Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Neonatal Dubin–Johnson syndrome: biochemical parameters, characteristics, and genetic variants study

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BACKGROUND: The clinical characteristics and gene mutation characteristics of children with Dubin–Johnson syndrome (DJS) need in-depth study.

METHODS: The clinical and genomic data of neonatal Dubin–Johnson syndrome (NDJS) and 155 cases with idiopathic cholestasis (IC) were analyzed from June 2016 to August 2020

RESULTS: ABCC2 gene variants were identified in eight patients, including one patient with homozygous variants and seven patients with compound heterozygous variants. A total of 13 different ABCC variants were detected in the NDJS patients, including three nonsense variants, six missense variants, three frameshift variants, and a splice site variant. The variant c.2443C > T (p.R815X), c.4237_4238insCT (p.H1414Lfs*17), c.960_961insGT (p.L322Cfs*3), c.4250delC (p.S1417Ffs*14), c.2224G > A (p.D742N), c.4020G > C (p.K1340N), and c.2439 + 5G > A were not reported in the Human Gene Variant Database. There was no significance in the sex, birth weight, and onset age between the NDJS and IC groups. Compared with the IC group, the NDJS group had significantly higher levels of total bilirubin (TB), but a significantly lower level of alanine transaminase and a ratio of direct bilirubin (DB) to TB. There is no significance in total bile acid, gamma-glutamyl-transpeptidase, albumin, or international normalized ratio between the two groups.

CONCLUSIONS: NDJS should be considered in prolonged neonatal intrahepatic cholestasis, especially in infants with normal or slightly elevated transaminase levels.

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IMPACT:

- Explore the biochemical parameters, characteristics, and genetic profile of NDJS.
- By summarizing the characteristics of biochemical indicators, seven new mutation types of the *ABCC2* gene were detected, which expanded the mutation spectrum of the *ABCC2* gene.
- NDJS should be considered in prolonged neonatal intrahepatic cholestasis, especially in infants with normal or slightly elevated transaminase levels.

INTRODUCTION

Dubin–Johnson syndrome (DJS) is caused by the mutation of the *ABCC2* gene, which leads to the functional defect of its encoded MPR2 protein. This causes endogenous and exogenous anionic conjugates, such as bile acid, conjugated bilirubin, and glutathione, to be blocked from hepatocytes to bile ducts. Patients with persistent or intermittent hyper-conjugated bilirubinemia suffer from lysosomal pigmentation in liver cells, increased excretion of coproporphyrin in urine, and other clinical manifestations.[1–3] DJS is an autosomal recessive genetic disease. The incidence in Spanish Jews is high. It is rare in other countries and regions, and DJS in newborns is also rare.[4] With the development of molecular biology diagnostic technology, reports of neonatal DJS (NDJS) have gradually increased. At present, domestic NDJS reports are all individual case reports.[5–7] The clinical characteristics and gene mutation characteristics of children need in-depth study. This study investigated eight patients with DJS syndrome at the onset of the neonatal period and analyzed and discussed the clinical characteristics and gene mutation characteristics.

METHODS

Research object

The study retrospectively analyzed the clinical data of NDJS diagnosed and treated in the Gastroenterology Department of Hebei Children's Hospital from June 2016 to August 2020, and clinical data of children with cholestasis of unknown origin who developed this within 3 months of age. Cholestasis is defined as when total bilirubin (TB) < 5 mg/L, direct bilirubin (DB) > 1.0 mg/L, TB ≥ 5 mg/L, and DB/TB > 20%.[8] Information of 388 children with cholestasis were totally recorded, and the causes for disease were following aspects: abnormal anatomy were found in 62 cases, including 58 cases of biliary atresia, four cases of choledochal cysts; infection were present in 61 cases; parenteral nutrition-related cholestasis were found in 37 cases; hypothyroidism were

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Table 1. The number of cases and the proportion of the entire population of patients were classified by the causes of neonatal cholestasis.

	Causes of neonatal cholestasis	*n*	%
Abnormal anatomy	Biliary atresia	58	15.98
	Choledochus cyst	4	
Infectious disease		61	15.72
Parenteral nutrition-associated cholestasis		37	9.53
Endocrine disease	Hypothyroidism	3	0.78
Autoimmune disease	Neonatal erythematosus lupus	2	0.52
Genetic and metabolic liver disease		68	17.53
	Citrin deficiency	28	
	Alagille syndrome	9	
	Progressive familial intrahepatic cholestasis	*ABCB11* gene variant in 8, TJP2 gene variant in 1, *ABCB4* gene variant in 1	
	Dubin–Johnson syndrome	8	
	Niemann–Pick disease type C	2	
	Galactosemia	2	
	Transient acute liver failure caused by *TRMU* gene mutation	2	
	Nephronophthisis	1	
	Cystic fibrosis	2	
	Caroli disease and congenital fibrosis	3	
	Fatty acid oxidation defects	1	
Unexplained cholestasis		155	39.95

present in three cases; infantile lupus were found in two newborn cases; inherited metabolic liver disease were present in 68 cases, including 28 cases of Citrin deficiency, nine cases of Algille syndrome, ten cases of progressive familial intrahepatic cholestasis (eight cases of ABCB11 gene mutation, one case of TJP2 gene mutation, one case of ABCB4 gene mutation), eight cases of DJS, two cases of Niemann–Pick's disease type C, two cases of galactosemia, two cases of transient infantile acute liver failure caused by TRMU gene mutation, two cases of renal wasting disease (liver renal pancreatic dysplasia) in one case, cystic fibrosis in two cases, Caroli disease with congenital liver fibrosis in two cases, and fatty acid oxidation deficiency in one case. All the above were also summarized in Table 1.

Inclusion criteria for NDJS were as follows: (1) cholestasis in the neonatal period; (2) biallelic variation of the *ABCC2* gene; (3) cholestasis caused by other factors is excluded.

Inclusion process for children with cholestasis of unknown cause: (1) etiological examination: five items of hepatitis B, hepatitis A and C antibodies, HIV antibodies, Treponema pallidum antibodies, herpes simplex virus I and II, rubella virus-IgM antibody, cytomegalovirus, Epstein–Barr virus (EBV) antibody and EBV–DNA, hematuria and stool culture, and other pathogenic examinations. (2) Alpha-fetoprotein, blood sugar, lactic acid, blood ammonia, blood lipids, thyroid function, cortisol level, immunoglobulin, autoimmune hepatitis antibodies, blood tandem mass spectrometry, urine organic acid spectrum analysis, abdominal ultrasound, and other examinations to exclude endocrine, immunity, metabolic diseases. (3) Bone marrow puncture smear examination for children with a suspected blood disease. (4) Children with suspected B biliary atresia undergo laparoscopic or laparotomy, and intraoperative cholangiography assists in the diagnosis. (5) Genetic testing should be performed on children who cannot be diagnosed by the above examinations. After excluding biliary tract dysplasia, infection, hematological tumor diseases, liver damage caused by drug poisons, and autoimmune diseases, children whose genetic testing still fails to determine the cause are considered cholestasis of unknown cause.

Research methods

Clinical data collection. Clinical data of children with NDJS and unexplained cholestasis were collected. The data included age, birth history, family history, serum alanine transaminase (ALT) and aspartate transferase (AST), TB, DB, gamma-glutamyl-transpeptidase (GGT), total bile acid (TBA), serum albumin (Alb), blood glucose, and other laboratory indicators measured on the day of admission or the next morning on an empty stomach for 4 h. The general conditions and biochemical indexes of the two groups of children were compared. In the study, at <1 month of age, all the sick children had symptoms and their biological serum samples were collected. Moreover, they were not treated before the blood collection. Before blood collection, patients had to come on an empty stomach at least 4 h before on the day of admission or the next morning of hospitalization.

Gene detection and mutation analysis. With the consent of the child's guardian, 2 mL of peripheral venous blood (ethylenediaminetetraacetic acid anticoagulation) from the child and parents is collected and sent to MyGenostics (Beijing, China) for high-throughput sequencing. Genomic DNA is extracted with QIAamp Whole Blood DNA Extraction Kit (Qiagen, Germany), and GenCap Liquid Capture Kit (Mykino) is used to capture the exons of 210 genes, including the *ABCC2* gene (NM_000392) related to cholestasis and the 50 bp upstream and downstream regions. Paired-end sequencing on IlluminaNextSeq 500 is performed. After sequencing the target area, the adapters and low-quality data in the sequencing data are removed. BWA software (http://bio-bwa.sourceforge.net/) was used to compare the reference genome (hg19 version). GATK software (https://software.broadinstitute.org/gatk/) was used to detect the polymorphic sites of the comparison data of the samples and perform statistical analysis of single-nucleotide polymorphisms (SNPs) and InDels

Table 2. Comparison of general conditions and laboratory indicators between the two groups.

	NDJS group ($n = 8$)	Unexplained cholestasis group ($n = 155$)	Statistics Z value	P value
Male:female	5:3	104:51	–	1.000
Birth weight (kg)	3.2 (2.9, 3.4)	3.0 (2.5, 3.4)	−1.192	0.239
Age of onset (days)	6 (3, 14)	13 (4, 32)	−1.368	0.175
TB (μmol/L)	196.8 (140.0, 306.2)	132.8 (86.1, 174.6)	−2.320	0.018
DB (μmol/L)	81.6 (72.8,111.6)	91.5 (55.9, 123.2)	−0.261	0.800
DB/TB	0.55 (0.25, 0.62)	0.76 (0.62, 0.83)	−2.619	0.007
ALT (U/L)	33.5 (25.0, 36.8)	79.0 (32, 138)	−2.543	0.009
AST (U/L)	34.1 (26.7, 39.5)	126.0 (78, 206)	−4.033	0.000
ALT/AST	0.92 (0.67, 1.27)	0.64 (0.40, 0.83)	−2.308	0.019
TBA (μmol/L)	71.9 (36.9, 108.8)	98.9 (71.6, 135.8)	−1.564	0.120
GGT (U/L)	103.9 (58.1, 170.3)	131 (83, 243)	−1.337	0.186
Alb (g/L)	38.8 (36.7, 40.3)	36.5 (38, 39)	−0.529	0.605
INR	1.13 (1.08, 1.15)	0.96 (1.01, 1.04)	−1.476	0.143

and other data. All SNPs and InDels were annotated with ANNOVAR software (http://annovar.openbioinformatics.org/en/latest). According to the annotation information, the frequency of screening the normal population database is <0.02; screening SIFT(http://sift.jcvi.org/). PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) predicts harmful sites. The pathogenicity of the variation is judged according to the American College of Medical Genetics and Genomics (ACMG) sequence variation interpretation standards and guidelines. Finally, Sanger sequencing and family verification are performed on the potential pathogenic sites selected.

The Ethics Committee of Hebei Children's Hospital (2016034) approved this study, and the children's parents provided informed consent.

Statistical analysis. SPSS 21.0 statistical software is used for data analysis. Count data are expressed as a percentage, and the comparison between groups is tested by Fisher's exact probability method. Measurement data are expressed as the median (interquartile range), and the Wilcoxon rank-sum test is used for comparison between groups. $P < 0.05$ indicates that the difference is statistically significant.

RESULTS

General information

All 155 children with unexplained cholestasis have undergone genetic testing. DJS has been excluded from these 155 children. Eight children with NDJS and 155 children with cholestasis of unknown cause were included in the study. None of the children's parents were consanguineous. Two children with DJS were siblings (P7, P8). The remaining children denied having special diseases and similar family history (Tables 2 and 3).

In the NDJS group, there were five males and three females, with a birth weight of 3.2 (2.9, 3.4) kg, and the age of onset of jaundice was 6 (3, 14) days. There were two cases of Acholic stools (DJS) and 28 cases of children with cholestasis of unknown origin. Fisher's exact test was used, the P value was 1.0, and the difference between the two groups was not statistically significant. Due to cholestasis, the urine color of the two groups of children was yellow, and no detailed clinical records were recorded. Because vomiting is a common symptom in children within 3 months of age, gastric volvulus, gastroesophageal reflux, and other factors can cause vomiting and poor weight gain, so these two indicators have not been collected. But thanks to the reviewer's suggestions, we will collect data, follow-up with these children, and conduct further research in the next step.

There were 104 males and 51 females in the unexplained cholestasis group, with a birth weight of 3.0 (2.5, 3.4) kg, and the age of onset of jaundice was 13 (4, 32) days. There was no significant difference between the two groups of children in sex, birth weight, and age of onset (Table 2).

Comparison of biochemical indicators between the two groups

At the first diagnosis in the NDJS group, the TB level was 196.8 (140.0, 306.2) μmol/L, the highest value was 351.3 μmol/L, and the lowest value was 79.9 μmol/L. This was higher than that of children in the unexplained cholestasis group. The DB/TB ratio was 0.54 (0.27, 0.61), which was lower than that of children in the cholestasis group of unknown cause. ALT and AST were 33.5 (25.0, 36.8) and 34.1 (26.7, 39.5) U/L, respectively, which were lower than those of the idiopathic cholestasis (IC) group. The ALT/AST ratio was 0.92 (0.67, 1.27), which was higher than that of children in the IC group. The differences between the two groups were statistically significant. There were no statistically significant differences in the TBA, GGT, Alb, and international normalized ratio (INR) between the NDJS group and the IC group (Table 2).

Follow-up of children with NDJS

The oldest child with NDJS at follow-up is 7 years (P4), and the youngest child is 2 months old (P7). Five children are currently <1 year, and neither TB nor DB has returned to normal levels. The highest value of TB is 104.8 μmol/L (P2, 2 months), and the lowest value is 37.7 μmol/L (P6, 9 months). The highest value of DB is 87 μmol/L (P2, 2 months), and the lowest value is 32.7 μmol/L (P1, 6 months). Except for child P7 (2 months), whose GGT is 88 U/L, the GGT levels of the remaining children have returned to normal. The TBA of P7 (2 months) and P3 (7 months) have not returned to normal. However, the TBA of the remaining children has returned to the normal range (Table 3).

Three children are older than 1 year. The TB, DB, TBA, and GGT levels of P4 (7 years) are normal. For P5 (2 years), TB is 25.5 μmol/L, and DB is 23.4 μmol/L. For P8 (5 years), TB is 40.7 μmol/L and DB is 19.5 μmol/L. However, TBA and GGT of the two children are normal (Table 3).

Gene mutation analysis

There are biallelic variants in eight children with NDJS, of which one case is homozygous and seven cases are compound heterozygous variants. P7 and P8 are siblings. They have compound heterozygous variants, with the same variant types. A total of 13 variant types are detected, including nonsense variants c.2443C > T (p.R815X), c.1939G > T (p.E647X), c.3825C > G (p.Y1275X); missense variants c. 2302C > T (p.R768W), c.1177C > T

Table 3. Clinical and laboratory indicators of children with NDJS.

	Gender	Family history	Onset (d)	Age	TB (μmol/L)	DB (μmol/L)	DB/TB (%)	ALT (U/L)	AST (U/L)	TBA (μmol/L)	GGT (U/L)	Alb (g/L)	INR
					3.4–17.1	1.7–6.8		5–40	5–40	0–20	7–32	35–55	0.8–1.2
P1	Male	No	15	1 m	156.8	84.8	54.1	28	40	105.7	58.3	39.2	1.14
				6 m	43.5	32.7	75.2	24	37	18.4	26	40.6	1.09
P2	Female	No	20	1 m	79.9	72.5	90.7	37	56	129.6	37	38.4	1.12
				7 m	56.7	55.2	97.3	36	62	19.9	24	41.2	0.92
P3	Male	No	3	1 m	351.3	118.0	33.6	35	32	109.8	213	35.1	1.07
				7 m	56.1	47.7	85.0	23	47	48.5	11	45.4	1.15
P4	Male	No	4	1 m	135.2	78.4	57.9	24	27	77.8	58	41.5	0.98
				7 y	17.9	16.1	89.9	11	19	14.9	12	39.7	1.10
P5	Male	No	13	1 m	167.8	92.4	55.1	36	38	42.1	121	36.5	1.15
				2 y	25.5	23.4	91.7	15	25	2.1	10	38.8	1.12
P6	Female	No	3	1 m	225.7	144.1	63.8	32	24	35.2	182	40.6	1.36
				9 m	37.7	35.0	92.8	17	42	1.4	11	48.2	0.96
P7	Male	Yes	8	20d	324.3	73.5	22.7	12.2	26.6	20.4	135	39.1	1.13
				2 m	104.8	87.4	83.4	17	25	29.5	88	41.2	1.11
P8	Female	Yes	10	1 m	251.7	35.4	14.06	61.6	36.2	66	86.9	37.4	1.13
				5 y	40.7	19.5	47.9	10	23	3.7	7	41.5	0.98

BW birth weight, *W* week, *d* day, *m* month old.

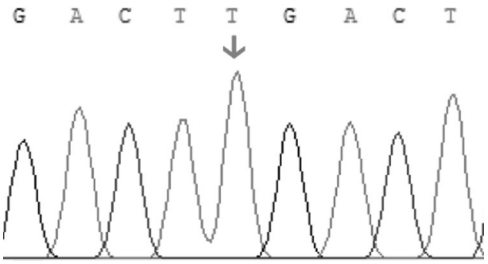

Fig. 1 Sanger sequencing diagram of *ABCC2* gene mutation of patient No 1. Homozygous mutation of the *ABCC2* gene in child P1: c.2443C > T (p.R815X).

(p.R393W), c.2026G > C (p.G676R), c.2125T > C (p.W709R), c.2224G > A (p. D742N), c.4020G > C (p.K1340N); splicing region variation: c.2439 + 5G > A; frameshift variation c.4237_4238insCT (p.H1414Lfs*17), c.960_961insGT (p.L322Cfs*3), and c.4250delC (p.S1417Ffs*14) (Figs. 1–4 and Table 4). The variation is distributed in exons 8, 9, 15, 16, 17, 18, 19, 27, 29, and 30. A search for the professional version of Human Gene Variant Database (HGMD) database shows c.2443 C > T (p.R815X), c.4237_4238insCT (p.H1414Lfs*17), c.960_961insGT (p.L322Cfs*3), c.4250delC (p.S1417Ffs*14), C.2224 G > A (p.D742N), c.4020 G > C (p.K1340N), c.2439 + 5 G > A and seven other variants have not been reported before.

The mutation c.2443C > T (p.R815X) is a nonsense mutation, which changes from cytosine to thymine at nucleotide 2443, resulting in a stop codon at position 815 of amino acid. The synthesis of the protein polypeptide chain is terminated early, and c.4237_4238insCT (p.H1414Lfs*17) is a frameshift variation. Cytosine and thymine are inserted between the 4237 and 4238 nucleotides, and the amino acid is frameshifted. The stop codon appears after the 17th amino acid after the 1414th amino acid, leading to premature termination of protein synthesis; c.960_961insGT (p.L322Cfs*3) is the insertion of guanine and thymine at the 960th and 961st nucleotides. This results in an amino acid frameshift. A termination codon appeared after the third amino acid after 322 amino acid, which caused the protein to be truncated ahead of time, resulting in structural abnormalities. In c.4250delC (p.S1417Ffs*14), due to the deletion of cytosine, the amino acid is shifted, and the stop codon appears after the 14th amino acid after the 1417th amino acid. This leads to the premature termination of protein synthesis. The above four types of mutations are judged as pathogenic variants according to ACMG guidelines. c.2224G > A (p.D742N), the heterozygous mutation at nucleotide 2224, changes from guanine to adenine, resulting in amino acid 742 changing from aspartic acid to asparagine. c.4020G > C (p.K1340N) changes from guanine to cytosine at nucleotide 4020, which causes the 1340th amino acid to change from lysine to asparagine; c.2439 + 5G > A (splicing) is the splicing region variation (Table 4).

The relationship between genotype and clinical phenotype was as follows: the main types of mutations in children with DJS in this study were truncating mutations (7/16) and missense mutations (7/16), followed by splice site mutations (2/16). One case was a homozygous variant (truncated variant), seven cases were a compound heterozygous variant, of which two cases were a compound heterozygous truncation variant, two cases were a compound heterozygous missense variant, two cases were a missense variant and splice site compound heterozygous variants, and one case was truncated variant and missense variant compound heterozygous variant. Previous studies have suggested that missense mutations and truncation mutations were identified among the two alleles of DJS patients in the early onset of infancy. The expression of MRP2 protein on the bile duct epithelium was influenced by the mutations mentioned above, which was a necessary condition for DJS phenotype identification in the neonatal period. In this study, two cases in neonatal-onset DJS children were compound heterozygous variants, suggesting that biallelic missense mutations can also manifest as the neonatal-onset DJS phenotype. It was worth noting that the two mutation sites in one of these two children were located in exons, which encoded the ABCC multidrug resistance-associated protein domain and ABC membrane region, respectively; the other mutation occurred in exons that encoded the ABCC multidrug resistance-associated protein domain. Missense mutations that

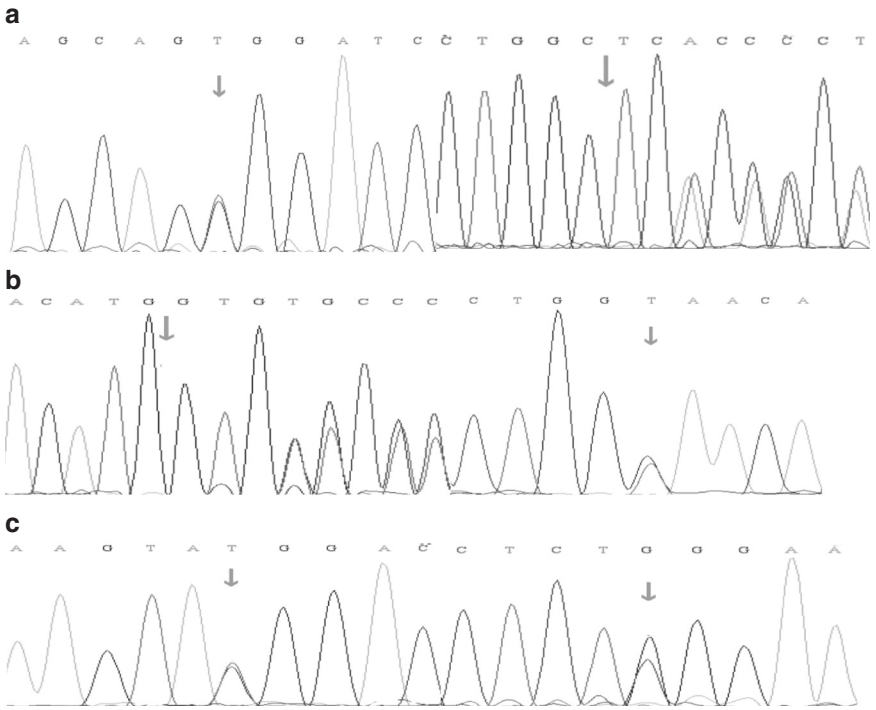

Fig. 2 Sanger sequencing diagram of *ABCC2* gene mutation of patients No 2, 3, and 4. a Compound heterozygous mutation of the *ABCC2* gene in child P2: c.4237_4238insCT (p.H1414Lfs*17); c.2302C > T (p.R768W). **b** Compound heterozygous mutation of the *ABCC2* gene in child P3: c.960_961insGT (p.L322Cfs*3); c.1939G > T (p.E647X). **c** Compound heterozygous mutation of the *ABCC2* gene in child P4: c.1177C > T (p.R393W); c.2026G > C (p.G676R).

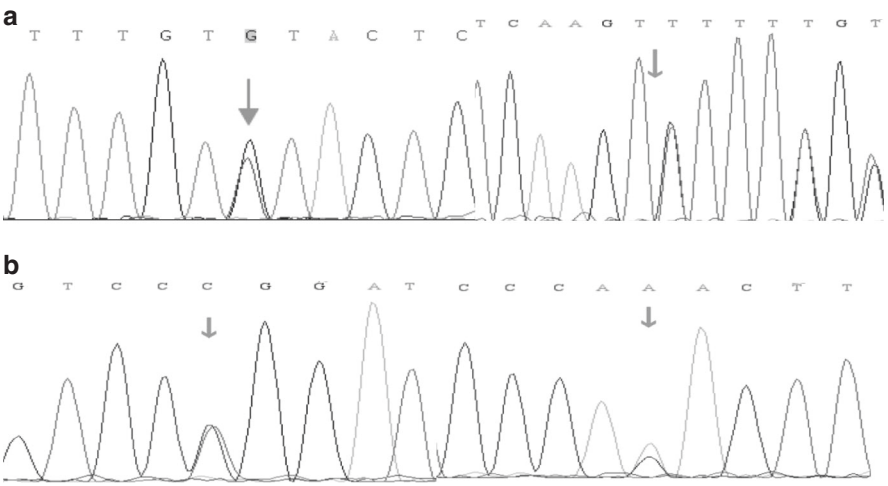

Fig. 3 Sanger sequencing diagram of *ABCC2* gene mutation of patients No 5 and 6. a Compound heterozygous mutation of the *ABCC2* gene in child P5: c.3825C > G (p.Y1275X); c.4250delC (p.S1417Ffs*14). **b** Compound heterozygous mutation of the *ABCC2* gene in child P6: c.2125T > C (p.W709R), c.2224G > A (p.D742N).

occurred in this region were also likely to be characteristic of the neonatal onset DJS phenotype.

DISCUSSION

With the rapid development of molecular biology detection technology, reports of DJS in the neonatal period have gradually increased.[4,9–13] At present, the description of DJS in the neonatal period in China is only a case report, and there is no clinical characteristic research with large sample sizes. In this study, eight children with NDJS were diagnosed by genetic testing methods, including one homozygous variant and seven compound heterozygous variants. A total of 13 variants were detected, including missense variants, nonsense variants, and frameshift variants. On searching the HGMD database professional version, c.2443C > T (p.R815X), c.4237_4238insCT (p.H1414Lfs*17), c.960_961insGT (p.L322Cfs*3), c.4250delC (p.S1417Ffs*14), c.2224G > A (p.D742N), c.4020G > C (p.K1340N), c.2439 + 5G > A, and other seven mutations have not been reported, which expands the ABCC2 gene mutation spectrum.

The *ABCC2* gene is located on chromosome 10q24 and contains 32 exons. There have been 74 mutations reported, and the mutation sites are distributed in all exons of the *ABCC2* gene. c.2302C > T (p.R768W) is currently considered to be one of the ABCC2 gene

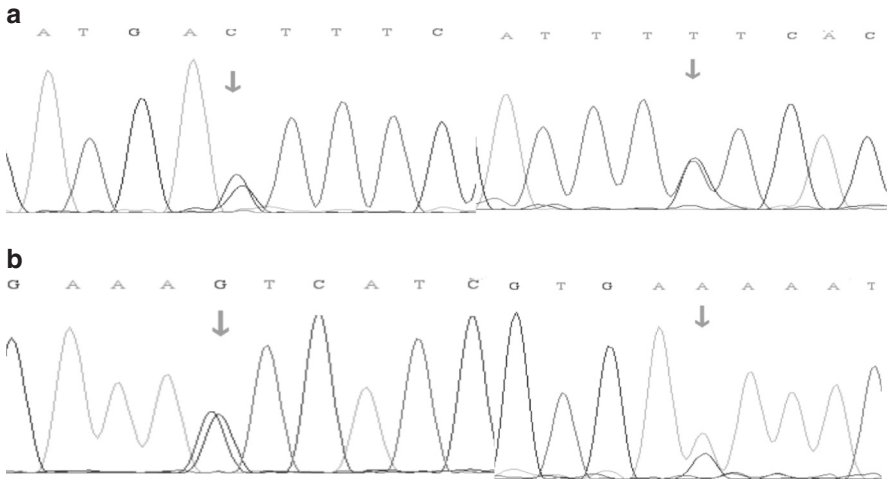

Fig. 4 Sanger sequencing diagram of ABCC2 gene mutation of patients No 7 and 8. a Compound heterozygous mutation of the *ABCC2* gene in child P7: c.4020G > C (p.K1340N), c.2439 + 5G > A (splicing). **b** Compound heterozygous mutation of the *ABCC2* gene in child P8: c.4020G > C (p.K1340N), c.2439 + 5G > A (splicing).

mutation hotspots. It has been reported in infants and adults with DJS. The patients were from different races in the Caucasus, France, North Africa, and other regions. Parts of Asia, such as Japan and South Korea,[11,14] Turkey, and other countries have also reported cases.[11,15,16] This mutation is located in exon 18 and occurs in the nucleotide functional region and the highly conserved region. It interferes with the maturation of MRP2 and affects its entry from the endoplasmic reticulum to the Golgi apparatus, causing serious damage to protein activity. Togawa et al.[11] detected c.2302C > T (p.R768W) mutation rate of 20% (4/20) in ten children with NDJS. Kim et al.[14] found that the rate of this in six children with NDJS was 33.4% (4/12). In this study, only child P2 had a heterozygous mutation in the c.2302C > T (R768W) gene, and the mutation rate was 6.25% (1/16). This mutation has not been found in previous reports of patients with NDJS in China.[4–6,13,17–19] This is lower than other East Asian countries. The types of gene mutations may have different geographic distribution characteristics. The characteristics of the NDJS gene mutation spectrum in China need to be further expanded in the sample size study.

At present, the biochemical indicators of these children have gradually recovered during the period of patient follow-up, and the values in detail have been illustrated in the paper. Two of the eight children (P1, P3) developed fever, cough, and rapid breathing at the age of 9 and 11 months, respectively, and were diagnosed as pneumonia. The first-generation cephalosporin drug was used for anti-infection and budesonide atomization. After comprehensive therapy, the pneumonia in these children was controlled. During the treatment, the level of bilirubin was not increased, and the levels of transaminase, TBA, and GGT were normal. None of the children had chronic liver disease such as cirrhosis. We would conduct long-term patient follow-up to observe the changes in various indicators of liver function during the growth and development of these children, as well as whether the bilirubin raised or cholestasis occurred again under the conditions of infection, drugs, pregnancy, hormone changes, etc.

DJS was mostly diagnosed in adults in the past. The clinical manifestations were chronic or intermittent cholestasis. The biochemical index changed to a slight increase in conjugated bilirubin, and the levels of bile acid and transaminase were normal. With the increasing application of molecular biology in clinical practice, reports of NDJS have gradually increased. In this study, the highest serum TB in children with NDJS at the first diagnosis was 351.3 μmol/L, the lowest was 79.9 μmol/L, and the average was 196.8 μmol/L, which was higher than the adult DJS bilirubin level.[12,16] Studies have found that the expression level of MRP2 protein in newborn rats is only 50–60% of that of adult rats, and it only develops to the normal level of adult rats when they are 1–3 weeks and close to weaning.[20] Human MRP2 gene expression is similar.[21] Its level is only 1/200 of that of adults in the neonatal period and 1/100 of the adult level in infancy.[22] The reduction of MRP2 expression levels leads to higher TB levels in children with NDJS than in adults with DJS. At the same time, because the activity of the UGT1A1 enzyme in newborns is still <1% of that of adults, its activity does not reach adult levels until 3 months of age. Nearly 50% of full-term infants and 80% of premature infants will develop jaundice.[23] The onset of NDJS in children is in the neonatal period, and the above-mentioned effects work together with the low level of MRP2 gene expression, making the TB level of NDJS children significantly higher than that of adult DJS. During follow-up, the child's jaundice gradually subsided, and the levels of TB and DB decreased, but the DB/TB ratio was higher than that at the initial diagnosis. The reason was that with the age increasing, the childrens' liver gradually matured and their liver glycosylation process improved; thus, the level of indirect bilirubin was decreased and the DB/TB ratio was enhanced. However, there was a mutation in the *ABCCC* gene. So pregnancy, infection, or the effect of certain drugs can induce the patient to reappear jaundice. This suggests that direct bilirubin metabolism is impaired and indicates that the dysfunction of MRP2 protein in the process of direct bilirubin transport from liver cells to bile ducts may persist.

Therefore, when the patient enters adolescence or adulthood, jaundice may reappear under the conditions of pregnancy, infection, or certain drugs.[12,24,25] In this study, the TBA level of children with NDJS was increased by 3.5 times the normal level at the first visit, and the GGT was more than two times higher than the normal level. During follow-up, the TBA and GGT indicators of the child gradually decreased. After 1 year, the TBA and GGT of the child completely returned to normal levels, suggesting the benign self-limiting process of DJS.[26]

Cholestasis has a complex etiology. Infection, autoimmunity, metabolism, hematological tumors, gene mutations, and other factors can all cause bile acid metabolism disorders, liver cell damage, and increase ALT and AST levels. Compared with children with unexplained cholestasis, the level of transaminase in children with NDJS is normal or only slightly elevated. The risk of liver cirrhosis and tumors is extremely low, and the prognosis is good. In this study, the ALT and AST levels of children with NDJS were significantly lower than those of children with IC. The AST at the initial diagnosis of P2 at 56 U/L and the follow-up at 7 months of

Table 4. Gene mutations and pathogenicity judgments in eight children with NDJS.

	Homozygous/heterozygous	Mutations	Exon	Normal frequency	HGMD	SIFT	PolyPhen_2	Source	ACMG score
P1	Homozygous	c.2443C > T (p.R815X)	19	0.00002	No	Harmful	Harmful	Parents	Pathogenic
P2	Compound heterozygous	c.4237_4238insCT (p.H1414Lfs*17)	30	0.00010	No	Unknown	Unknown	Father	Pathogenic
		c.2302C > T (p.R768W)	18	0.00030	Yes	Harmful	Harmful	Mother	Suspected of causing illness
P3	Compound heterozygous	c.1939G > T (p.E647X)	15	0.00001	Yes	Unknown	Unknown	Father	Pathogenic
		c.960_961insGT (p.L322Cfs*3)	8	–	No	Unknown	Unknown	Mother	Pathogenic
P4	Compound heterozygous	c.1177C > T (p.R393W)	9	0.00060	Yes	Harmful	Harmful	Father	Pathogenic
		c.2026G > C (p.G676R)	16	–	Yes	Harmful	Harmful	Mother	Pathogenic
P5	Compound heterozygous	c.3825C > G (p.Y1275X)	27	0.0009	Yes	Harmful	Harmful	Father	Pathogenic
		c.4250delC (p.S1417Ffs*14)	30	–	No	Harmful	Harmful	Mother	Suspected of causing illness
P6	Compound heterozygous	c.2125T > C (p.W709R)	17	0.0001	Yes	Harmful	Harmful	Mother	Suspected of causing illness
		c.2224G > A (p.D742N)	17	0.0003	No	Harmful	Harmful	Father	Unknown
P7-8	Compound heterozygous	c.4020G > C (p.K1340N)	29	–	No	Harmful	Harmful	Mother	Unknown
		c.2439 + 5G > A (splicing)	18	0.0001	No	–	–	Father	Unknown

The ABCC2 gene transcript is: NM_000392.
ACMG American Society of Medical Genetics and Genomics, *PVS1* zero-effect mutation (frameshift mutation or nonsense mutation or splicing site mutation), which may cause loss of function, *PM2* the frequency in the normal population database is a low-frequency variant; *PP3* multiple bioinformatics protein function prediction software predicts harmful, *PM3* recessive genetic disease, existing in trans with another pathogenic variant (compound heterozygous with another pathogenic variant), *PS1* the human genome database has reported the pathogenicity of this locus, *PP4* the patient's phenotype or family history is highly specific to a disease with a single genetic basis.

age at 62 U/L was slightly higher than the normal value. However, the AST levels of other children with NDJS were all within the normal range. This is one of the good prognostic indicators of NDJS. In infants with moderate to severe high direct bilirubinemia, if the transaminase continues to be normal and other causes of cholestasis can be excluded, those looking for conditions like NDJS should be highly vigilant.

The limitations of this study were: (1) the harmfulness of gene variants was predicted by an online software. It was necessary to make a supplement on functional verification for identifying the impact of newly discovered variants on proteins. (2) Since most children with DJS recovered well after treatment. Liver puncture was not performed, and pathological observation and immunohistochemistry for neonatal early-onset DJS were missing. (3) The number of cases was small, and the quantity of samples and long-term follow-up of recovered patients were still needed. To observe the improvement of biochemical indicators in children with DJS, the effect of medication and pregnancy on DJS, and the possibility of developing chronic liver disease and cirrhosis were what we put energy into paying attention to in the future.

CONCLUSION

In this study, eight children with NDJS were diagnosed through genetic testing. By summarizing the characteristics of biochemical indicators, seven new mutation types of the *ABCC2* gene were detected, which expanded the mutation spectrum of the *ABCC2* gene. However, this study has limitations and is a retrospective study. The research is limited to patients in northern China, and further research is needed to expand the sample size to people in more regions and of different races, explore new gene variants, and understand the relationship between genotypes and clinical phenotypes.

DATA AVAILABILITY

Data are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

H.F. and R.Z. contributed to conception and design. X.J. and X.L. contributed to acquisition, analysis, and interpretation of data. G.L. and C.Y. wrote the first draft of the paper. All authors reviewed, critically revised, and approved the final version of the manuscript.

ADDITIONAL INFORMATION

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