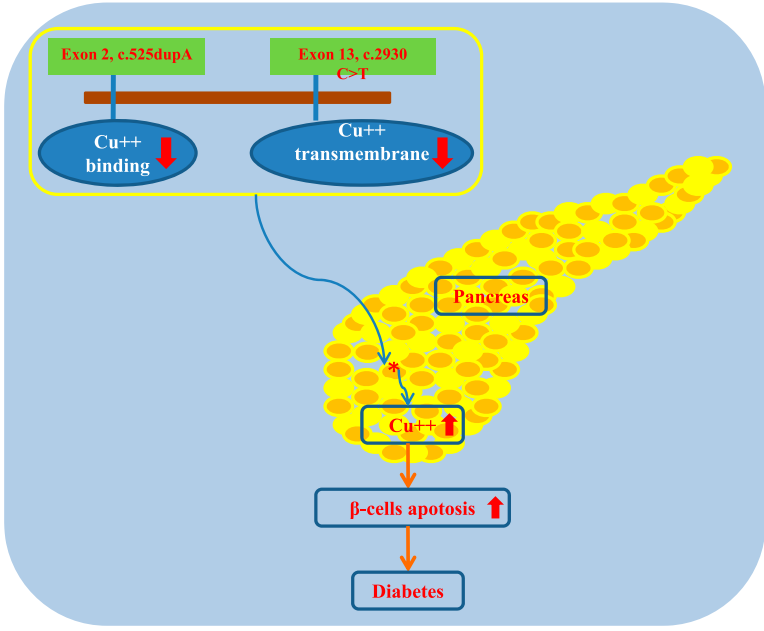

Figure 1—The mutations in *ATB7P* may cause absent or reduced function of ATP7B protein, leading to accumulation of copper in the pancreas and causing apoptosis of β-cells and the progressive features of diabetes.

CONCLUSIONS

Previous studies have reported that *ATP7B* is most highly expressed in the liver, but is also found in the brain, kidney, placenta, mammary glands, and lung (5), and is rarely expressed in the pancreas. However, no reports are available on the correlation between copper and the pancreas. In the current study, compound heterozygous variants Val176Serfs*28 and p.Thr977Met were identified in the *ATP7B* gene of a WD patient, and the above-mentioned mutations were located in copper-binding sites and transmembrane domains of the copper channel (6), respectively. After treatment with D-PCA, the serum ceruloplasmin level was elevated, and the levels of FPG and HbA_{1c} were reduced, while FPG was increased when D-PCA treatment was terminated. In addition, the levels of FPG and HbA_{1c} were reduced when dimercaptosuccinic acid capsules were used. Furthermore, other factors, including various hepatitis viruses causing liver dysfunction, autoimmune diabetes, and type 1 diabetes were excluded. We therefore speculated that the mutations in *ATB7P* may cause absent or reduced function of the ATP7B protein, leading to the accumulation of copper in the pancreas and causing apoptosis of β-cells and progressive features of diabetes (Fig. 1). To our knowledge, this is the first report of diabetes caused by WD; however, the underlying molecular mechanism(s) involved in the influence of copper accumulation on the development of diabetes remain to be elucidated in future studies.

Currently, little is known about the association between the *ATP7B* genotype and the phenotype of disease. It has been proposed that non-H1069Q mutations, such as Arg778Leu or W939C are associated with liver disease, whereas the H1069Q mutation was mainly associated with neurologic disease. In addition, mutations c.2299insC and p.Ala1003Thr were associated with both liver and neurologic syndromes (7–10). In this study, the WD patient with compound heterozygous variants Val176Serfs*28 and p.Thr977Met in the *ATP7B* gene presented with severe neurologic disease, severe diabetes, and slight liver disease; therefore, we suggested that the compound heterozygous variants Val176Serfs*28 and p.Thr977Met mutations were mainly associated with neurologic disease and diabetes as well as slightly recurrent liver damage.

WD is among a limited number of inherited diseases for which symptoms can be prevented if affected individuals can be identified and treated at an early stage (11). Thus, early diagnosis and treatment are critical to prevent neuropsychiatric, hepatic, and systemic disabilities. Currently, direct Sanger sequencing of *ATP7B* is the most sensitive and gold standard testing method for WD. Therefore, our findings provide insights into novel mutations in the *ATP7B* of WD patients with novel phenotypes, which may help develop better and accurate diagnosis and treatment of individual WD-affected patients.

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