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RESEARCH ARTICLE

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Genetic studies discover novel coding and non-coding mutations in patients with Wilson's disease in China

Chenjun Huang[1] | Meng Fang[2] | Xiao Xiao[1] | Zhiyuan Gao[1] | Ying Wang[2] | Chunfang Gao[1]

[1]Department of Clinical Laboratory Medicine Center, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

[2]Department of Laboratory Medicine, Shanghai Eastern Hepatobiliary Surgery Hospital, Shanghai, China

Correspondence

Ying Wang, Department of Laboratory Medicine, Shanghai Eastern Hepatobiliary Surgery Hospital, Shanghai 200438, China.
Email: nadger_wang@139.com

Chunfang Gao, Department of Clinical Laboratory Medicine Center, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China.
Email: gaocf1115@163.com

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Abstract

Objectives: Wilson disease (WD) is a rare autosomal recessive genetic disorder associated with various mutations in the ATP7B gene and leads to significant disability or death if untreated. Early diagnosis and proper therapy usually predict a good prognosis, especially in pre-symptomatic WD. Genetic testing provides an accurate and effective diagnostic method for the early diagnosis of WD.

Methods: We recruited 18 clinically diagnosed WD patients from 16 unrelated families and two independent individuals. The next-generation sequencing of the ATP7B gene was performed. The 293T cell lines were divided into wild-type (WT) ATP7B and mutated ATP7B groups. Cell proliferation was determined by Cell Counting Kit-8 (CCK-8) assay and apoptosis was detected by Annexin V/propidium iodide (PI) assays.

Results: Pedigree analysis showed that compound heterozygous variants (17/18, 94.44%) were present in the majority of WD patients. A total of 33 ATP7B gene variants were identified, including three variants with uncertain significance (VUS) [two splice mutations (c.51+2T>G, c.1543+40G>A) and one frameshift mutation (c.3532_3535del)]. The CCK-8 and apoptosis assays demonstrated that the VUS of ATP7B could significantly affect the transportation of copper.

Conclusions: The study revealed genetic defects of 16 Chinese families and two independent individuals with WD, which enriched the mutation spectrum of the ATP7B gene worldwide and provided valuable information for studying the mutation types of ATP7B in the Chinese populations. Genetic testing in WD patients is necessary to shorten the time to initiate therapy, reduce damage to the liver and improve the prognosis.

KEYWORDS

ATP7B, Chinese population, mutation, next-generation sequence, Wilson disease

Chenjun Huang and Meng Fang have contributed equally to this work.

Correction added on 30th April 2022, after first online publication: the contributor details `Chenjun Huang and Meng Fang have contributed equally to this work' has been updated.

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1 | INTRODUCTION

Wilson disease (WD), first reported by Kinnear Wilson in 1912, is a rare autosomal recessive inherited disease involving copper metabolism disturbance due to the mutations of ATP7B gene that encodes the P-type ATPase.[1,2] Defects of ATP7B will reduce the ceruloplasmin (CP) plasma levels and affect the transport of copper to plasm CP with pathological copper accumulation in different organs (liver, kidney, and other tissues). [3,4] Excess copper exposure in these tissues can lead to secondary organ damage, including liver cirrhosis, limbal Kayser–Fleischer (K-F) ring, neurologic degeneration, and other clinical symptoms.[2-5]

Early diagnosis and intervention of WD are critical to limit disease progression, and the disease is lethal if the patients is not treated early. The diagnosis of WD is usually conducted by combining the signs, symptoms, laboratory, and imaging information.[5-7] However, each of the diagnostic strategies has its limitations.[2] It is reported that due to the clinical heterogeneity of WD, only about 30% of WD patients were accurately diagnosed of the beginning medical consultation.[8]

The detection of ATP7B gene mutation may be powerful tools for WD accurately diagnosis, especially for patients who have mild-to-moderate disease.[8,9] The frequency of p.r778l (c.2333g>t, exon 8) reported by China is 17.3%–31.9%, which is the most common of ATP7B mutations.[10-12] However, because some studies have reported that the frequency of heterozygotes is much higher than that of homozygotes, WD disease shows genetic heterogeneity.[10,13,14]

Previous studies have usually focused on unrelated individuals,[10,15,16] case reports,[17,18] or a limited number of pedigrees.[19-21] In addition, the functional consequences of these mutations still lack direct experimental evidence. Therefore, we recruited 18 WD Chinese patients and their 43 first-degree relatives from 16 families and two independent individuals for DNA sequencing to systematically analyze the genotypes of Chinese WD patients. We also conducted a series of experiments to elucidation of possible functional consequences of these ATP7B mutations.

2 | METHODS

2.1 | Patients and diagnostic criteria

Patients with WD or suspected WD and their first-degree relatives were recruited from the Shanghai Eastern Hepatobiliary Surgery Hospital (EHBH) and Yueyang Hospital of Integrated Traditional Chinese and Western Medicine of Shanghai University of Traditional Chinese Medicine between January 2019 and June 2021. The diagnosis of WD was established according to the scoring system provided by the 8th International Meeting on Wilson disease and Menkes disease[5] and the EASL Clinical Practice Guidelines for Wilson disease.[22] The biochemical parameters, clinical presentation, and medical history were recorded in the Department of Laboratory Medicine of EHBH. All the patients and their relatives provided written informed consent, and this study was approved by the Ethics Committee of the EHBH (EHBHKY2020-02–013).

2.2 | Identification of ATP7B gene variants

2.2.1 | DNA extraction and sequencing

Blood DNA Extraction Kit (Cwbio, CWY049S) was used for isolating genomic DNA from peripheral blood. In the presence of high salt, DNA binds to the surface of silicon-coated magnetic beads. The isolated DNA concentration was determined by the Qubit dsDNA HS Assay Kit (Life Technologies). For each serum ATP7B gene of the samples, ATP7B was sequenced through the MiSeq sequencer using the Illumina paired-end sequencing protocol using the MiSeq Reagent Kit, V3 (Illumina, San Diego, CA, USA), as we previously established and optimized.[23] The reads were aligned with the hg19 (UCSC) reference genome. All codes for WGBS data analysis are available on GitHub (https://GitHub.com/cemordarun/WilsonDiseaseEpigenome).

2.2.2 | Variant identification

The sequencing results were aligned to referred ATP7B sequence (NM_000053.3) to figure out the mutations. When a genetic variation meets all the following criteria, the variation with uncertain significance (VUS) is identified: (1) no reports in PubMed literatures; (2) no records in Human Gene Mutation Database (HGMD, http://www.hgmd.org/) and WD Mutation Database (http://www.wilsondisease.med.ualberta.ca/database.asp).

2.3 | The experiment of functional consequences

2.3.1 | Cell culture

The 293T cell lines were obtained from the National Collection of Authenticated Cell Cultures (Shanghai, China) and cultured in basic DMEM (GIBCO, California, USA) supplemented with 10% fetal bovine serum and 100 U / ml penicillin/streptomycin (Invitrogen). All cultures were stored in a humidified incubator at 37 °C and 5% CO_2.

2.3.2 | Lentiviral transduction

The construction of lentivirus vector expressing ATP7B and ATP7B mutation is the same as previous studies.[24,25] Then, the 293T cell lines were transfected with GV341-ATP7B and ATP7B mutant expression vectors. The relative expression of the ATP7B gene was detected by quantitative real-time PCR. The expression of ATP7B protein in 293T cells was detected by Western blot. The 293T cells

transfected with naked GV341 vector without ATP7B sequence and non-transfected 293T cell lines were used as controls.

2.3.3 | ATP7B protein secretion assays

The total protein of 293T cell line was extracted using sample loading buffer (Cat No. P0015; beyotime Biotechnology Institute), and the proteins were determined using BCA Protein Assay Kit (Cat No. P0010S; beyotime Biotechnology Institute). The proteins were separated by SDS-PAGE (10% separation gel and 4% concentrated gel) and transferred to a polyvinylidene fluoride membranes. After blocking with non-fat milk dissolved in Tris-buffered saline with Tween-20 buffer (Tris HCl, NaCl, and Tween-20) at 37℃ for 1h, the membrane was incubated with anti-ATP7B (1:1000) antibody at 4℃ overnight. The membrane was then incubated with 15 mL of horseradish peroxidase-labeled secondary antibody (1:2000, Cat No: 31490, Thermo Fisher Scientific, Inc.) at room temperature for 1 h. Signals were visualized with the Odyssey Infrared Imaging System (LI-COR® Odyssey® 700 or 800 channels).

2.3.4 | Cytotoxicity assay

Cytotoxicity of $CuCl_2$ in 293T cell line was assessed using the Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies Inc., Rockville, MD, USA). Briefly, $4{\times}10^3$ cells were seeded into 96 well plates and allowed to rest for 24 h. $CuCl_2$ was diluted to concentrations ranging from 300 to 500 μM. Cells were incubated with increasing doses of $CuCl_2$ and after 48, 72, and 96 h, cell viability was measured using the CCK-8 kit according to the manufacturer's protocol.

2.3.5 | Visible assay of apoptosis

According to the manufacturer's instructions, the apoptosis of 239T cell lines was analyzed by Annexin V-APC propidium iodide (PI) staining (Invitrogen). Briefly, the cells were washed with PBS and resuspended in 1 × binding buffer at a 1 × 106 cells/mL concentration. Two hundred microliters of the cell suspension, 10 μl of Annexin V-APC, and 5 μl of PI were added. After 15 min of incubation, the cells were analyzed by flow cytometry. Living cells (Annexin V-APC-/PI-), early apoptotic cells (Annexin V-APC+/PI-), late apoptotic cells (Annexin V-APC+/PI+), and necrotic cells (Annexin V-APC-/PI+) were distinguished and quantified. The apoptotic ratio refers to the percentage of cells with Annexin V-APC+/PI- fluorescence and Annexin V-APC+/PI+fluorescence.

2.4 | Statistical analysis

Continuous variables were reported as mean ±standard deviation (SD). The unpaired two-tailed t-test was used for comparison between the two groups. P values <0.05 were considered statistically significant. The data were analyzed using Prism 8.0 (GraphPad, USA) and R language for Windows (http://www.r-project.org/).

3 | RESULTS

3.1 | Variants identified in the ATP7B Gene

By detecting the ATP7B mutation of patients and their immediate family members, 18 WD patients and 43 first-degree relatives (including 33 males and 28 females) were recruited. They were recruited from 16 unrelated families and two independent individuals aged from 2 to 78 years. Table 1 and Figure 1 summarized the clinical characteristics of these patients. Among these, 11 patients had some clinical manifestations of hepatic origin, such as hepatomegaly, splenomegaly, thrombocytopenia, and elevation of serum bilirubin. Three patients had different neurological abnormities, such as tremor, dysarthria, dystonia, epilepsy, or muscle weakness. Nine patients had K-F rings. All patients had decreased serum ceruloplasmin levels.

3.2 | Mutation analysis

By performing the genetic screening on the 18 WD probands and their relatives, a total of 33 ATP7B gene variants were identified, including 24 missense mutation (c.695C>T, c.1168A>G, c.2111C>T, c.2267C>T, c.2333G>T, c.2605G>A, c.2621C>T, c.2662A>C, c.2804C>T, c.2924C>A, c.2930C>T, c.2975C>T, c.3074T>G, c.3104G>T, c.3316G>A, c.3443T>C, c.3459G>T, c.3517G>A, c.3532A>G, c.3646G>A, c.3836A>G, c.3889G>A, c.3956G>A, c.3960G>C), four splice mutation (c.51+2T>G, c.1543+1G>T, c.1543+40G>A, c.1708-1G>C), four frameshift mutation (c.2304dupC, c.3532_3535del, c.3767_3768insCA, c.525dupA), and one large fragment deletion (c. 52544898_52558635del defined as delEXON2), showed in Table 1. Seventeen patients harbored compound heterozygous variants and one patient had one homozygous variant (c.3074T>G).

As shown in the Table 1, the mutations located in different protein domains, including the ATP bind domains (p.V1216 M, p.D1279G, p.Q1256Hfs*74, p.R1320S, p.V1106I, p.I1148T, p.W1153C, p.E1173K, p.T1178Pfs*12, p.T1178A, p.V1297I), Cu^+-binding domains (p.V176Sfs*27, delEXON2, p.P232L, p.I390V), phosphorylation domain (p.M1025R, p.G1035V), transduction domain (p.G869R, p.A874V, p.T888P), and the transmembrane domains (p.T704I, p.A756V, p.M769Hfs*25, p.R778L, p.T935 M, p.S975Y, p.T977 M, p.P992L). We reviewed HGMD, WD Mutation Database, and PubMed literatures in our WD cohort, the two splice mutations (c.51+2T>G, c.1543+40G>A) and one frameshift mutation (c.3532_3535del), being reported for the first time, were defined as variants with uncertain significance (VUS) according to the American College of Medical Genetics and Genomics (Table 1).[26]

TABLE 1 Mutations identified in ATP7B gene

Patients	Mutation	Change of Nuclear acid	Chromosome	Mutation position	types of mutation	Change of amino acid		Domain of protein
1	c.3316G>A	GGTGCAAAGT	Ch13	Exon 15	Missense	p.V1106I	GIGCKVSNVE	ATP loop
	c.51+28788_c.1272	-	Ch13	Exon 2	Deletion	-	-	Cu2/Cu3/Cu4
2	c.2333G>T	GGGCCGGTGG	Ch13	Exon 8	Missense	p.R778L	FVFIALGRWL	TM4
	c.2267C>T	CTGAGAAGGC	Ch13	Exon 8	Missense	p.A756V	AVAEKAERSP	TM3/TM4
3	c.2662A>C	AGCTACCCAC	Ch13	Exon 11	Missense	p.T888P	GSVLIKATHV	Td/TM5
	c.695C>T	ACTAACCCAA	Ch13	Exon 2	Missense	p.P232L	NPKRPLSSAN	Cu3
	c.3960G>C	AGGATACGCA	Ch13	Exon 19	Missense	p.R1320S	IHLSKRTVRR	ATP hinge/TM7
4	c.2621C>T	TTGCGGGGTC	Ch13	Exon 11	Missense	p.A874V	TVIARSINAH	Td/TM5
	c.2333G>T	GGGCCGGTGG	Ch13	Exon 8	Missense	p.R778L	FVFIALGRWL	TM4
5	c.3889G>A	CGTCGTCCTT	Ch13	Exon 18	Missense	p.V1297I	AIEAADVVLI	ATPhinge/TM7
	c.3532_3535del	ACAGACAGCC	Ch13	Exon 16	Frame shift	p.T1178Pfs*12	DHEMKGQTAI	ATP loop
	c.2930C>T	CACGGTGCTG	Ch13	Exon13	Missense	p.T977 M	AFQTSITVLC	TM6
6	c.2662A>C	AGCTACCCAC	Ch13	Exon 11	Missense	p.T888P	GSVLIKATHV	Td/TM5
	c.51+2T>G	GTGAGTTTTG	Ch13	Intron 1	Splice	-	-	before Cu1
7	c.3316G>A	GGTGCAAAGT	Ch13	Exon 15	Missense	p.V1106I	GIGCKVSNVE	ATP loop
	c.2333G>T	GGGCCGGTGG	Ch13	Exon 8	Missense	p.R778L	FVFIALGRWL	TM4
8	c.3767_3768insCA	TCCACAGAAT	Ch13	Exon 18	Frame shift	p.Q1256Hfs*74	KVQELQNKGK	ATP bind
	c.3517G>A	ACAGACCACG	Ch13	Exon 16	Missense	p.E1173K	DHEMKGQTAI	ATP loop
9	c.2605G>A	GAAACCCGGA	Ch13	Exon 11	Missense	p.G869R	AMPVTKKPGS	Td
	c.1543+1G>T	GTAAGAGATG	Ch13	Intron 3	Splice	-	-	Cu5
	c.3646G>A	GTGTGGACGT	Ch13	Exon 17	Missense	p.V1216 M	SMGVDVVLIT	ATP bind
10	c.1543+40G>A	GAATGCTGCG	Ch13	Intron 3	Splice	-	-	Cu5
	c.3836A>G	CCAGGCAGAC	Ch13	Exon 18	Missense	p.D1279G	DSPALAQADM	ATP hinge
11	c.1708-1G>C	TAATGACAAG	Ch13	Intron 5	Splice	-	-	Cu6
	c.1168A>G	ATATCGGTGT	Ch13	Exon 2	Missense	p.I390V	ISQLEGVQQI	Cu3/Cu4
	c.3956G>A	GACTGTCCGA	Ch13	Exon 19	Missense	p.R1319Q	IHLSKRTVRR	ATP hinge/TM7
	c.2975C>T	GCCACGCCCA	Ch13	Exon 13	Missense	p.P992L	TPTAVMVGTG	TM6/Ph
12	c.3074T>G	CTGTGATGTT	Ch13	Exon 14	Missense	p.M1025R	IKTVMFDKTG	Ph
13	c.2975C>T	GCCACGCCCA	Ch13	Exon 13	Missense	p.R1319Q	IHLSKRTVRR	TM6/Ph
	c.2111C>T	GTACCTTTGT	Ch13	Exon 7	Missense	p.T704I	ILCTFVQLLG	TM2
14	c.3532A>G	ACAGACAGCC	Ch13	Exon 16	Missense	p.T1178A	DHEMKGQTAI	ATP loop
	c.2975C>T	GCCACGCCCA	Ch13	Exon 13	Missense	p.P992L	TPTAVMVGTG	TM6/Ph

TABLE 1 (Continued)

Patients	Mutation	Change of Nuclear acid	Chromosome	Mutation position	types of mutation	Change of amino acid		Domain of protein
15	c.2333G>T	GGGCCGGTGG	Ch13	Exon 8	Missense	p.R778L	FVFIALGRWL	TM4
	c.525dupA	GAGTCAAAGT	Ch13	Exon 2	Frame shift	p.V176Sfs*27	VVRVKVSLSN	Cu2
16	c.2804C>T	CTTTGACGTT	Ch13	Exon 12	Missense	p.T935 M	MSTLTLVVWI	TM5
	c.2304dupC	GCCCCCCATG	Ch13	Exon 8	Frame shift	p.M769Hfs*25	VTFFDTPPML	TM4
17	c.2924C>A	AGACGTCCAT	Ch13	Exon 13	Missense	p.S975Y	AFQTSITVLC	TM6
18	c.3459G>T	GGCTGAGGCG	Ch13	Exon 16	Missense	p.W1153C	REWLRRNGLT	ATP loop
	c.3316G>A	GGTGCAAAGT	Ch13	Exon 15	Missense	p.V1106I	GIGCKVSNVE	ATP loop
19	c.3104G>T	GTTTTATCGA	Ch13	Exon 14	Missense	p.G1035V	FDKTGTITHG	Ph
	c.3443T>C	TCAGGAAGGT	Ch13	Exon 16	Missense	p.I1148T	PQTFSVLIGN	ATP loop

3.3 | Effect of mutations on copper transport

However, the functional and clinical relevance of many VUS identified through clinical genetic testing is unclear. In order to determine the effect of mutation on ATP7B transport function, we analyzed the biological consequences of the ATP7B variant by constructing lentivirus vector expression plasmid containing wild-type (WT) ATP7B or its mutant variant. It is difficult to construct the lentiviral vector expression plasmids with the splice mutations. Therefore, the fragment deletion mutation of p.T1178fs was used to evaluate the functional consequences of ATP7B variants. The known pathogenic variant delEXON2 was selected as positive control.[27]

The expression of ATP7B from 293T cell line transformed mutant strains was confirmed by Western blot. $ATP7B^{delEXON2}$ and $ATP7B^{T1178fs}$ showed weaker expression compared with the WT ATP7B (Figure 2A). The CCK-8 assay was used to determine whether $CuCl_2$ caused cytotoxicity in 293T cells after 48, 72, and 96 hours of $CuCl_2$ exposure, respectively. $CuCl_2$ did not cause cytotoxicity in the CCK-8 assay after 96 hours at a concentration of 300 μM. When exposed to 500 μM $CuCl_2$ for the same amount of time, $ATP7B^{delEXON2}$ and $ATP7B^{T1178fs}$ significantly reduced the viability of cultured cells when compared to WT groups for 72 and 96 hours (Figure 2B).

To clarify the mechanism of $CuCl_2$-induced 293T cell death, annexin V/PI staining was performed to determine quantitatively the apoptotic cell percent by flow cytometry (Figure 2C). The early apoptotic cell rate in the WT group was 2.90 ± 0.17%t after 72 hours at 300 μM concentrations. In contrast to the WT group, the $ATP7B^{delEXON2}$ (4.37 ± 0.23) and $ATP7B^{T1178fs}$ (5.45 ± 0.21%) groups had higher rates of early apoptotic cells ($p < 0.05$). Similarly, in the 300 μM concentrations, the rate of late apoptotic cells was significantly higher in the $ATP7B^{delEXON2}$ (5.60 ± 0.26%) and $ATP7B^{T1178fs}$ (6.30 ± 0.00%) groups compared to the WT group (3.37 ± 0.15%; $p < 0.05$). The $CuCl_2$ at concentrations of 500 μM induced early apoptotic and late apoptosis of the 293T cells at a higher proportion compared with the $CuCl_2$ at concentrations of 300 μM (early apoptotic cell of $ATP7B^{delEXON2}$: 11.20 ± 0.44%, $ATP7B^{T1178fs}$: 12.30 ± 0.28%; late apoptotic cell of $ATP7B^{delEXON2}$: 21.67 ± 0.86%, $ATP7B^{T1178fs}$: 15.20±0.28%). These results show that $CuCl_2$ caused a remarkable increase in cellular apoptosis in a concentration-dependent manner.

4 | DISCUSSION

WD is a treatable monogenic disease.[15] Its main symptoms include liver and nervous system diseases, ranging from mild abnormalities to severe progression.[28,29] Traditionally, the diagnosis of WD mainly depends on clinical manifestations and routine biochemical indexes, including hepatic copper content, 24-hour elevated urinary copper, and decreased serum CP.[30] However, patients with mild symptoms may be misdiagnosed, resulting in delayed treatment.[20] Gene detection of ATP7B gene mutation may lead to reliable early diagnosis and treatment to prevent copper accumulation and tissue

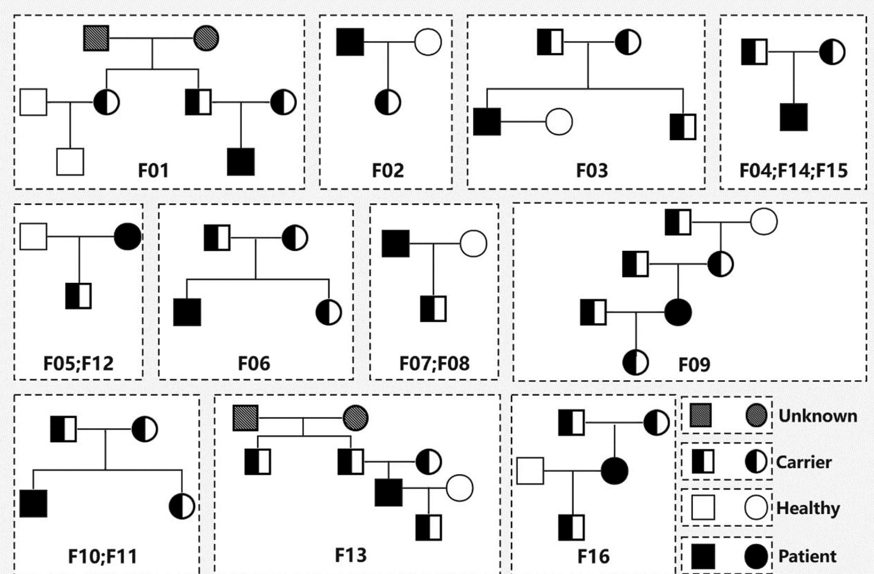

FIGURE 1 Representative pedigree analysis of 16 families

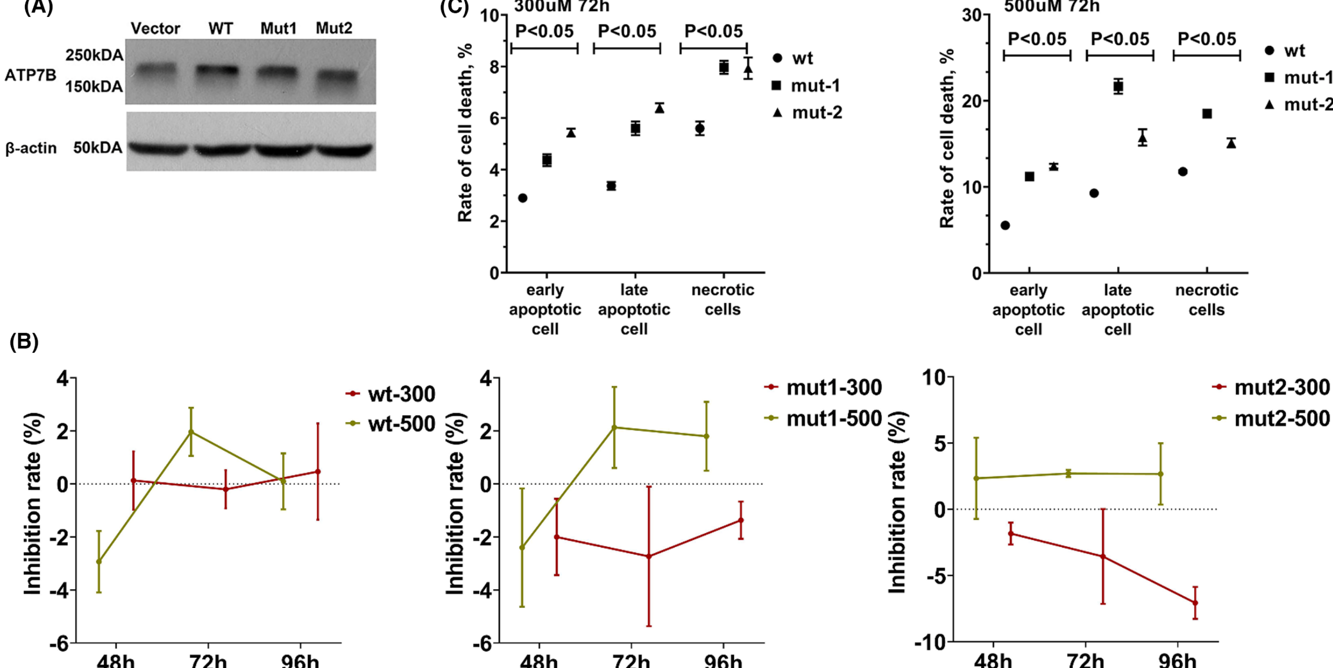

FIGURE 2 Effect of mutations on copper transport. (A) Western blot analysis and quantification of ATP7B in 297T cell lines. $ATP7B^{delEXON2}$ and $ATP7B^{T1178fs}$ showed weaker expression compared with the WT ATP7B. (B) Effect of the mutations in ATP7B on the proliferation of 293T cell lines, as determined by CCK-8 assay. (C) Effect of the mutations in ATP7B on the apoptosis of 293T cell lines

damage.[31,32] Nowadays, ATP7B gene detection is suitable for prenatal diagnosis and neonatal screening.[33]

At present, more than 500 mutations of ATP7B have been described.[34] In our study, when we finished sequencing, a total of 33 different mutations were identified in 18 Chinese WD patients, including VUS [two splice mutations (c.51+2T>G, c.1543+40G>A) and one frameshift mutation (c.3532_3535del)]. The mutations were characterized by pervasive compound heterozygous and rare homozygous, and common missense and point mutations.[34] In general, the mutation frequency of exon 8 was 12.00% (9 / 75), ranking the first, followed by exon 16, 10.67% (8 / 75). This result is consistent with the previous study.[34] The p.R778L mutation on exon 8 was the most common in our study populations, accounting for 9.33% (7/75) of the alleles studied. Most studies have shown that p.R778L is the first hot spot mutation in the Chinese population.[35-38] Exon 8 is located in the transmembrane domain (TM4) of the ATP7B gene.[16] Therefore, the p.R778L may destroy the proper anchoring of transporters in the membrane and eventually damage copper transport.[39]

The second hotspot mutations remained controversial in Chinese populations.[35,36] Our study demonstrated that p.V1106I on exon 15 was the second frequent mutation, accounting for 6.67% (5/75). Nevertheless, Wu et al. suggested that the p.T935 M was the second hotspot.[35,36] A study of 62 Chinese WD patients showed that p.P992L was the second most frequent mutation instead of p.T935 M.[11] Another study also demonstrated that p.P992L was the second hotspot through a study of 65 southern Chinese WD patients.[37] Our study also found a high mutation frequency of p.P992L on exon 13, accounting for 5.33% (4/75). Li et al. sequenced the DNA from 114 WD patients from northern Chinese populations and showed that p.R778L(21.5%), p.A874V (7.5%), and p.P992L (6.1%) were the most frequent mutations.[38]

The c.51+2T>G, c.1543+40G>A, and c.3532_3535del (p.T1178Pfs) mutations were newly found. The VUS of c.3532_3535del (p.T1178Pfs) on exon 16 and the known pathogenic variant of delEXON2[27] were used to evaluate the functional consequences of ATP7B variants. The mutation of p.T1178Pfs occurred in the ATP loop region and presumably disrupted ATP binding to affect the transport of copper.[16] Similarly, the dexEXON2 located in the copper-binding domain from ATP7B is implicated in WD.

The CCK-8 assays showed that the mutations of ATP7B groups, including $ATP7B^{delEXON2}$ and $ATP7B^{T1178fs}$, could significantly inhibit the proliferation than WT control cells when treated with 500μM $CuCl_2$ for more than 72 h. Apoptosis is a complicated process that involves multiple genes.[40] Our results indicated that the mutations of ATP7B could induce 293T cell apoptosis with the treatment of $CuCl_2$ (300 μM and 500 μM) for 72 h. The results matched with the outcome with CCK-8 assays.

In conclusion, this study revealed the genetic pattern of WD in 16 Chinese families and summarized the pathogenic genotypes. Compound heterozygous mutations of different alleles are the most common genotype in WD patients. Three VUS [two splice mutations (c.51 + 2T > G, c.1543 + 40g > A) and one frameshift mutation (c.3532_3535del)] were found, and the functional changes associated with the new ATP7B mutation were evaluated by CCK-8 and apoptosis analysis. Our study enriches the mutation spectrum of the ATP7B gene worldwide and provides valuable reference data for studying the mutation type and genetic mode of ATP7B in the Chinese population. However, to understand the pathogenesis of WD more comprehensively, it is necessary to analyze the patients and families with clinically confirmed WD on a larger scale, and it is urgent to study the molecular mechanism of WD pathogenic mutation.

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CONFLICT OF INTEREST

All authors claim that there is no conflict of interest including a desire for financial gain, prominence, professional advancement, or a successful outcome.

DATA AVAILABILITY STATEMENT

Data sources and handling of the publicly available datasets used in this study are described in the Materials and Methods. Further details and other data that support the findings of this study are available from the corresponding authors upon request.

ORCID

Chenjun Huang https://orcid.org/0000-0003-3836-6262
Xiao Xiao https://orcid.org/0000-0002-9260-4519
Chunfang Gao https://orcid.org/0000-0002-4891-2944

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SUPPORTING INFORMATION

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