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Phenotypic and genetic characterization of children with Wilson Disease from Northeast China

Tianhe Zhang[1†], Wenliang Song[1†] and Zhiqin Mao[1*]

Abstract

Background Wilson disease (WD) is an autosomal recessive inherited disease caused by ATP7B variants and characterized by copper metabolism defects. However, children with WD are often asymptomatic, making the clinical diagnosis difficult. Therefore, more accurate methods are required for clinical diagnosis. The objective of this study was to highlight the phenotypic and genetic characteristics of children with WD in northeast China.

Methods We retrospectively analyzed the clinical data and gene sequencing results of 65 children with WD from January 1, 2014, to December 31, 2022, at the Shengjing Hospital of China Medical University. All data refer to the time of diagnosis before treatment.

Results The median age at diagnosis was 5 years (range 1.2–15 years). In 50 cases (50/65, 76.9%) patients, routine physical examinations revealed only abnormal liver function. However, they had a significantly negative ($p < 0.05$) Kayser–Fleischer ring (KF). Children with acute liver failure had significantly increased 24 h urinary copper excretion ($p < 0.05$). We detected 46 genetic variants of ATP7B, including seven novel variants. The most frequent variant was p.R778L with an allele frequency of 38.7%. Phenotype-genotype correlation analysis suggested that p.R778L was significantly associated with lower serum ceruloplasmin levels and higher zinc levels ($p < 0.05$). The loss-of-function (LOF) variant was associated with significantly lower albumin levels ($p < 0.05$).

Conclusion Most children with WD are asymptomatic, which makes early diagnosis of WD difficult. Therefore, clinical and laboratory characteristics as well as genetic testing are essential. p.R778L is the most frequent variant of ATP7B in China and may play an important role in lowering serum ceruloplasmin levels.

Keywords Wilson disease, Gene, Children, China

[†]Tianhe Zhang and Wenliang Song contributed equally to this work.

*Correspondence:
Zhiqin Mao
maozq6590@126.com
[1]Department of Pediatrics, Shengjing Hospital of China Medical University, Shenyang City, Liaoning Province, People's Republic of China

Background

Wilson disease (WD) is a rare autosomal recessive disorder caused by mutations in ATP7B [1]. ATP7B is located on the short arm of chromosome 13 and encodes a copper-transporting P-type ATPase [2, 3]. ATP7B is responsible for transporting copper from intracellular chaperone proteins into the secretory pathway, both for excretion into bile and incorporation into apo-ceruloplasmin for the synthesis of functional ceruloplasmin [3]. The absence or reduced function of the ATP7B protein leads to copper accumulation and liver injury. Eventually, copper is released into the bloodstream and deposited in other organs, notably the brain, kidneys, and cornea [4].

WD can develop at any age and often occurs between 5 and 35 years of age [3]. WD is increasingly diagnosed in children younger than 5 years of age, and the clinical findings may be nonspecific in children younger than 2 years [5]. Most children present with liver dysfunction, ranging from incidental findings of elevated serum transaminases in otherwise asymptomatic patients, acute hepatitis, and hepatomegaly, to acute liver failure (ALF) or cirrhosis [6]. However, children with WD are often asymptomatic, making the clinical diagnosis difficult. Therefore, more accurate methods are required for clinical diagnosis.

Given these diagnostic challenges, genetic testing has become increasingly important for early, rapid, and accurate diagnosis of WD. To date, more than 700 ATP7B variants have been identified [7]. The most common variant in patients from Europe is the point variant H1069Q (exon 14), and that in Asia is R778L (exon 8) [8]. More than 50% of the variants are missense and nonsense, and the rest may be insertion/deletion or splice-site variants [9, 10]. Studies have shown that variants affecting critical portions of the protein, including copper-binding domains or the ATPase loop, may lead to the early onset of liver disease [11]. Other studies have indicated that p.H1069Q is more frequently associated with neurological presentation and later symptom onset [12, 13]. Loss-of-function (LOF) of ATP7B gene variants has been associated with ALF and an earlier age of onset [14, 15]. However, there are no confirmed genotype–phenotype correlations [16].

The objective of our study was to summarize the clinical manifestations and laboratory and genetic characteristics of children with WD in northeast China. We aimed to determine the spectrum and frequency of ATP7B variants and the genotype–phenotype correlation in pediatric patients with WD.

Methods

Patients and groups

A total of 65 pediatric patients with WD admitted to Shengjing Hospital of China Medical University between January 1, 2014, and December 31, 2022, were included in this study. The diagnosis of WD was established according to the scoring system provided by the Eighth International Meeting on Wilson Disease [17] and a Leipzig score of >3 was considered appropriate for the diagnosis of WD. The clinical data included medical history, physical examination results, laboratory examination results, and imaging findings. All data refer to the time of diagnosis before treatment. This study was conducted in accordance with the protocols of the Ethics Committee of Shengjing Hospital of China Medical University for a clinical retrospective study (No. 2022PS107K). Verbal consent was obtained from the legal guardians in this study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

According to the main clinical symptoms of WD in childhood, all children with WD were divided into four groups according to the type of presentations as follows: Group 1 ($n=50$): Asymptomatic cases with only an increase in serum transaminases diagnosed during the routine physical examination. Group 2 ($n=5$): Patients with biochemical abnormalities and abnormal haptic manifestations including jaundice, ascites, or liver ultrasound findings suggestive of cirrhosis. Group 3 ($n=4$): Patients with ALF were diagnosed according to the criteria of the Pediatric Acute Liver Failure Study [18, 19]. Group 4 ($n=6$): Patients with neurological, behavioral, or psychiatric disorders.

LOF variants were classified as nonsense (substitution in the coding base pair predicted to result in a premature stop codon), frameshift (insertion or deletion predicted to result in reading frame shift), and splice site (substitutions predicted to involve a splice site), which produce a nonfunctional protein product [20, 21].

Variant analysis of the ATP7B gene

Genomic DNA was extracted from the peripheral blood samples. All patients were tested using ATP7B-targeted gene panel sequencing or whole-exome sequencing. All testing protocols, including DNA extraction, gene library construction, high-throughput sequencing, data analysis, Sanger sequencing verification, and bioinformatics analysis, were performed by the Clinical Genetics Laboratory at Shengjing Hospital of China Medical University and commercial companies, including My Genostic (Beijing, China), Chigene Corp (Beijing, China), and Kingmed (The Guangzhou Genomics Institute, Shenyang, China).

Statistical analysis

All continuous numerical values are expressed as medians with ranges. Discrete variables were presented as patient numbers or percentages (%). Statistical comparisons between groups were performed using analysis of variance (ANOVA) for parametric variables and the Kruskal–Wallis test for non-parametric variables.

Analyses were conducted using Statistical Product and Service Solutions (SPSS 22.0). A *p*-value of less than 0.05 was considered statistically significant.

Results

Clinical features of pediatric WD patients

At the time of diagnosis, 65 children had WD, including 34 males and 31 females, with a median age of 5 years (range 1.2–15 years). Five patients had family histories of WD.

In 50 cases (50/65, 76.9%), routine physical examinations revealed only abnormal liver function. Cases of children with symptoms included neuropsychiatric symptoms (6/65, 9.2%), ascites (3/65, 4.6%), jaundice (2/65, 3.1%), lower limb edema (2/65, 3.1%), and hemolytic anemia (1/65,1.5%). Fourteen patients (14/65, 21.5%) showed a positive Kayser–Fleischer ring (KF) under a slit lamp, and the youngest child with a positive KF ring was 6 years old. All asymptomatic patients exhibited a negative KF ring, which was significantly different from that in the other three groups ($p<0.05$). Of all 65 patients, 92% of children had a 24 h urinary Cu value > 40 μg, and 64.4% of children had a 24 h urinary Cu value > 100 μg. Patients with WD and ALF in Group 3 exhibited increased total bilirubin, decreased albumin, increased white blood cell count, and decreased hemoglobin. Group 3 also had significantly increased 24 h urinary Cu excretion compared to the other three groups ($p<0.05$). Groups 3 and 4 had significantly lower blood platelets than the other two groups ($p<0.05$) (Table 1).

Brain MRI abnormalities were observed in six patients with neurological symptoms in Group 4, including abnormal signal intensity in the basal ganglia, thalamus, brainstem, and corpus callosum (6/65, 9.2%). Liver ultrasonographic abnormalities included hepatomegaly, splenomegaly, hepatic steatosis, fibrosis, and cirrhosis. Patients in Group 3 had liver cirrhosis on ultrasonography.

Gene variant analysis

ATP7B sequencing was performed in 65 patients. Four patients had only one allelic variant, eight had homozygous variants, and 53 had compound heterozygous variants. In total, 126 alleles were included in the analysis. We identified 46 gene variants in ATP7B (Table 2; Fig. 1), seven of which were novel. Novel variants included c.2572 A > C, c.-362G > A, c.51 + 4 A > C, c.1543 + 40G > A, c.3903 + 2T > C, c.2664-2665delCC, and c.3524-3528delAAGGA. The most frequent variant was p.R778L, with an allele frequency of 38.7%, followed by p.P992L

Table 1 Clinical and laboratory characteristics of 65 pediatric WD patients at diagnosis

Parameter	G1(*n* = 50)	G2(*n* = 5)	G3(*n* = 4)	G4(*n* = 6)	*p* value
Male/female	26/24	3/2	2/2	3/3	0.986
Medianageofonset (yrs) (Range)	5(3–6)	8(4–10)	12(9–13)	12(11–13)	< 0.01
Family history	5.0(10.0)	0(0.0)	0(0.0)	0(0.0)	0.654
Jaundice	0(0.0)	0(0.0)	2(50.0)	0(0.0)	< 0.01
Edema	0(0.0)	0(0.0)	3(75.0)	1(16.7)	< 0.01
Ascites	0(0.0)	0(0.0)	2(50.0)	0(0.0)	< 0.01
Hemolytic anemia	0(0.0)	0(0.0)	1(25.0)	0(0.0)	< 0.01
Neuropsychiatric deficit	0(0.0)	0(0.0)	0(0.0)	6(100.0)	< 0.01
KF ring (+)	0(0.0)	4(80.0)	4(100.0)	6(100.0)	< 0.01
Brain involvement(MRI)	0(0.0)	0(0.0)	1(25.0)	6(100.0)	< 0.01
Hepatomegaly(USG)	14(28.0)	3(60.0)	1(25.0)	2(33.3)	0.519
Splenomegaly(USG)	1(2.0)	1(20.0)	3(75.0)	1(16.7)	< 0.01
Hepatosteatosis(USG)	14(28.0)	0(0.0)	0(0.0)	1(16.7)	0.428
Fibrosis(USG)	0(0.0)	3(60.0)	0(0.0)	1(16.7)	< 0.01
Cirrhosis(USG)	0(0.0)	2(40.0)	4(100.0)	3(50.0)	< 0.01
Ceruloplasmin (mg/dl)	2.6(2.0-5.8)	3.8(2.2–12.3)	4.1(3.4–10.4)	3.8(2.6–6.02)	0.686
Urinary copper (μg/24 h)	109.6(75.5-152.9)	221.0(104.0-461.6)	440.4(224.1-907.2)	242.1(158.8-383.8)	< 0.01
ALT (IU/L)	227.0(135.8–308.0)	131.0(69.0-173.0)	110.0(62.5-221.3)	30(22.3-152.5)	0.013
AST (IU/L)	110.5(62.0-151.8)	72.0(68.0-117.5)	131.0(71.3-303.3)	55.0(31.0-112.8)	0.109
TB (mg/dL)	6.4(5.3–8.2)	7.4(5.2–34.3)	64.7(57.0-104.8)	14.1(7.0–22.0)	0.010
ALB (g/L)	42.9(40.8–44.1)	42.9(30.0-44.3)	25.8(23.9–28.6)	39.2(32.1–42.8)	0.030
WBC (10^3/ml)	6.8(5.6–8.1)	6.0(4.7–6.4)	4.8(3.2–7.1)	4.0(3.0-6.1)	0.003
Hb (g/dL)	125.5(120.5-133.3)	122.0(113.5-132.5)	105.5(93.7-109.8)	127.5(115.8-135.5)	< 0.01
PLT(10^3/ml)	314.5(279.0-386.3)	116.0(84.5–272.0)	73.5(53.3-104.3)	110.5(224.8)	< 0.01
Zinc (mg/dl)	73.4(61.2–85.6)	63.0(74.1–85.2)	62.3(56.9–78.6)	70.9(58.8–83.2)	0.114

G1: Asymptomatic cases with only increase in serum transaminases; G2: Patients with biochemical abnormalities and hepatic presentation; G3: WD patients with acute liver failure at diagnosis; G4: WD patients with neurological/psychiatric symptoms. Zinc values is not used before Zinc treatment.

Table 2 Spectrum of gene variants in the ATP7B gene of this study

Exon	Nucleotide variant	Amino acid change	Variation type	Pathogenicity	Domain	Allele frequency (%)
1	**c.51 + 4 A > C***	-	splice site	Uncertain	Before Cu1	0.81
2	c.122 A > G	p. N41S	Missense	Likely Pathogenic	Before Cu1	0.81
2	**c.-362G > A***	**-**	**Nonsense**	**Uncertain**	**Cu1**	**0.81**
2	c.525DupA*	p. V176Sfs*28	Frameshift	Pathogenic	Cu2	0.81
3	c.1488 C > T*	p. G496=	Synonymous	Uncertain	Cu2	0.81
4	**c.1543 + 40G > A***	**-**	**splice site**	**Uncertain**	**Cu5**	**0.81**
6	c.1924G > T	p. D642Y	Missense	Pathogenic	bet Cu6/TM1	1.61
8	c.2128G > A	p. G710S	Missense	Pathogenic	TM2	1.61
8	c.2132G > A	p. G711E	Missense	Pathogenic	TM2	0.81
8	c.2145 C > T	p. Y715=	Synonymous	Uncertain	TM4	0.81
8	c.2252 C > A	p. A751D	Missense	Likely Pathogenic	TM4	0.81
8	c.2333G > T	p. R778L	Missense	Pathogenic	TM4	38.71
8	c.2337G > A*	p. W779X	Nonsense	Pathogenic	TM4	0.81
8	c.2293G > A	p. D765N	Missense	Pathogenic	TM4	0.81
10	**c.2572 A > C**	**p. T858P**	**Missense**	**Likely Pathogenic**	**Td**	**0.81**
11	c.2621 C > T	p. A874V	Missense	Pathogenic	bet Td/TM5	4.84
11	c.2633 A > C	p. N878T	Missense	Pathogenic	bet Td/TM5	0.81
11	c.2662 A > C	p. T888P	Missense	Pathogenic	bet Td/TM5	0.81
11	**c.2664-2665delCC***	**p. H889fs**	**Frameshift**	**Pathogenic**	**bet Td/TM5**	**0.81**
11	c.2668G > A	p. V890M	Missense	Pathogenic	bet Td/TM5	0.81
12	c.2755 C > G	p. R919G	Missense	Pathogenic	bet Td/TM5	4.84
12	c.2785 A > G	p. I929V	Missense	Pathogenic	TM5	0.81
12	c.2790-2792delCAT	p. I930del	In-frame deletion	Likely Pathogenic	TM5	0.81
13	c.2924 C > A	p. S975Y	Missense	Pathogenic	TM6	1.61
13	c.2930 C > T	p. T977M	Missense	Pathogenic	TM6	0.81
13	c.2975 C > T	p. P992L	Missense	Pathogenic	bet TM6/Ph	6.45
13	c.3029 A > C	p. K1010T	Missense	Pathogenic	bet TM6/Ph	0.81
14	c.3140 A > T	p. D1047V	Missense	Pathogenic	ATP loop	0.81
14	c.3168delT*	p. L1057fs	Frameshift	Pathogenic	ATP loop	0.81
15	c.3316G > A	p. V1106I	Missense	Pathogenic	ATP loop	1.61
16	c.3459G > T	p. Trp1153Cys	Missense	Pathogenic	ATP loop	0.81
16	c.3517G > A	p. E1173K	Missense	Pathogenic	ATP loop	1.61
16	**c.3524-3528delAAGGA***	**-**	**Frameshift**	**Pathogenic**	**ATP loop**	**0.81**
16	c.3532 A > G	p. T1178A	Missense	Pathogenic	ATP loop	0.81
17	c.3634 A > G	p. M1212V	Missense	Likely Pathogenic	ATP bind	0.81
17	c.3646G > A	p. V1216M	Missense	Pathogenic	ATP bind	3.22
18	c.3797G > A	p. G1266E	Missense	Likely Pathogenic	ATP hinge	1.61
18	c.3809 A > G	p. N1270S	Missense	Pathogenic	ATP hinge	2.42
18	c.3818 C > T	p. P1273L	Missense	Pathogenic	ATP hinge	0.81
18	c.3851T > A	p. I1284N	Missense	Likely Pathogenic	ATP hinge	0.81
18	**c.3903 + 2T > C***	**-**	**splice site**	**Likely Pathogenic**	**bet ATP hinge/TM7**	**0.81**
19	c.3955 C > T*	p. R1319X	Nonsense	Pathogenic	bet ATP hinge/TM7	0.81
19	c.4006delA*	p. I1336fs	Frameshift	Pathogenic	TM7	1.61
20	c.4064G > T	p. G1355V	Missense	Likely Pathogenic	TM8	0.81
20	c.4112T > C	p. L1371P	Missense	Pathogenic	TM8	0.81
20	c.4114 C > T*	p. Q1372X	Nonsense	Pathogenic	TM8	2.42

Novel variants are highlighted in bold

*: LOF variants were defined as produced a non-functional protein product

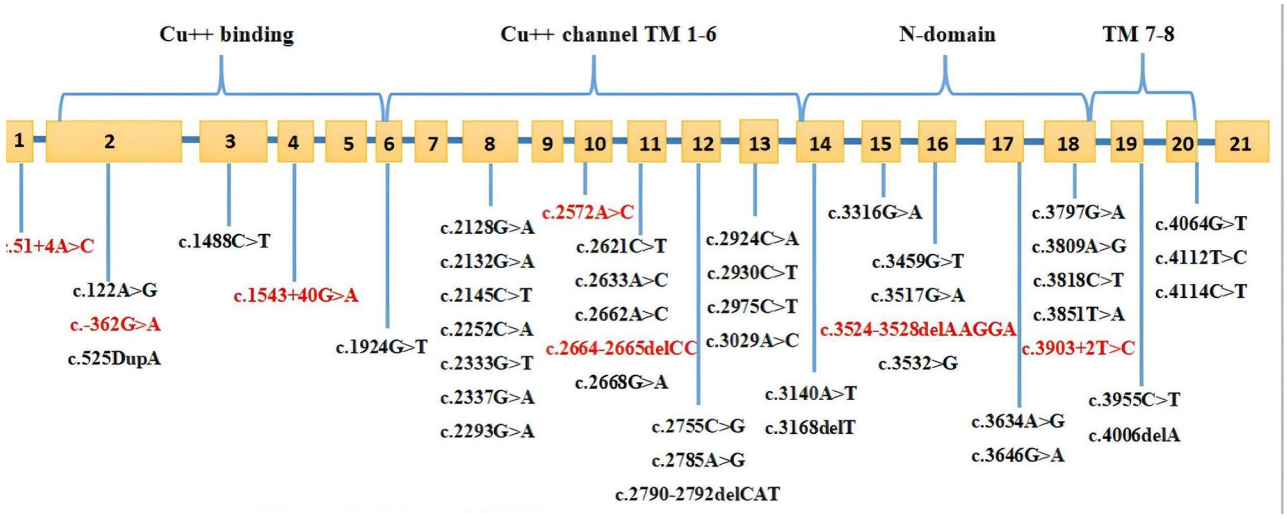

Fig. 1 Spectrum of gene variants in ATP7B gene of this study. Novel variants detected in this study are in red

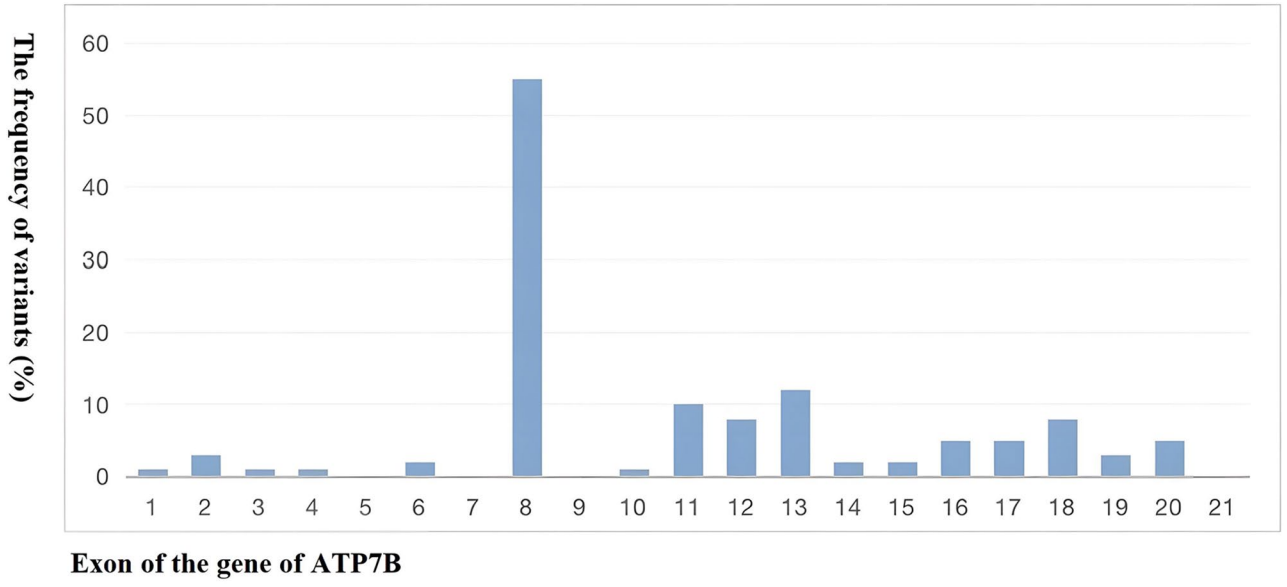

Fig. 2 Distribution of variants within 21 exons of ATP7B.

(6.5%), p.R919G, p.A874V (4.9% each), p.V1216M(3.2%), p.N1270S, and p. Q1372Ter (2.4% each).

Of the 46 identified variants, 33 were classified as mild variants, including 31 missense variants, one synonymous variant, and one in-frame deletion variant. Thirteen were LOF variants, including five frameshift variants, four nonsense variants, three splice-site variants, and one synonymous splice-related variant.

The 46 variants were distributed throughout all exons of ATP7B except for exons 5, 7, 9, and 21. The exons harboring the highest percentage of variants were exons 8, 11, 12, 13, and 18 (Fig. 2). The overall variant detection rate for these five exons was 75%, suggesting that these exons may be important regions for detecting variants.

Assessment of phenotype and genotype correlation

We compared the two most common variants, c.2333G>T (p.R778L) and c.2975 C>T (p.P992L), with other variants in terms of the age at symptom onset and clinical parameters. Of the 65 patients, 41 (63.1%) had the p.R778L variant, eight were homozygous, and 33 were heterozygous. We observed that patients carrying the p.R778L variant had lower serum ceruloplasmin levels and higher zinc levels than patients with other variants of both alleles ($p<0.05$) (Fig. 3a, b). Eight (12.3%) patients had the p.P992L variant, all of whom were heterozygous, and there was no significant association between the variant types and phenotype.

Of the 65 patients, 16 (24.6%) harbored a LOF variant on their allelic chromosomes. We observed that

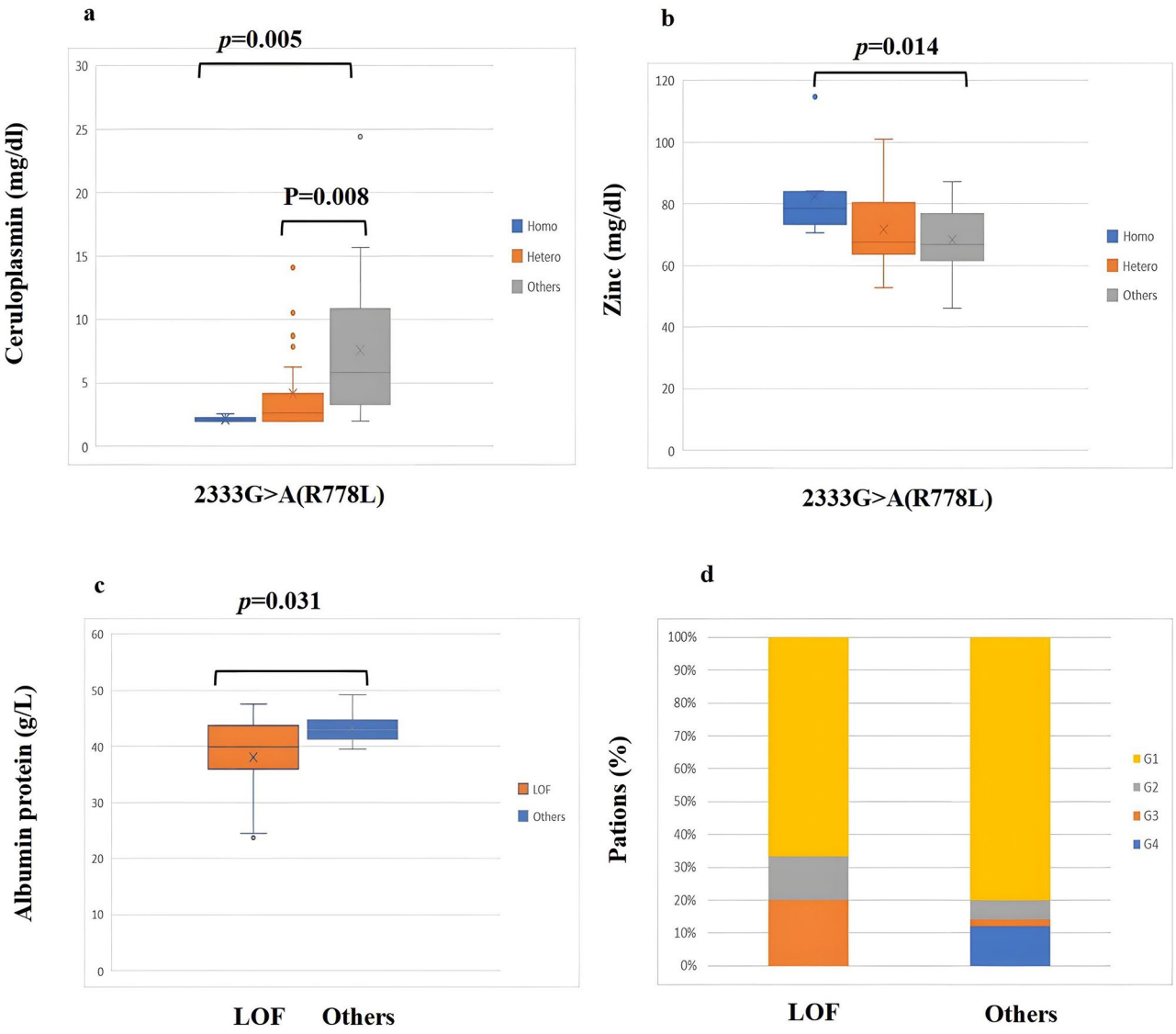

Fig. 3 Correlation of phenotype and genotype of this study. **(a)** Correlation of 2333G > T (R778L) and serum ceruloplasmin level; **(b)** Correlation of 2333G > T (R778L) and zinc level; **(c)** Correlation of LOF variant and albumin protein level; **(d)** Correlation of LOF variant and clinical manifestations

patients carrying the LOF variant had lower albumin levels for both alleles than patients without the LOF variant ($p < 0.05$) (Fig. 3b, c). Four patients with WD and ALF were identified (Fig. 3d; Table 3). All patients had a LOF variant on one allelic chromosome and a missense variant on the other.

Discussion

Most children (50/65, 76.9%) in our study were asymptomatic with elevated serum transaminase levels observed only on routine physical examination. The mean age of onset in asymptomatic children was 5 (3–6) years and it was therefore significantly younger than that of the other groups. Asymptomatic children only manifested hepatomegaly and liver steatosis, which were detected using liver ultrasonography. If left untreated, asymptomatic patients with signs of organ damage typically develop symptomatic WD [22]. Therefore, the early diagnosis and treatment of WD are crucial to achieve better outcomes. However, the early diagnosis of asymptomatic children with WD is challenging. Therefore, clinical and laboratory characteristics, as well as support for genetic testing, are essential.

ALF is the leading cause of death in children with WD, with a mortality rate of approximately 95% if left untreated [3]. In our study, four pediatric patients with WD and ALF were identified, two of whom showed improvement after appropriate treatment, one after liver transplantation, and one who died because she did not receive a liver transplant in time. Prompt diagnosis is crucial, as these patients urgently require liver transplantation to survive [4].

Table 3 Clinical and variant data from four pediatric WD patients with ALF

Patient	ATP7B variants	Variantion type	Sex	Age at diagnosis (y)	KF ring	Neuropsychiatric deficit	Hemolytic anemia	Outcome
1	c.2333G>T	missense	M	13	+	-	-	Survival
	c.3524-3528delAAGGA*	frameshift						
2	c.2333G>T	missense	F	11	+	-	-	Death
	c.3955 C>T*	nonsense						
3	c.1924G>T	missense	M	8	+	-	-	Liver transplant
	c.3903+2T>C*	splice site						
4	c.2128G>A	missense	F	12	+	-	+	Survival
	c.1488 C>T*	Synonymous						

*: LOF variants were defined as producing a nonfunctional protein product.

Six patients presented neurological symptoms. In addition to involuntary movements, tremors, and dysarthria because of extrapyramidal involvement, they may be associated with cognitive difficulties, depressive disorders, sleep disorders, and other psychiatric symptoms. All patients had brain MRI abnormalities, including abnormal signal intensities in the basal ganglia, thalamus, brainstem, and corpus callosum. In addition, all patients had abnormal liver ultrasound findings, and 50% of the patients had liver cirrhosis on liver ultrasound. Therefore, attention should be paid to the possibility of WD in children with neurological symptoms, particularly those with concomitant ultrasound abnormalities of the liver.

Previous studies have suggested that KF rings are usually absent in children with WD and liver disease [23, 24]. In our study, they were absent in asymptomatic children and were present in children with ALF and neurological symptoms. This suggests that the positivity rate of the KF ring is very low in the early stages, especially in asymptomatic children. The KF ring is often associated with disease progression, particularly neurological and severe hepatic manifestations, in WD. Therefore, the KF ring cannot be used as a basis for early diagnosis.

In our study, 92% of children had 24 h urinary Cu excretion>40 μg, and 64.4% of children had 24 h urinary Cu excretion>100 μg. Thus, these results suggest that >40 μg appears to be more sensitive to the abnormality of 24 h urinary Cu. This is consistent with previous studies [25]. A recent study showed that cholestasis prevents biliary copper excretion and may lead to systemic copper overload with markedly increased urinary copper excretion, particularly in children [26]. In our study, children in Group 3 with ALF also had significantly increased 24 h urinary Cu excretion compared to the other three groups ($p<0.05$). We suspected that cholestasis occurred during ALF, resulting in abnormally high 24 h urine levels.

Previous studies have mainly focused on southern China, and there are few reports on northern China. In our study, the high frequencies were p.R778L, p.P992L, p.R919G, and p.A874V, which accounted for 54.8% of all variants, which differs from previous studies in southern China, where the three most common variants were p.R778L, p.P992L, and p.T935M, accounting for 50–60% of all variants [24, 27]. Furthermore, in southern China, the major ATP7B variants are concentrated in exons 8, 12, 13, and 16 [28, 29], whereas the variants detected in our study were mainly present in exons 8, 11, and 13. The results regarding the exons are the same in Qingdao and are also prevalent in northern China [30], suggesting that these regions may be hotspots for variants in northern China.

Seven novel variants have not been previously reported, including c.2572 A>C, c.-362G>A, c.51+4 A>C, c.1543+40G>A, c.3903+2T>C, c.2664-2665delCC, and c.3524-3528delAAGGA. These had not yet been included in the Exome Aggregation Consortium or 1000 Genomes Project databases. Bioinformatics software analysis assumes that they are pathogenic or probably pathogenic.

The relationship between the clinical phenotypes and genotypes is complex. Most studies suggest that the phenotypic variability in WD may be caused by additional genetic, epigenetic, and even environmental factors that play a role in the timing of disease onset and clinical phenotype [31–33]. LOF variants result in severe impairment of ATP7B gene function owing to the lack of a full-length gene product. European studies have suggested that patients with LOF variants have lower serum ceruloplasmin oxidase activity and earlier onset of WD than those with missense variants [14, 15, 34, 35]. The current research suggests that the presence of at least one LOF variant in patients receiving chelation therapy is associated with poor transplant-free survival over a long follow-up [20]. In our study, the LOF variant was significantly associated with lower albumin levels, which may be associated with ALF, similar to previous studies [14, 15]. Furthermore, patients carrying the p.R778L variant had lower serum ceruloplasmin levels and higher zinc levels for both alleles than patients with other variants. Recent studies showed that p.R778L was also associated with lower ceruloplasmin levels [27, 29]. Some observations suggest that serum Zn levels could be related to the clinical phenotype of patients with WD and its severity;

patients with excessive Cu overload have lower serum Zn levels [36]. The toxicity of Cu observed in WD might be caused by the disruption of numerous Zn-dependent and Zn-responsive proteins, including transcription factors [37]. However, the role of Zn in WD remains unclear.

Our study had several limitations. The sample size of our study was small. Therefore, errors and outliers may have a greater impact on the statistical results. Therefore, multicenter and large-scale studies are needed to test these conclusions and investigate the relationship between different genotypes and phenotypes of WD.

Conclusion

In conclusion, the diagnosis of WD in children can be challenging, particularly during the early stages of liver disease and neurological symptoms. Therefore, supportive clinical and laboratory characteristics and genetic testing are essential.

Abbreviations

WD	Wilson Disease
KF ring	Kayser–Fleischer ring
ALF	acute liver failure
LOF variant	Loss of function variant
MRI	magnetic resonance imaging
USG	ultrasonogram
ALT	alanine aminotransferase
AST	aspartate aminotransferase
TB	total bilirubin
DB	directly bilirubin
ALB	Albumin protein
WBC	white blood cell
Hb	hemoglobin
PLT	platelets

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Author contributions

Tianhe Zhang (zth)contributed to conceptualization, methodology and original draft preparation. Wenliang Song contributed to visualization, software, data curation and editing. Zhiqin Mao contributed to conceptualization, validation, review and supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of the manuscript(All authors read and approved the final Manuscript).

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This work was in accordance with the protocols of the Ethics Committees of Shengjing Hospital of China Medical University for clinical retrospective study (No. 2022PS107K). Verbal consent was obtained from legal guardians in this study. This study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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