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Case report

Plasma transfusion combined with chelating therapy alleviates fulminant Wilson's disease with a single Arg778Leu heterozygote mutation

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ABSTRACT

Wilson's disease (WD), resulting from homozygote and compound heterozygote mutations in ATB7B, is an autosomal recessive disease. WD associated acute liver failure (ALF) is fatal, and a revised Wilson's disease prognostic index (RWPI) >11 is a reliable indication of liver transplantation (LT) or artificial liver support (ALS). We described a WD patient who initially presented with ALF and severe hemolytic anemia. A single heterozygote c.2333G>T mutation (p. Arg778Leu, R778L) in ATP7B was screened by whole exome sequencing and validated by Sanger sequencing. Rapid diagnostic criteria (ALP/TBIL <4 and AST/ALT >2.2) are suitable for early diagnosis. Although the RWPI amounted to 15, the patient recovered after intermittent plasma transfusion and subsequent chelating therapy without LT or ALS. In conclusion, WD patients with a single R778L heterozygote mutation can present with ALF as the initial clinical manifestation, and intermittent plasma transfusion combined with chelating therapy may alleviate fulminant WD without LT or ALS.

1. Introduction

Wilson's disease (WD) is an autosomal recessive disease, resulting from ATP7B gene mutations and copper accumulation in the liver and extrahepatic tissues. WD's manifestations may range from an asymptomatic state to life-threatening acute liver failure (ALF), with or without neurological presentations. The prevalence of WD is estimated at 1:30,000 to 5.87:100,000, with a heterozygote carrier frequency of approximately 1% [1]. It was reported that c.2333G>T (p.Arg778Leu, R778L) homozygote and compound heterozygote mutations of ATP7B are the most common alterations in the Chinese population [2]. However, the associations of genotypes with phenotypes in WD patients with single R778L heterozygote mutations remain undefined.

Moreover, ALF mortality due to WD approaches 100%, and liver transplantation (LT) is the only effective treatment for patients with WD who present with ALF [1,3,4]. Revised Wilson's disease prognostic index (RWPI) > 11 [5,6] is a reliable indication for LT. Meanwhile, combining plasma exchange and chelating therapy has been reported to rescue ALF in Wilson's disease without liver transplantation [7]. Several reports showed that advances of artificial liver support (ALS) may improve the outcome of ALF [8]. However, LT is limited by donor shortage, and ALS remains unavailable in some areas because of costly equipment and the lack of plasma.

We herein described a case of WD harboring a single R778L heterozygote mutation, and initially presenting with acute liver failure and severe hemolytic anemia. The patient recovered after intermittent plasma transfusion and subsequent chelating therapy without LT or ALS.

2. Case presentation

The patient was a 13-year-old Chinese girl admitted to the Liver Department of the Third People's Hospital of Changzhou on 17 January 2017. She presented with a 10-day history of fatigue, nausea, and loss of appetite. She had no history of drug or alcohol abuse. Family history was negative for metabolic and inherited liver diseases. Her parents had no consanguineous marriage. On physical examination, the girl had pronounced jaundice on the skin and sclera, with ecchymosis at the injection sites. Palpable liver and splenomegaly were found by abdominal ultrasound. Cornea Kayser-Fleischer rings were found using slit lamp examination. The clinical data of the patient were obtained during one year of

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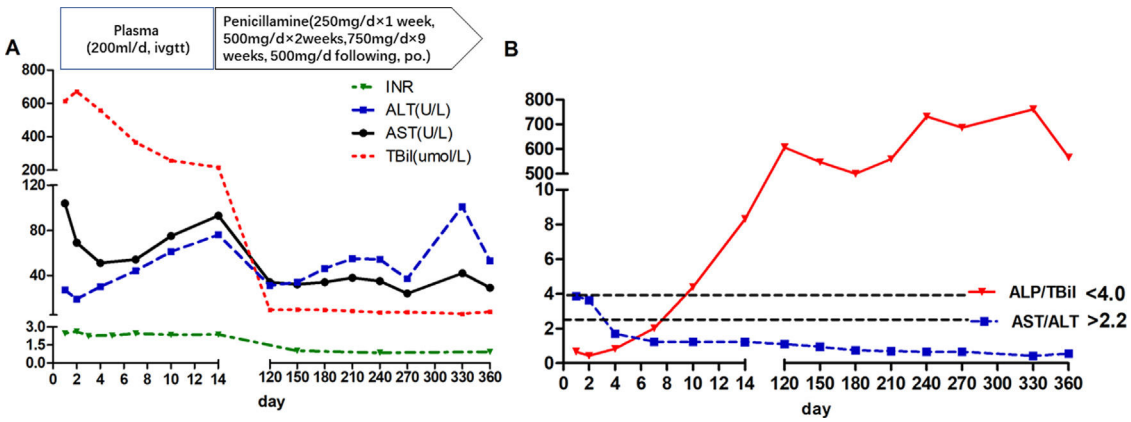

Fig. 1. Dynamic changes in biochemical parameters (A) and respective ratios (B).

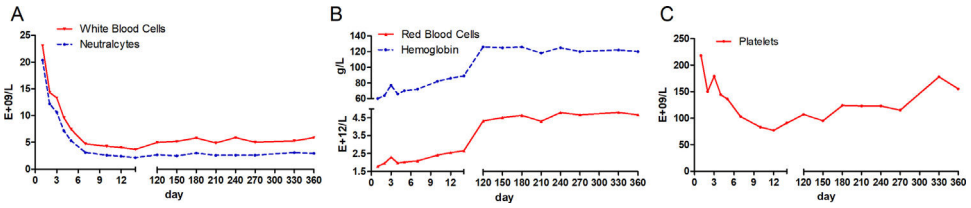

Fig. 2. Dynamic changes in white blood cells (A), red blood cells (B) and platelet (C).

follow-up. The current study was approved by the ethics committee of the Third People's Hospital of Changzhou and performed according to the Declaration of Helsinki in 1975. Informed consent was obtained from the girl and her parents.

At the time of admission, laboratory assessment revealed abnormal liver function tests, Coomb's negative hemolytic anemia and coagulopathy. Bone marrow morphology showed hyperplastic anemia. She had aspartate amino-transaminase (AST) levels nearly 2 times the upper limit of the normal range (104 U/L), whereas alanine aminotransferase (ALT) amounts were normal (27 U/L). Total bilirubin (TBIL) was more than 35 times the upper limit of the normal range (613.1 μmol/L), with the majority being unconjugated bilirubin (416.1 μmol/L). Liver failure was diagnosed according to overtly altered TBIL (613.1 μmol/L), international normalized ratio (INR, 2.62) and prothrombin activity (24.27%); the RWPI score amounted to 15 [6]. Moreover, the hemogram test showed high levels of white blood cells (23.3E+9/L) and severe anemia (hemoglobin, 60 g/L). Serological markers for HBV, HAV, HCV, HDV and HEV were all negative except for anti-HBs.

During the first 14 days after onset, fresh plasma was administered intravenously every day at a dose of 200 ml, and erythrocytes were transfused every other day at a dose of 2 U. TBIL was overtly decreased at the 14th day after admission (Fig. 1A). In addition, white blood cell count was reduced, while anemia was ameliorated (Fig. 2). Ceruloplasmin was low (0.04 g/L) and WD was considered; then, the patient was transferred to the First Hospital of Anhui Traditional Chinese Medicine University. Penicillamine was used as a copper-chelating drug and administrated on the 15th day after admission (250 mg/d for the 1st week, then 500 mg/d for 2 weeks, 750 mg/d for 9 weeks, and 500 mg/d for subsequent months, continued till now).

During the one-year follow-up, TBIL was maintained below 10 μmol/L, whereas alkaline phosphatase (ALP) levels increased to 258 U/L at the 4th month and lasted for several months. On December 19, 2017 (330th day after onset), further tests revealed a slight elevation of ALT (101 U/L), which declined a month later.

The ALP (U/L) to TBIL (mg/dl) ratio was lower than 4, and that of AST (U/L) to ALT (U/L) was higher than 2.2 at admission; meanwhile, the ALP/TBIL ratio increased to above 4 at day 10 after admission, and AST/ALT declined to below 2.2 at day 4. After one year of follow-up, ALP was maintained at a high level (above 258 U/L) upon recovery, while the ALP/TBIL ratio was increased to 607 and lasted for several months. The AST/ALT ratio remained <2.2 until the end of follow-up (Fig. 1B).

As whole exome sequencing is attractive in diagnosing patients with life-threatening liver diseases of indeterminate etiology [9], it was performed for this patient. Genome DNA was extracted with QIAamp DNA Blood Kit (Qiagen, Tokyo, Japan). The entire genomic DNA was amplified with adapter-modified fragments; then, a DNA library was established according to the manufacturer's instructions (Agilent, Santa Clara, CA, USA) and captured using Agilent's SureSelect Human All Exon V6 Kit. DNA fragments were sequenced on an Illumina HiSeq X Ten for paired-end 150 bp reads. Raw reads were processed to remove adaptor sequences and trim low-quality reads (base quality <30), and aligned to the reference genome sequence (UCSC hg19) with Bowtie2 version 2.2.6 [10]. Mutations were then detected with GATK [11], Lofreq [12] and VarScan [13] from aligned BAM files. Databases, including dbSNP, OMIM and ClinVar, and annotation software, including SIFT, PolyPhen, MutationTaster, Provean and MetaSVM, were used to screen potential disease associated mutations. The ATP7B gene fragments which contained mutations screened in whole exome sequencing were amplified by PCR and sequenced after purification.

As shown in Table 1, a total of 44 disease causing variations were screened. A single R778L heterozygote mutation in the ATP7B gene was identified by whole exome sequencing and validated by Sanger sequencing (Fig. 3).

3. Discussion

The present study assessed a WD patient harboring a single R778L heterozygote mutation and initially presenting with ALF and severe hemolytic anemia. Interestingly, the patient recovered after

Table 1
Disease causing variations ($n = 44$) screened by whole exome sequencing[a].

Chromosome	Site	Base change	Amino acid change	Mutation frequency	SNP	Gene
chr1	20931474	G>A	p.A70T	0.5	rs60369023	CDA
chr1	32149760	C>T	p.G745S	0.5	rs34770879	COL16A1
chr1	201021733	C>T	p.R1302Q	0.5	rs200042281	CACNA1S
chr2	44078728	G>A	p.G110R	0.5	rs754458742	ABCG8
chr2	71894551	G>A	p.R1735H	0.5	rs531935195	DYSF
chr2	129075877	G>T	p.D87E	0.5	rs200979099	HS6ST1
chr2	132021946	G>A	p.G973D	0.5	rs62178369	POTEE
chr3	37053562	C>T	p.R217C	0.5	rs4986984	MLH1
chr3	129195166	G>T	p.K164N	0.5	rs117517364	IFT122
chr4	126372111	G>T	p.G3316C	0.5	rs776046433	FAT4
chr4	178355548	C>A	p.R265L	0.5	rs375663828	AGA
chr4	190874234	C>T	p.P91S	0.5	rs200620299	FRG1
chr6	161159625	G>A	p.A620T	0.5	rs121918027	PLG
chr7	56087300	C>T	p.G90S	0.5	rs75395437	PSPH
chr7	56087365	A>G	p.L68P	0.5	rs78067484	PSPH
chr7	56087374	C>T	p.R65H	0.5	rs200442078	PSPH
chr7	151927021	C>A	p.C988F	0.5	rs28522267	KMT2C
chr7	151927025	A>G	p.Y987H	0.5	rs183684706	KMT2C
chr8	101721817	T>C	p.E372G	0.5	rs201076736	PABPC1
chr8	125115444	G>A	p.C1728Y	0.5	rs75319208	FER1L6
chr9	33386465	A>G	p.Y115H	0.5	rs74668961	AQP7
chr9	33796785	C>G	p.A55G	0.5	rs199600414	PRSS3
chr9	33798042	G>C	p.C132S	0.5	rs141382822	PRSS3
chr9	140777306	C>G	p.N167K	0.5	rs4422842	CACNA1B
chr10	50667028	C>G	p.A1439P	0.5	rs530673596	ERCC6
chr10	101157438	G>C	p.Q370E	0.5	rs76850691	GOT1
chr11	5153329	A>G	p.C182R	0.5	rs182211570	OR52A5
chr11	64083293	G>T	p.R376L	0.5	rs201971362	ESRRA
chr11	64083320	T>C	p.L385P	0.5	rs201072913	ESRRA
chr11	64083328	C>T	p.L388F	0.5	rs79204587	ESRRA
chr11	64083331	C>T	p.R389C	0.5	rs80310817	ESRRA
chr11	64950341	C>T	p.R57C	0.5	rs770891480	CAPN1
chr12	21795008	C>T	p.R158H	0.5	rs200163319	LDHB
chr12	45751080	T>C	p.Y291H	0.5	rs760031599	ANO6
chr12	52308249	C>T	p.R218W	0.5	rs199874575	ACVRL1
chr12	53343069	G>T	p.G38C	0.5	rs77999286	KRT18
chr12	53343084	G>C	p.G43R	0.5	rs75441140	KRT18
chr12	53343209	G>A	p.M84I	0.5	rs79346135	KRT18
chr13	52532469	C>A	p.R778L	0.5	rs28942074	ATP7B
chr14	52494000	C>T	p.A865T	0.5	rs181688285	NID2
chr14	104645057	C>T	p.R1761W	0.5	rs200460079	KIF26A
chr15	50288937	G>A	p.R176C	0.5	rs116334504	ATP8B4
chr15	72638961	G>A	p.R413W	0.5	rs762494949	HEXA
chr16	1291669	G>T	p.W156C	0.5	rs779296557	TPSAB1

[a] All the 44 variations are disease causing variations predicted by SIFT, PolyPhen, MutationTaster, Provean and MetaSVM libraries.

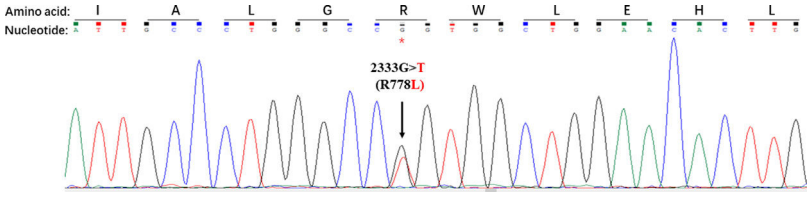

Fig. 3. Arg778Leu heterozygote mutation in the ATP7B gene.

intermittent plasma transfusion and subsequent chelating therapy without LT or ALS.

Approximately 5% of WD patients develop ALF, and up to 50% of pediatric patients with ALF do not show encephalopathy [14–16]. The current patient had evidence of severe liver injury (TBIL 613.1 μmol/L) and coagulopathy (INR = 2.62) at admission, and she met ALF criteria according to a previous report [6], in spite of the absence of encephalopathy. The RWPI score of the patient amounted to 15 (TBIL > 301 μmol/L; INR > 2.5; AST > 100 U/L; albumin < 33 g/L; white blood count > 15.4E+09/L), with >11 representing a reliable indicator of LT [6]. Of these parameters, elevated white blood cell count is considered a marker of occult infection, a stress response to liver injury or an unidentified factor that predicts liver failure severity. Considering that procalcitonin is normal and no definite infectious focus exists, there was insufficient evidence of infection or sepsis for this patient.

The Leipzig [17] and rapid diagnostic [18,19] criteria can be used for ALF patients with suspected WD. According to Leipzig criteria, this patient had a cumulative score of 6, with a score >4 indicating reliable diagnosis of WD. The rapid diagnostic criteria [17], including ALP/TBIL below 4 and AST/ALT above 2.2, have been reported to have 100% sensitivity and specificity in WD diagnosis. For this patient, ALP/TBIL and AST/ALT ratios met these criteria at admission, while ALP/TBIL increased to above 4 at day 10 after admission; meanwhile, AST/ALT fell below 2.2 at day 4 and lasted until the end of follow-up. Notably, the diagnostic criteria are suitable for early diagnosis since these ratios change during the disease course.

Disease causing homozygote mutations can support WD diagnosis with certainty, whereas heterozygote mutations with clinical symptoms are scarce [17]. To the best of our knowledge, a single R778L heterozygote mutation in the ATP7B gene in patients with ALF has not been reported. In the present study, only a single R778L heterozygote mutation in the ATP7B gene was detected by whole exome sequencing and validated by Sanger sequencing. These findings confirmed that a WD patient carrying a single R778L heterozygote mutation can present with ALF as initial clinical manifestations.

In addition, liver transplantation is considered the only life-saving option for fulminant WD patients after failure of copper-chelating therapy [4]. Remission can also be achieved by combining ALS and chelating therapy in some cases [7,8]. Considering that liver function and the general condition were overtly improved before copper-chelating therapy, we presume that plasma transfusion combined with penicillamine may alleviate fulminant WD in the transitional period when LT or ALS is unavailable. However, the role of plasma transfusion in WD treatment remains elusive and requires further investigation to support the above notion. Undoubtedly, liver transplantation and ALS are still the preferred approaches for fulminant WD.

In conclusion, a WD patient carrying a single R778L heterozygote mutation can present with ALF as initial clinical manifestations, and rapid diagnostic criteria (ALP/TBIL < 4 and AST/ALT > 2.2) are suitable for early diagnosis. Moreover, intermittent plasma transfusion and copper-chelating therapy may rescue fulminant WD when LT or ALS is unavailable.

Abbreviations

WD	Wilson's disease
ALF	acute liver failure
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ALP	alkaline phosphatase
TBIL	total bilirubin
INR	international normalized ratio
LT	liver transplantation
ALS	artificial liver support
RWPI	revised Wilson disease prognostic index

Authors' contributions

Yuan Xue conceived and designed the study. Longgen Liu, Qing Gong, Juan Liu, Hongyu Shen and Hongyu Zhang collected and confirmed the data. Longgen Liu and Qing Gong analyzed the data and drafted the manuscript. Yuan Xue revised of the manuscript. All authors read and approved the final manuscript. Longgen Liu and Qing Gong contributed equally to this work.

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