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Original Article

Literature review and report of three cases of Dubin-Johnson syndrome related to ABCC2 gene mutations in children

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Abstract: Objective: The aim of the present study was to analyze the clinical features of Dubin-Johnson syndrome (DJS) related to ABCC2 gene mutations in children and to review the relevant literature to improve understanding of this type of genetic disease and reduce misdiagnosis. Methods: Three children with clinically suspected DJS who were treated at Beijing Children's Hospital of Capital Medical University between 2017 and 2020 were enrolled in the study. The target genes were captured and sequenced using GenCap target gene capture technology and a new generation of high-throughput sequencing technology (Beijing Mykino Company). The clinical and genetic characteristics were analyzed and summarized. Results: Two of the cases were female and one was male. All three cases were in early infancy and in good general health. Case 1 was complicated with unilateral hypertrophy, Case 2 was complicated with pneumonia, anemia, myocardial injury, and bilateral inguinal hernia, and Case 3 was complicated with patent foramen ovale and a ventricular septal defect. In all three cases, total bilirubin was elevated, with the main increase being in direct bilirubin (DBIL) and varying degrees of elevated alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), and total bile (TBA). Genetic testing indicated that there were seven gene mutations in ABCC2, two mutation sites of which had not been reported previously. Conclusion: The clinical manifestations of DJS are non-specific and are mainly characterized by elevated DBIL. Some children might have different degrees of hepatic function abnormality and cholestasis. Due to the lack of serological markers, the diagnosis of DJS is difficult, but genetic testing, along with the formation of pedigree analysis and verification, could be used for accurate diagnosis. Novel mutations might enrich the spectrum of ABCC2 gene mutation.

Keywords: Dubin-Johnson syndrome, ABCC2 gene, infant cholestasis

Introduction

Dubin-Johnson syndrome (DJS) is a rare autosomal recessive genetic disease with insidious onset. It was discovered and first reported by Dubin and Johnson in 1954 [1]. It is easily misdiagnosed and mistreated. The incidence of DJS is the same in males and females and usually has an onset in adolescence or young adulthood. Although it has been found among all ethnicities, it is more common among Spanish, French, and German Jews; the overall incidence of the disease among Jews has been reported as about 1/3000 [2]. The main clinical manifestation of DJS is continuous or intermittent jaundice of the skin, sclera, and urine [3]. Studies have shown that the dysfunction or deletion of the multidrug resistance-associated protein 2 (MRP2) caused by ABCC2 gene mutations may affect the secretion of bile and the transport of bilirubin, which is the pathogenesis of the disease [4]. The incidence of DJS is low, with rare related literature reports, and domestic and foreign reports are mostly limited to case reports. In the present study, high-throughput sequencing was used to perform genetic

analysis on three children with clinically suspected DJS, which detected mutations in the pathogenic gene ABCC2.

Subjects and methods

Subjects

Three children with clinically suspected DJS who were treated at Beijing Children's Hospital of Capital Medical University between 2017 and 2020 were enrolled in the study. Their ages ranged from 23 to 55 days, with an average of 39 days. All were hospitalized and followed up after discharge in the outpatient clinic or by telephone. The study was conducted in accordance with the Declaration of Helsinki (as was revised in 2013). The study was approved by Ethics Committee of Beijing Children's Hospital, Capital Medical University and informed consent was taken from all the patients, participants under the age of 16 have obtained written informed consent from their parents.

Methods

Clinical characteristics, data from laboratory examinations, and other data of the three children were collected, after which the detection of mutant genes and pedigree verification was undertaken. With the approval of the Ethics Committee of the hospital and the informed consent of family members, 2 mL of the peripheral venous blood of the children and their parents was drawn and stored in ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. The samples were then sent to the medical laboratory of Beijing Mykino Gene Technology Co., Ltd. for relevant gene capture, enrichment, and high-throughput sequencing.

Results

General characteristics

Two cases were female and one was male. All were in early infancy and had no family history of jaundice. The mother of Case 1 had gestational diabetes, the mother of Case 2 had pregnancy-induced hypertension and the mother of Case 3 suffered from bipolar disorder for more than nine years before her pregnancy, for which she took sodium valproate, quetiapine fumarate, and tranquilizer medication (this treatment was stopped six months before her pregnancy).

The birth history of Case 1: the birth weight was 3750 g, the birth length was unknown, and cesarean section was performed at 40 weeks of pregnancy. Immediately after birth, there was crying, rosy skin, hypertrophy of the right limb, normal limb posture, without resuscitation, breastfeeding after birth. Case 1 was found to have slight jaundice of the skin and sclera and dark-yellow urine on the 19th day after birth. The jaundice and urine had become slightly worse by the 28th day after birth. A skin test identified the jaundice value as 17.6 mg/dl. Phenobarbital, multivitamin B1 tablets, and multi-dimensional lactic acid bacteria were administered orally. The jaundice did not disappear significantly, so the child was hospitalized. The results of the blood biochemistry were albumin (ALB) 34.5 g/L, alkaline phosphatase (ALP) 521 U/L, aspartate aminotransferase (AST) 26 U/L, alanine aminotransferase (ALT) 17.3 U/L, γ-glutamyltransferase (GGT) 227.6 U/L, total bilirubin (TBIL) 232.22 umol/L, direct bilirubin (DBIL) 79.06 umol/L, and total bile acid (TBA) 21.4 μmol/L, and electrolyte levels were normal. The results of abdominal ultrasonography showed no abnormality in the shape and echo of the gallbladder and the contraction. The liver was located approximately 4 cm below the ribs. There was no abnormality in the spleen and pancreas. The average liver elasticity was 5.2 kpa. The diagnosis at admission was infant cholestasis and unilateral hypertrophy. The child was discharged following improvement after treatment. The levels of transaminase and bilirubin decreased during regular outpatient follow-ups.

The birth history of Case 2: the birth weight was 1200 g, the birth length was unknown, and cesarean section was performed at 31 weeks of pregnancy. After birth, the child had moans, foaming at the mouth and jaundice. He was admitted to the local hospital, given CPAP respiratory support, intravenous nutrition symptomatic treatment, and oral ursodeoxycholic acid symptomatic treatment. After 24 days in the hospital, he was improved and discharged. Intermittent treatment with blue light in the outpatient department showed no significant improvement in skin yellow staining. After birth, the premature baby was fed with milk for 45

Table 1. The clinical characteristics in 3 cases with Dubin-Johnson syndrome in children

Cases	Gender	Age at onset	Age at diagnosis	Chief complaint	Jaundice	Stool color	Hepatomegaly	Splenomegaly	Other specific signs	Family history	Complication
Case 1	Female	19 days	51 days	Jaundice in the skin and sclera for 20 days	Moderate	Yellow	4 cm under the rib	2 cm under the rib	Facial asymmetry, unilateral limb hypertrophy, milk coffee spots	Gestational diabetes, Taking 50 ml of bird's nest every day during pregnancy	Unilateral limb hypertrophy
Case 2	Male	42 days	61 days	Finding of jaundice in the skin for 55 day	Moderate	Yellow	2 cm under the rib	No	No	At the 31st week, Administration of Labetrol during pregnancy	Pneumonia, anemia, myocardial injury, inguinal hernia
Case 3	Female	2 days	32 days	Finding of jaundice in the skin for 21 day	Mild	Yellow	No	No	Breast nodules	Maternal bipolar disorder, Administration of magnesium valproate, quetia fumarate tablets, stabilizers, and withdrawal of the drugs half of a year before pregnancy	Ventricular septal defect

Table 2. The laboratory indicators in 3 cases with Dubin-Johnson syndrome in children

Cases	ALT	AST	GGT	ALP	TP	ALB	GLB	TBIL	DBIL	TBA	PT	APTT	FIB	Blood glucose
Case 1	17.3	26.0	227.6	521.0	45.7	34.5	11.2	232.2	79.1	21.4	11.6	53.4	2.4	4.3
Case 2	25.9	67.0	827.1	572.0	45.1	23.9	11.3	175.4	131.5	86.6	12.2	59.8	1.9	4.3
Case 3	72.9	73.6	288.6	519.0	53.4	38.7	14.7	198.4	54.0	7.2	12.5	62.4	2.8	4.9

Note: ALT: Alanine aminotransferase, normal reference range: 9~50 U/L; AST: Aspartate aminotransferase, normal reference range: 5~60 U/L; GGT: γ-glutamyltransferase, normal reference range: 13~57 U/L; ALP: Alkaline phosphatase, normal reference range: 5-350 U/L; TBIL: Total bilirubin, normal reference range: 2~17 μmol/L; DBIL: Direct bilirubin, normal reference range: 0~7 μmol/L; TBA: bile acid, normal reference range: 0~13 μmol/L; TP: Total cholesterol, normal reference range: 3.4~5.2 mmol/L; PT: Prothrombin time, normal reference range: 9.4~12.5 s; APTT: Activated partial thromboplastin time, normal reference range: 25.1~38.4 s; FIB: Fibrinogen quantification, normal reference range: 2~4 g/L; Blood glucose: normal reference range: 3.5~5.7 mmol/L.

days and then changed to oiru milk powder. Case 2 was hospitalized having suffered from jaundice of the skin for 1 month and 25 days. The results of the biochemistry were AST 67 U/L, ALT 25.9 U/L, GGT 827.1 U/L, TBIL 175.4 umol/L, DBIL 131.5 umol/L, and TBA 86.6 μmol/L. The ultrasonography of the liver and gallbladder showed that the liver was located 3 cm below the ribs and had an elasticity of 5.7 kpa. Infant hepatobiliary imaging showed that the nuclide failed to reach the intestine within 24 hours, so the first consideration was biliary atresia. The diagnosis was infant cholestatic hepatic disease, possible biliary atresia, possible congenital metabolic disease, pneumonia, anemia, and myocardial injury. After hospitalization, the patient was treated with hepatoprotection, choleretic therapy, anti-infection, and myocardial preservation treatments, with decreased levels of transaminase and bilirubin.

The birth history of Case 3: the birth weight was 2980 g, the length of the birth body was unknown, the pregnancy was 39^{+1} week, the crying immediately after birth, the skin color was ruddy, the limb posture was normal, and there was no resuscitation. Breastfeed 2 hours after birth. Moderate jaundice appeared on the second day after birth and did not subside. The umbilical cord fell off 16 days after birth. The first time of defecation is 6 hours after birth and 2 days after birth. Passed binaural hearing test. Case 3 was hospitalized having suffered from jaundice of the skin for 21 days. The results of the biochemistry were ALP 519 U/L, AST 73.6 U/L, ALT 72.9 U/L, GGT 288.6 U/L, TBIL 198.5 umol/L, DBIL 54 umol/L, and TBA 7.2 μmol/L. The ultrasonography of the liver and gallbladder found that the liver was located 2.4 cm below the ribs and had a slightly thickened Glisson's sheath, with no signs of biliary obstruction. The liver elasticity was 4.26 kpa. There were no abnormalities in the hepatotropic virus. The diagnosis was neonatal hyperbilirubinemia, cholestasis, hepatic dysfunction, patent foramen ovale, and a ventricular septal defect. After hospitalization, the patient was treated with hepatoprotection and choleretic therapy, with a decrease in transaminase and bilirubin levels.

The clinical characteristics of the three patients are illustrated in **Tables 1**-**3**.

Capture and sequencing of the target gene and Sanger verification

Results of genetic tests and pathogenicity analysis: Seven nucleotide changes were found in the three subjects. Relevant literature was reviewed and the Human Gene Mutation Database (HGMD Pro) was checked, which revealed that two of the mutation sites have not been reported previously. Using the American Society for Medical Genetics and Genomics (ACMG) Gene Mutation Interpretation Guidelines, the pathogenicity was graded. The details are shown in **Table 4**.

Two mutation sites were detected in the ABCC2 gene in Case 1: a frameshift mutation c.116delA (p.Y39Sfs*39) and a missense mutation c.2882A>G (p.K961R). There was no report on the c.116delA (p.Y39Sfs*39) in the HGMD. Pedigree verification suggested that the mutation was derived from the child's mother. The HGMD did have reports of the pathogenicity of DJS at the c.2882A>G (p.K961R) locus, in which the mutation was derived from the father. Based on the ACMG guidelines, the pathogenicities of the two loci were initially determined as the pathogenic variant and suspected pathogenic variant, respectively.

Table 3. The image features of 3 cases with Dubin-Johnson syndrome in children

Cases	Abdominal ultrasonography	Average hepatic elasticity
Case 1	Liver located approximately 4 cm below the rib	5.2 kpa
Case 2	Liver located 3.6 cm below the rib, splenomegaly, Lack of contraction of the gallbladder cavity after feeding, considering the presence of biliary atresia	5.7 kpa
Case 3	Liver located 2.1 cm below the rib, Poor gallbladder filling	4.26 kpa

Table 4. The results of genetic tests and pathogenicity grading in 3 cases with Dubin-Johnson syndrome in children

Cases	Mutant gene	Chromosome position	Exon	Nucleotide/ Amino Acid	Type	Homozygous/ heterozygous	Frequency in the normal population	Prediction	Pathogenicity analysis	HGMD	Hereditarymode	Origin
Case 1	$ABCC_2$	chr10-101544446-101544447	exon2	c.116delA (p.Y39Sfs*39)	Frameshift	het	No	-	Pathogenic variants	No	AR	Mother
	$ABCC_2$	chr10-101590607	exon21	c.2882A>G (p.K961R)	Missense	het	0.0003	B	Suspected of pathogenic variants	Yes	AR	Father
Case 2	$ABCC_2$	chr10-101563869	exon10	c.1303A>C (p.T435P)	Missense	het	No	P	Unknown clinical significance	Yes	AR	Mother
	$ABCC_2$	chr10-101563892	exon10	c.1326G>A (p.W442X)	Nonsense	het	No	-	Suspected of pathogenic variants	Yes	AR	Mother
	$ABCC_2$	chr10-101567196	exon12	c.1586G>A (p.R529Q)	Missense	het	0.0003	P	Unknown clinical significance	No	AR	Father
Case 3	$ABCC_2$	chr10:101603639	exon27	c.3825C>G (p.Y1275X)	Nonsense	het	0.0019	-	Pathogenic variants	Yes	AR	Mother
	$ABCC_2$	chr10:101572833	exon16	c.2026G>C (p.G676R)	Missense	het	No	D	Suspected of pathogenic variants	Yes	AR	Father

Note: Prediction: Protein function prediction software REVEL (rare exome variant ensemble learner), P: Predicted to be harmful; B: Predicted to be benign; -: Unkonwn.

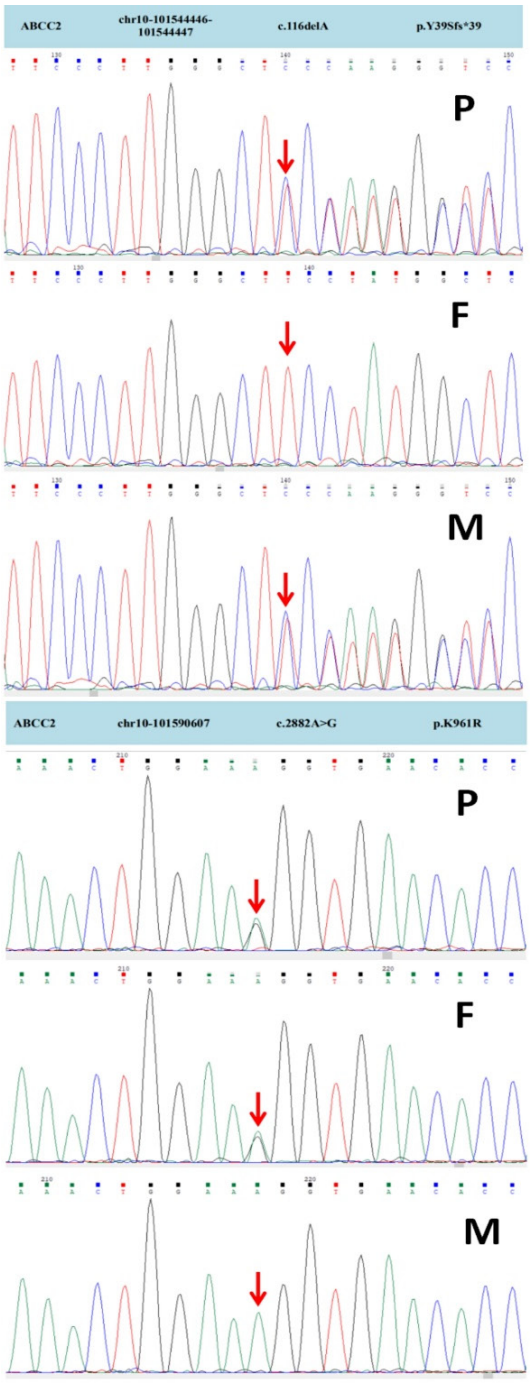


Figure 1. Results of the gene sequence in Case 1. P: Proband; F: Father of the proband; M: Mother of the proband; Arrow indicates the site of mutation.

Three mutation sites were detected in the ABCC2 gene in Case 2: missense mutations c.1303A>C (p.T435P) and c.1586G>A (p.R529Q) and a nonsense mutation c.1326G>A (p.W442X). The HGMD had reports of the c.1303A>C and the c.1326G>A loci, in which both heterozygous loci were derived from the mother. There was no report of c.1586G>A, which is derived from the father, in the database. Based on the ACMG guidelines, the pathogenicities of the three loci were initially determined as clinically unclear (twice) and suspected pathogenic variant, respectively.

Two mutation sites were detected in the ABCC2 gene in Case 3: a nonsense mutation c.3825C>G (p.Y1275X) and a missense mutation c.2026G>C (p.G676R). Reports on the two loci were found in the HGMD. Pedigree verification showed that the variant of c.3825C>G derived from the mother, while c.2026G>C derived from the father. Based on the ACMG guidelines, the pathogenicities of the two loci were initially determined as the pathogenic variant and suspected pathogenic variant, respectively.

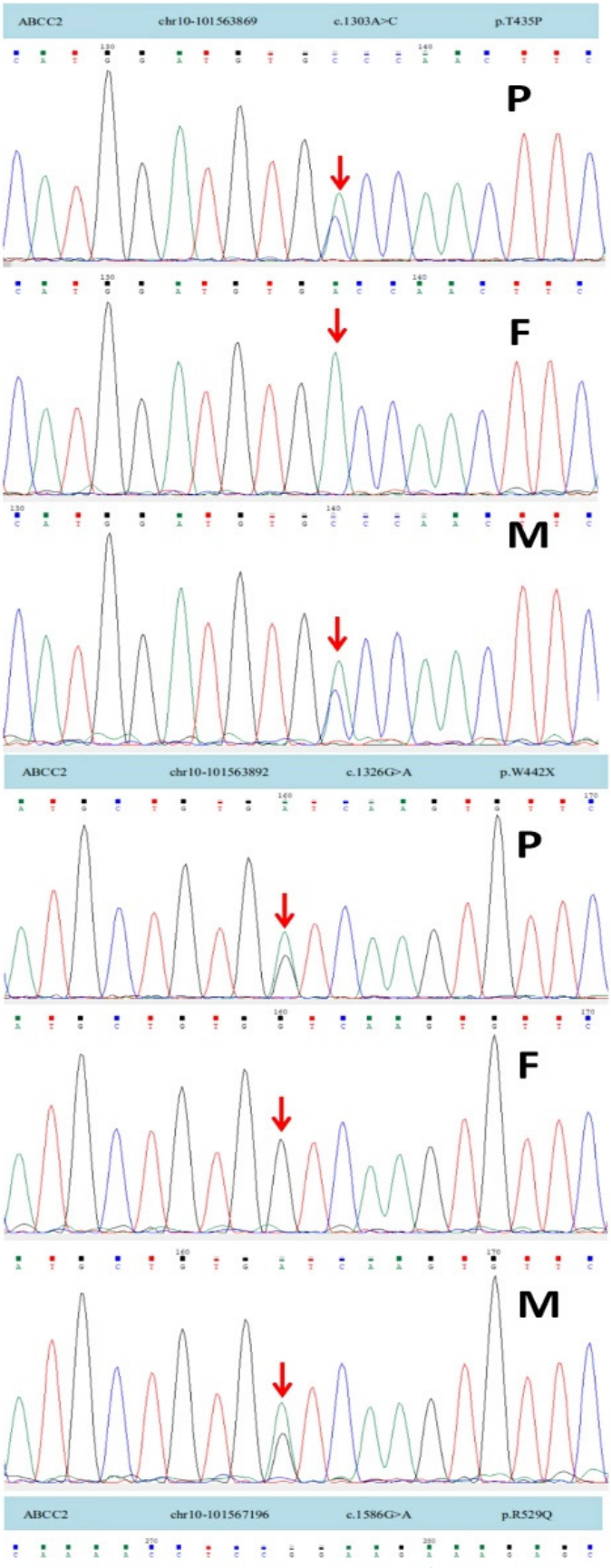
Pedigree verification: Case 1 had a compound heterozygous mutation in the ABCC2 gene. Sanger sequencing verified that the frameshift mutation c.116delA (p.Y39Sfs*39) was derived from the mother and the missense mutation c.2882A>G (p.K961R) was derived from the father. The results of the pedigree verification are shown in **Figure 1**.

Case 2 had a compound heterozygous mutation in the ABCC2 gene. Sanger sequencing verified that the missense mutation c.1303A>C (p.T435P) and the nonsense mutation c.1326G>A (p.W442X) were derived from the mother, while the missense mutation c.1586G>A (p.R529Q) was derived from the father. The results of the pedigree verification are shown in **Figure 2**. Case 2's older brother did not have the disease, and the results of his gene sequencing are shown in **Figure 3**.

Case 3 had a compound heterozygous mutation in the ABCC2 gene. Sanger sequencing verified that the nonsense mutation c.3825C>G (p.Y1275X) was derived from the mother and the missense mutation c.2026G>C (p.G676R) was derived from the father. The results of the pedigree verification are shown in **Figure 4**.

Discussion

The ABCC2 gene is located in the autosomal 10q24 and encodes the MRP2 [5]. Since the discovery of the gene mutation that results in DJS in 1997 [6], an increasing number of patients with DJS have been diagnosed through



genetic diagnosis. According to the HGMD (www.hgmd.cf.ac.uk), 68 ABCC2 gene mutations related to DJS have been reported so far, including missense mutations, nonsense mutations, splicing mutations, regulatory region mutations, deletion/loss mutations, insertion mutations, small insertion/deletion mutations, and complex rearrangement mutations. ABCC2 genetic analysis has become a reliable basis for the diagnosis of DJS.

ABCC2 encodes the MRP2, which contains two ATP-binding cassettes and 17 transmembrane sequences. It is mainly distributed in the capillary membranes of hepatocytes and the apical membranes of other polarized cells. It relies on the energy generated by the hydrolysis of ATP to actively transport a variety of organic anions, such as conjugated bilirubin, bile acid, glutathione, and leukotriene, outside the cell [7, 8]. MRP2 is the main transporter of bilirubin. Therefore, the abnormal expression or functional injury of MRP2 may lead to bilirubin excretion disorders. As an important driving force for bile flow, MRP2 can also promote the secretion of bile and increase the fat solubility of bile salts, while fat-soluble bile salts can reduce hepatic injury [9]. The abnormality of MRP2s may affect bile flow and cause cholestasis. However, the down-regulation of MRP2 expression can cause excessive release of inflammatory cytokines, which can lead to hepatic injury. Inflammatory cytokines like IL-1β can also inhibit the extracellular regulatory kinase of the hepatocytes, which in turn down-regulates the expression of MRP2, thus forming a vicious circle [10]. Studies have confirmed that the ABCC2 gene mutation in patients with DJS is correlated with the loss of MRP2 function, which may lead to hyperbilirubinemia [11].

MRP2 abnormality causes clinical jaundice and DBIL elevation in pediatric patients with DJS; it can also

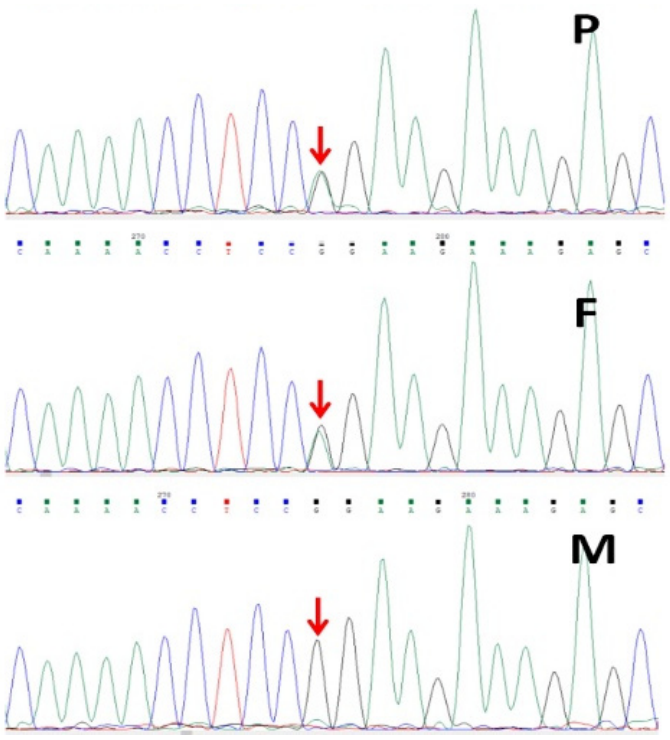


Figure 2. Results of the gene sequence in Case 2. P: Proband; F: Father of the proband; M: Mother of the proband; Arrow indicates the site of mutation.

lead to cholestasis, hepatocyte injury, and elevated hepatic enzymes. As such, in clinical practice the diagnosis of DJS for pediatric patients with cholestasis or hyper-direct bilirubinemia should be considered. The present study found manifestations of cholestasis, such as elevated GGT and ALP, so the diagnosis should be differentiated from other diseases that cause cholestasis. It is worth noting that the TBA level in Case 3 was normal, in Case 1 it was only slightly elevated, and in Case 2 is was slightly to moderately elevated. Therefore, when pediatric patients with clinically consistent infant cholestasis (DBIL >17.1 umol/L) have normal or slightly elevated TBA, the possibility of DJS should be considered. In differential diagnosis, congenital bile acid synthesis disorder (CBAS) may also have cholestasis with normal TBA. However, the GGT of the three patients in the present study was significantly increased, which was obviously different from the GGT in CBAS, and the clinical manifestations were inconsistent with CBAS. Thus, the diagnosis of CBAS might be excluded.

In the present study, all three patients experienced the onset of jaundice in infancy. It was accompanied by hepatomegaly and increased levels of DBIL in the laboratory tests. The examinations of two of the patients revealed abnormal hepatic function, with increased levels of ALT, AST, GGT, and ALP and decreased ALB. Abnormality in hepatic function is relatively rare in reported adult cases. The elevated hepatic enzymes of two of the patients in the study might be correlated with hepatic injury caused by excessive release of inflammatory cytokines following the down-regulation of MRP2 expression.

A total of seven mutations in the ABBC2 gene were found in the three patients in the present study, of which two were not reported in the HGMD. Genetic analysis confirmed that Case 1 had a compound heterozygous mutation at two loci encoding ABCC2; one was a missense mutation derived from the father and the other was a frameshift mutation derived from the mother.

Case 2 had a compound heterozygous mutation at three loci of the ABCC2 gene; one was a missense mutation derived from the father, and the other two were a missense mutation and a nonsense mutation derived from the mother. It is worth noting that Case 2's mother had a compound mutation in the ABCC2 gene. An inspection of her medical history revealed that she had yellowish skin and suffered from occasional fatigue. The results of the mother's examination showed ALT of 53 U/L, ALP of 149 U/L, GGT of 14 U/L, and normal bilirubin levels. Thus, it was considered that the related manifestations of the mother might be correlated with the abnormal expression of MRP2. The results of nuclide inspection in Case 2 showed that the nuclide failed to reach the intestine within 24 hours in the infant hepatobiliary imaging, so the diagnosis of biliary atresia was initially considered. In clinical hepatobiliary scintigraphy, 99mTc-N-pyridyloxy-5-methyltryptophan (99mTc-PMT) is an effective radiotracer. In humans, the uptake of 99mTc-PMT by the liver is transferred through OATP1B1 and OATP1B3 and is excreted into the bile duct through MDR1 and MRP2 [12]. Therefore, the failure of the radiotracer to reach Case 2's

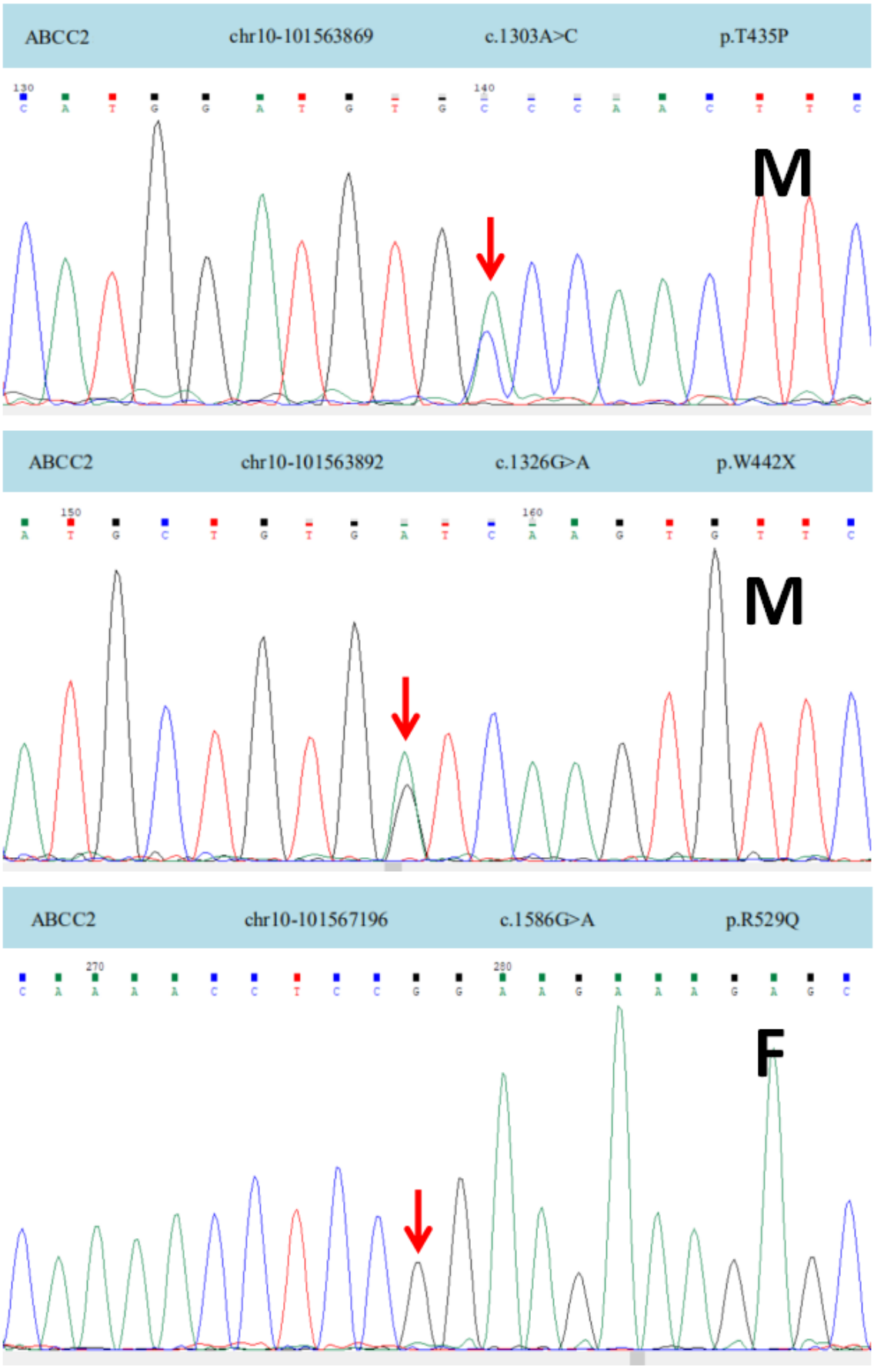

ABCC2
chr10-101563869
c.1303A>C
p.T435P
M
ABCC2
chr10-101563892
c.1326G>A
p.W442X
M
ABCC2
chr10-101567196
c.1586G>A
p.R529Q
F

Figure 3. Results of the gene sequence in Case 2's brother. F: Father of the proband; M: Mother of the proband.

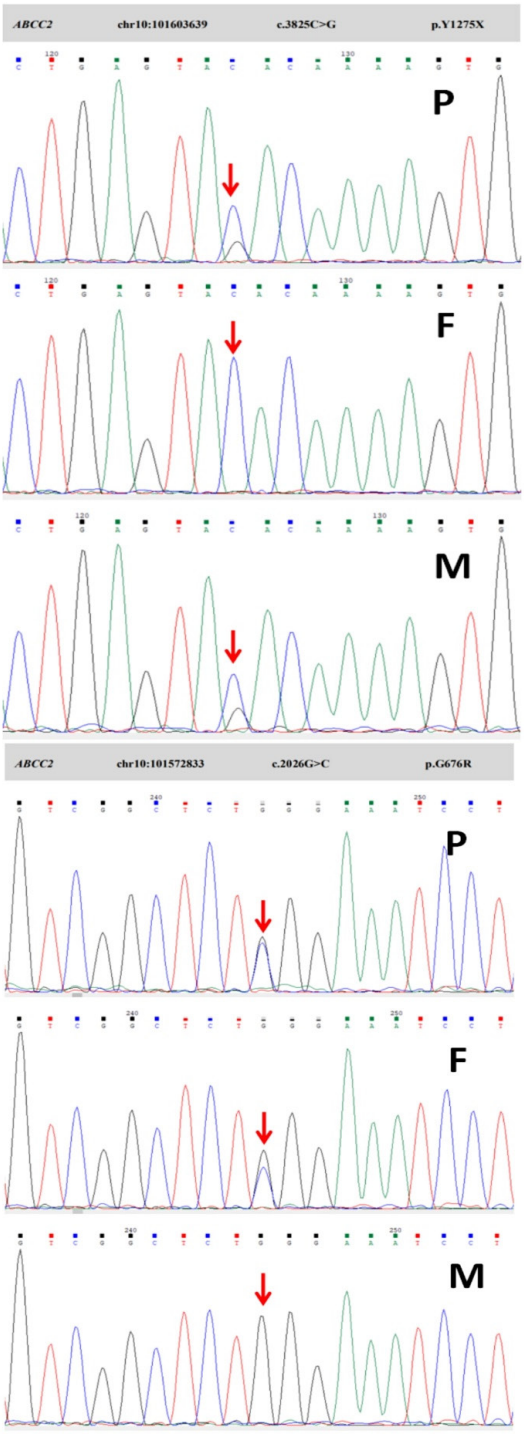


Figure 4. Results of the gene sequence in Case 3. P: Proband; F: Father of the proband; M: Mother of the proband; Arrow indicates the site of mutation.

intestine within 24 hours might be correlated with the abnormality of MRP2 rather than biliary atresia.

Case 3 had a compound heterozygous mutation at two loci encoding ABCC2; one was a missense mutation derived from the father and the other was a nonsense mutation derived from the mother, which was in accordance with the diagnosis of DJS. Therefore, for children with the onset of cholestasis in infancy, if persistent jaundice is found, the relatives should be asked to check whether they have similar symptoms. Genetic testing should also be performed to achieve an accurate diagnosis, thereby reducing the unnecessary invasive examinations and avoiding misdiagnosis and mistreatment.

The clinical symptoms of DJS are generally mild with a good prognosis [2]. The symptoms of DJS with onset in infancy can be prolonged into childhood but generally do not affect growth and development [13]. However, the long-term pigment deposition in the hepatocytes causes the rupture of the bile ducts in the liver, causing degeneration and necrosis of hepatocytes, the proliferation of fibrous tissue, pseudo-lobule formation, and a series of pathological changes. Therefore, for pediatric patients with severe jaundice and recurrent DJS, the jaundice should be actively treated to reduce the deposition of pigments in the hepatocytes and avoid the aggravation of injury to the hepatocytes. Phenobarbital [14], ursodeoxycholic acid [15], and other drugs should be considered as therapeutic treatments to reduce jaundice, protect the liver, and lower enzymes levels, thereby reducing clinical symptoms and the possibility of injury to the hepatocytes.

In June 2020, the latest follow-up with the three children in the present study indicated that Case 1 still had mild jaundice of the skin. Regular review of the biochemical indicators showed that the cholestasis had gradually improved and that growth and development were basically normal. Body height was 84 cm, which is in the 75th-90th percentile, and weight was 13 kg, which is in the 90th-97th percentile. In Case 2, the jaundice of the sclera and skin had almost disappeared, and the biochemical indicators of the regular review indicated that the cholestasis had gradually improved but that growth and development were delayed. Body height was 80 cm, which is in the 3rd-10th percentile, and weight was 10 kg, which is in the 3rd-10th percentile. In Case 3, the jaundice

Table 5. The follow-up of laboratory indicators in 3 cases with Dubin-Johnson syndrome in children

Cases	ALT	AST	GGT	ALP	TP	ALB	GLB	TBIL	DBIL	TBA	Date
Case 1	50.3	55.7	18.6	341.0	60.7	42.3	18.4	20.9	8.25	1.8	December 30, 2019
Case 2	26.0	46.0	-	-	3.6	48.3	-	39.0	14.1	4.1	May 15, 2020
Case 3	56.6	57.2	198.5	494.0	51.3	36.6	14.7	77.3	39.6	17.3	May 19, 2020

of the skin and sclera had mostly disappeared. Regular review of biochemical indicators showed that the cholestasis had gradually improved but that growth and development were delayed. Body height was 46 cm, which is in the 25th-50th percentile, and weight was 4.5 kg, which is below the 3rd percentile. The follow-up results of the clinical laboratory indicators of all three children are shown in **Table 5**.

Conclusion

Before the emergence of genetic testing technology, DJS was diagnosed based on the clinical manifestations of long-term or intermittent jaundice, the biochemical characteristics of elevated DBIL, and the pathological changes of hepatic tissue caused by deposition of lysosomal melanin [16]. A liver biopsy is an invasive operation accompanied by certain risks, whereas a genetic test using a blood sample is non-invasive and can achieve an accurate diagnosis at the genetic level; pedigree verification is also feasible. Therefore, mastering the clinical characteristics of DJS and the early application of genetic testing could reduce the medical burden of pediatric patients and promote the diagnosis and recovery of the disease.

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Disclosure of conflict of interest

None.

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