Received: 29 June 2021 | Revised: 31 December 2021 | Accepted: 6 January 2022

DOI: 10.1002/mgg3.1875

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

Mdr3 gene mutation in preterm infants with parenteral nutrition-associated cholestasis

Xiufang Yang[1,2] | **Guosheng Liu**[3] | **Bing Yi**[4]

[1]Department of Neonatology, Zhongshan Hospital Affiliated to Sun Yat-Sen University, Zhongshan, P.R. China

[2]Department of Neonatology, Guangdong Medical College, Zhanjiang, P.R. China

[3]Department of Neonatology, The First Affiliated Hospital of Jinan University, Guangzhou, P.R. China

[4]Molecular Inspection Center, Zhongshan Hospital Affiliated to Sun Yat-Sen University, Zhongshan, P.R. China

Correspondence
Xiufang Yang, Department of Neonatology, Zhongshan Hospital Affiliated to Sun Yat-Sen University, No. 2 of Sunwen Road, Zhongshan, Guangdong 528403, P.R. China.
Email: yamgxiufamg@aliyun.com

Funding information
This study was funded by the Project of the Science and Technology Bureau of Zhongshan city (2016B1016). The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Abstract

To investigate the relationship of multidrug resistance 3 (*Mdr3*) gene mutation and parenteral nutrition-associated cholestasis (PNAC) in preterm infants. Preterm infants who had received total parenteral nutrition for at least 14 days were enrolled: 76 preterm infants in the PNAC group and 80 preterm infants in the non-PNAC group. Genomic DNA was extracted from white blood cells. Twenty-eight exons of the *Mdr3* gene were amplified by polymerase chain reaction. PNAC infants of 1 month corrected age with the *Mdr3* gene mutation and abnormal liver biochemistry were selected for the experimental liver biopsy group. Five normal adult living liver transplantation donors were enrolled in a normal donor group. The *Mdr3* missense mutations c.1031G>A, c.3347G>A, and c.485T>A, and the *Mdr3* frameshift mutation c.2793_2794insA were found in the PNAC group. The allele frequency and genotype frequency of c.1031G>A, c.3347G>A, and c.485T>A in the *Mdr3* gene in the PNAC group were significantly higher than those in non-PNAC group ($p < 0.05$). The rate of *Mdr3* gene mutations c.1031G>A, c.485T>A, c.3347G>A, and c.2793_2794insA in the PNAC group was higher than in the non-PNAC group (21.05% vs. 1.25%, respectively, $\chi^2 = 15.747$, $p < 0.05$). *Mdr3* gene mutations c.2793_2794insA, c.1031G>A, c.3347G>A, and c.485T>A may be the genetic cause of PNAC.

KEYWORDS

Mdr3 gene mutation, parenteral nutrition-associated cholestasis, preterm infants

1 | INTRODUCTION

Parenteral nutrition-associated cholestasis (PNAC) is a serious complication in preterm infants receiving long-term parenteral nutrition (PN). PNAC can cause irreversible liver injury, liver cirrhosis, liver failure, and even death (Carter & Shulman, 2007; Duro et al., 2011; Kaufman et al., 2003). Research has shown that the incidence of PNAC in preterm infants with PN is as high as 50% (Suchy, 2001). However, the pathogenesis of PNAC is not clear. The multidrug resistance 3 glycoprotein (*Mdr3 P*-gp) is involved in lecithin secretion and bile transport and is encoded by the *Mdr3* gene. *Mdr3* gene mutation has been shown to be associated with multiple causes of intrahepatic cholestasis

including familial intrahepatic cholestasis, pregnancy cholestasis, and cholelithiasis (Denk et al., 2010; Harikar et al., 2009; Schneider et al., 2007; Wasmuth et al., 2007; Ziol et al., 2008). Mutations in the *Mdr3* gene may affect the quantity and function of the *Mdr3 P-gp* and this may lead to cholestasis as a result of a lack of phospholipids in the bile (Gotthardt et al., 2008; Oude Elferink & Paulusma, 2007). The purpose of this study was to investigate the relationship between PNAC and *Mdr3* through the detection of *Mdr3* gene mutations and the expression of *Mdr3 P-gp* in hepatocytes.

2 | METHODS

Patients were preterm infants admitted to the Department of Neonatology between 01 June 2011, and 30 November 2017. All infants had received total parenteral nutrition (TPN) for at least 14 days and were divided into two groups: there were 76 preterm infants in the PNAC group, and 80 preterm infants without PNAC in the non-PNAC group. Both groups received parenteral nutrition and enteral nutrition according to intestinal function. This study was approved by the ethics committee and written informed consent was obtained from the guardians of all participants. Each of the five adult liver transplant recipients had agreed to donate a small amount of liver tissue from their donor liver to our study and had signed a consent form.

The diagnostic criteria (Blau et al., 2007) for PNAC includes more than 14 days of PN therapy and jaundice, an enlarged liver, lightened stools, increased transaminase, increased total bile acid (TBA), a direct bilirubin (DBil) level higher than 2 mg/dL, and/or a DBil level accounting for more than 20% of total bilirubin (TBil).

Other causes of cholestasis need to be excluded, such as viral hepatitis, septicemia, hereditary metabolic disease, congenital biliary atresia, circumferential pancreas, and congenital choledochal cyst. This requires investigations including routine blood count, blood glucose, C-reactive protein, blood culture, hepatobiliary biochemistry, cytomegalovirus, and hepatitis virus serology, as well as hepatobiliary ultrasound, radiography, electrical capacitance tomography, magnetic resonance cholangiopancreatography, and metabolic disease screening (Cholestatic Liver Disease Diagnosis and Treatment Expert Committee, 2009).

3 | METHODS

On days 1, 14, 30, 60, and 90 after birth, serum hepatobiliary biochemistry levels were obtained and clinical manifestations were observed. Genomic DNA was extracted from peripheral venous white blood cells using the phenol chloroform method and samples were stored at −80°C (Barnett & Larson, 2012).

Twenty-eight exons of the *Mdr3* gene were amplified by polymerase chain reaction (PCR). *Mdr3* mutations were detected with restriction enzyme digestion and the DNA sequence was analyzed using the ABI Prism® 3100 genetic analyzer (Applied Biosystems). To extract genomic DNA, the following was used: isopropanol, DNAzol BD reagent (Invitrogen Life Technologies), a high-speed centrifuge (Eppendorf), 75% ethanol, and the QL-901 Vortex Mixer (Kylin-Bell Lab Instruments Co., Ltd.). A PCR machine (C1000; Bio-Rad) was used to detect the extracted DNA. The primers were synthesized using PCR-restriction fragment length polymorphism. Primer Software Version 5.0 (Premier Biosoft International) was used to design the primers, and the DNA concentration was quantified using a UV–VIS spectrophotometer (UV-5200, Shanghai Metash Instrument Co., Ltd.). The primers of the *Mdr3* gene exons are shown in Table 1.

In order to carry out PCR (Bio-Rad), 45 μl of Platinum® PCR SuperMix (Invitrogen Corporation) was used with 100 ng of genomic DNA and 200 μmol/l of deoxynucleoside-5-triphosphate (Takara). PCR steps included denaturation, annealing, and extension. PCR products (3 μl) were identified using electrophoresis of 1% Bio agarose gel (Bio-Rad) using an automated gel imaging system and DNA labeling (Takara). The *Mdr3* gene mutations were detected using the restriction enzyme (sex AI) digestion method and an ABI Prism® 3100 genetic analyzer (Applied Biosystems). The human genome sequence of the *Mdr3* gene in GenBank (GenBank, 2011) was used to compare the DNA sequences.

PNAC preterm infants with the *Mdr3* gene mutation and abnormal hepatobiliary biochemistry at 1 month corrected age were enrolled in the experimental group for percutaneous liver biopsy. Five normal adult living liver transplantation donors formed the normal liver donor group. Percutaneous liver biopsies were performed with informed consent. The expression level of *Mdr3 P-gp* in hepatocytes was determined using western blot analysis.

3.1 | Statistical analysis

SPSS 20.0 software (SPSS Inc.) was used for statistical analysis. The chi square test was performed to analyze the difference in the distribution of genotype frequency and allele frequency between the two groups. Comparing the data of the two groups with the Student's *t* test, a value of $p < 0.05$ was considered to be statistically significant. The BioEdit protein contrast software

TABLE 1 The primer sequences and amplification product length of exons 1–28 of *Mdr3* gene

Exons	Primers	Primer sequences	Annealing temperature (°C)	Amplification product length (bp)
Exon 1	Forward primer	5′-GGCTGCAACGGTAGGCGTTT-3′	65	434
	Reverse primer	5′-GGCGTGTAACGGAAAAGCCAGT-3′		
Exon 2	Forward primer	5′-GCGAGGTTCGAGGTGAGAGA-3′	65	404
	Reverse primer	5′-AACCGGATGCAAGACCCTTC 3′		
Exon 3	Forward primer	5′-CTTTCCTGTGTATGTGAGCTCTG-3′	65	453
	Reverse primer	5′-TCCAGGCTGGTCTCAAACTC-3′		
Exon 4	Forward primer	5′-GATTGATTTCTTCACAGAATACAAAA-3′	62	416
	Reverse primer	5′-TCTGGAGTCAACCAGATATCCA-3′		
Exon 5	Forward primer	5′-CCTAAACCCTTGGGCTCTTT-3′	62	432
	Reverse primer	5′-AATTGGGATTTGGGAGCAA-3′		
Exon 6	Forward primer	5′-TTGCAGTGAGCTGAGATGGT-3′	62	720
	Reverse primer	5′-TAGACATGGCTGCCAGATGA-3′		
Exon 7	Forward primer	5′-GGCTTGCAGTCAGTGAACAA-3′	62	488
	Reverse primer	5′-CCAGCCTGTGACATTTTGAA-3′		
Exon 8	Forward primer	5′-TGGCATTTGCTACATGACTTT-3′	62	415
	Reverse primer	5′-GCCATCAGTAAAGGGTGCTT-3′		
Exon 9	Forward primer	5′-GCCTGGCTGATCCTGAATTA-3′	58	433
	Reverse primer	5′-TGGACAGTGGAAAGATTCACC-3′		
Exon 10	Forward primer	5′-AAAGGAAAGGATAAACCTAAACTTAAT-3′	62	309
	Reverse primer	5′-TTTCATTTATTACTAACAGGTCATTCA-3′		
Exon 11	Forward primer	5′-CCAGGTCCTATTTTTGGAATTTGCTGA-3′	62	322
	Reverse primer	5′-AACCCCCAAAGGAAAAGGCACATAA-3′		
Exon 12	Forward primer	5′-GTGCCTTTTCCTTTGGGGGTTA-3′	62	423
	Reverse primer	5′-TGAAACCAGCCCAAGGGTGT-3′		
Exon 13	Forward primer	5′-TTTTTGTTCTTGCATATTGCTG-3′	62	403
	Reverse primer	5′-AACTGAGTCATTCAGGGGACTT-3′		
Exon 14	Forward primer	5′-TTTTATACAGCATGTGTCAGTTTTT-3′	62	500
	Reverse primer	5′-GAAATCAATACAGCTCCATGAGG-3′		
Exon 15	Forward primer	5′-TGATGCCTTTGCCATAATCA-3′	62	450
	Reverse primer	5′-TCCCCTATTTTCTCACCTGCT-3′		
Exon 16	Forward primer	5′-AATATTCCAAATTGCTTTATGATTC-3′	62	322
	Reverse primer	5′-TGGCTCATAGTAGCAGTCATCTG-3′		
Exon 17	Forward primer	5′-GCTTGTCATTCTCTGCACCT-3′	62	423
	Reverse primer	5′-TTAAGGACTTTGGCTTAGTTTAATTT-3′		
Exon 18	Forward primer	5′-CCACAATTACCAAAACCCTACA-3′	62	403
	Reverse primer	5′-TACCCTCCAGCAGAGCCTTA-3′		
Exon 19	Forward primer	5′-CTGCCATGTGGTGTCTGC-3′	62	500
	Reverse primer	5′-GCTCTTGAAGGACCAGGACA-3′		
Exon 20	Forward primer	5′-AGAGATGCCCTCCCTGCTAC-3′	62	450
	Reverse primer	5′-GCAGTGGGTCAATCAACCTT-3′		
Exon 21	Forward primer	5′-GGAATGAGAGTGTAAAGGCACA-3′	65	352
	Reverse primer	5′-CAATTATTAGTTGTAGTGGGCACAAA-3′		
Exon 22	Forward primer	5′-TCAGCCTTCCTGAGCATACA-3′	62	329
	Reverse primer	5′-TTTTCAGTGACAGAATTGTTGAAAA-3′		

(Continues)

TABLE 1 (Continued)

Exons	Primers	Primer sequences	Annealing temperature (°C)	Amplification product length (bp)
Exon 23	Forward primer	5′-TTGTCAAAACAATTCTGCATTACA-3′	54	468
	Reverse primer	5′-CCAGGATGGAAACTGTGGTAA-3′		
Exon 24	Forward primer	5′-GGGGAGAAAGGGGATGATTA-3′	65	339
	Reverse primer	5′-TGTCAGTCAAGTTGCCCAAA-3′		
Exon 25	Forward primer	5′-TCACCTTCATTTCACACCATAA-3′	62	344
	Reverse primer	5′-TGTGGTCATTGTATCAAACAGGA-3′		
Exon 26	Forward primer	5′-TGCTGGCCTCAATGGTATAA-3′	62	484
	Reverse primer	5′-ATCCTGAAGTGCCTTGTCCA-3′		
Exon 27	Forward primer	5′-TCCTCCTCCTATTTTAAAGAAAGTT-3′	62	382
	Reverse primer	5′-AATCGCTTGAACCCTGGAG-3′		
Exon 28	Forward primer	5′-AGCATGGGAACCCATTTGT-3′	62	641
	Reverse primer	5′-TCTCAAATTTCAAATGCCGTA-3′		

was used to analyze gene mutations (BioEdit protein contrast software, 2011).

4 | RESULTS

4.1 | PCR-amplified MDR3 fragments electrophoresis and DNA sequencing

The PCR amplification fragments of exons 1–28 of *Mdr3* through electrophoresis can be clearly seen (Figure 1). The *Mdr3* missense mutations c.1031G>A, c.3347G>A, and c.485T>A, and the *Mdr3* frameshift mutation c.2793_2794insA were found in the PNAC group (Figures 2). The allele frequency and genotype frequency of c.1031G>A, c.3347G>A, and c.485T>A in the *Mdr3* gene in the PNAC group were significantly higher than those in non-PNAC group ($p < 0.05$) (Table 2). The rate of *Mdr3* gene mutations c.1031G>A, c.485T>A, c.3347G>A, and c.2793_2794insA in the PNAC group was higher than in the non-PNAC group (21.05% vs. 1.25%, respectively, $\chi^2 = 15.747$, $p < 0.05$). These four types of *Mdr3* gene mutations may be high-risk factors for PNAC (OR = 21.067, $p < 0.05$).

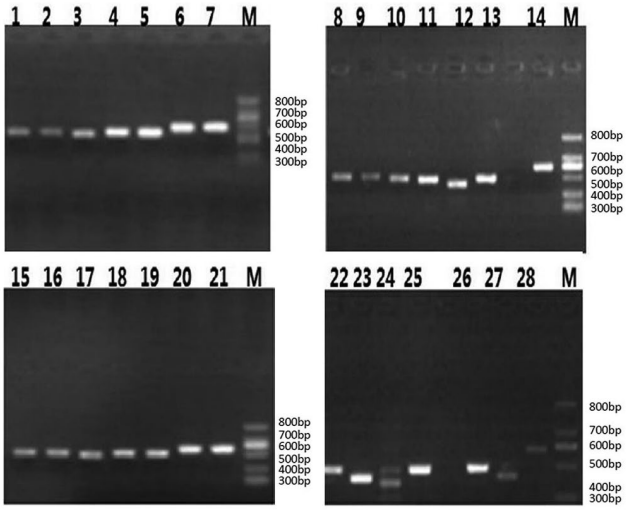

FIGURE 1 Amplification product of gene fragments of the *Mdr3* gene exons 1–28 (lanes 1–28); M: DNA Marker DL2000

4.2 | Hepatobiliary biochemistry

Hepatobiliary biochemistry on day 30 after birth in infants in the PNAC group with and without the *Mdr3* gene mutations c.485T>A, c.2793_2794insA, c.1031G>A, and c.3347G>A were compared. The serum levels of ALT, TBil, DBil, TBA, and γ-GT in infants with these four types of *Mdr3* gene mutations in the PNAC group (group A) were higher than infants without these mutations in the PNAC group (group B, $p < 0.05$) (Table 3). The duration of jaundice in group A was significantly longer than in group B (135 ± 32 vs. 84 ± 25 days, respectively, $p < 0.01$), and in group A, liver biochemistry at 1 month corrected age was abnormal.

4.3 | Pathological changes of the liver

The parents of the four infants in group A gave consent for their infants to undergo percutaneous liver biopsy. The four infants each had one of the *Mdr3* gene mutations c.485T>A, c.2793_2794insA, c.1031G>A, and c.3347G>A. The pathological changes of the liver of the PNAC infant with the *Mdr3* c.2793_2794insA gene mutation included the partial destruction of liver tissue, diffuse infiltration of

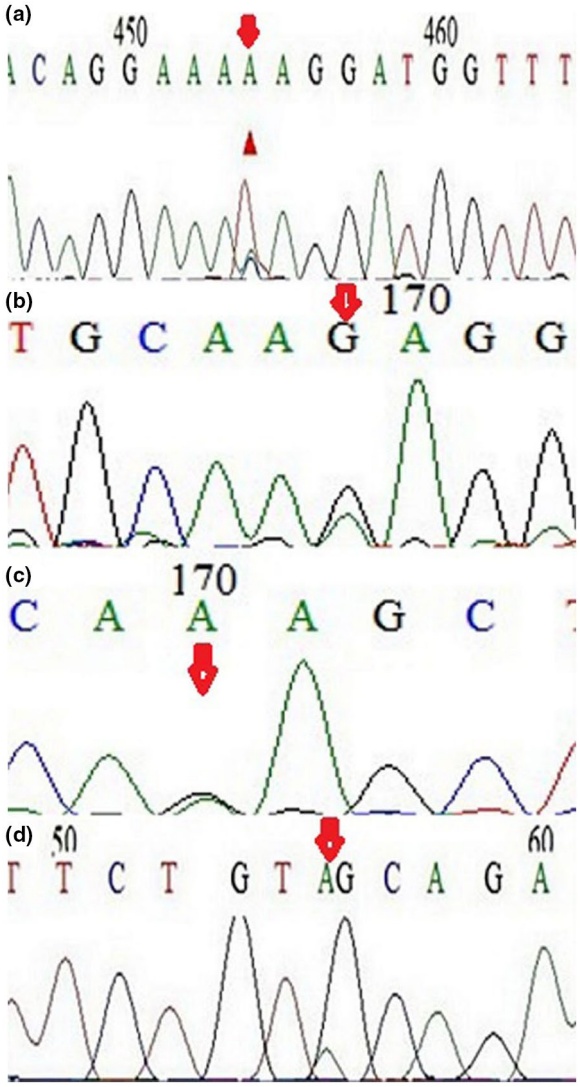

FIGURE 2 (a) DNA sequencing of the *Mdr3* c.485T>A gene mutation: The mutation site is GAAAAAGGA, the protein site is p.(Ile162Lys), missense mutation. (b) DNA sequencing of the *Mdr3* c.1031G>A gene mutation: The mutation site is AATGCAAGA, protein site p.(Glu344Lys), heterozygous mutation. (c) DNA sequencing of the *Mdr3* c.3347G>A gene mutation: the mutation site is CTCAGCT, protein site p.(Arg1116Lys), heterozygous mutation. (d) DNA sequencing of the *Mdr3* c.2793_2794insA frameshift gene mutation

lymphocytes and monocytes, degeneration of hepatocytes, dilation of hepatic sinusoids, and brown pigmentation. Hepatocyte degeneration, bile accumulation, eosinophil formation, and brownish pigmentation were found in the PNAC infants with the *Mdr3* gene mutations c.485T>A, c.1031G>A, and c.3347G>A. In the normal liver group, liver parenchyma showed an intact structure, with normal structure of lobules, central veins, hepatic cords, and hepatic sinuses. No lymphocyte or monocyte infiltration, hepatocyte degeneration, or eosinophilic bodies were seen. The histopathologic features of liver tissue in these cases are shown in Figures 3. Among them, the liver pathology of PNAC infant with *Mdr3* c.2793_2794insA frameshift mutation showed partial structural destruction of liver tissue, scattered lymphoid and monocyte infiltration, hepatocyte degeneration, hepatic sinus expansion, and Tan pigmentation, as shown in Figure 3a. The liver pathological manifestations of PNAC children with *Mdr3* c.485T>A mutation were hepatocyte degeneration, bile accumulation, eosinophilic body formation, and Tan pigmentation, as shown in Figure 3b. The liver pathology of PNAC children with MDR3c.3347G>A or c.1031G>A mutant showed hepatocyte degeneration, inflammatory cell infiltration, and Tan pigment in hepatocytes, as shown in Figure 3c,d). The liver histopathology of normal living liver donors showed that the liver tissue structure was complete, normal liver lobules were visible, central veins, hepatic cords, and hepatic sinuses were visible, and no lymphoid and monocyte infiltration were found, no hepatocyte degeneration and eosinophilic bodies are found, as shown in Figure 3e.

4.4 | MDR3 glycoprotein in hepatocytes

On liver biopsy, western blot analysis showed that the *Mdr3 P-gp* protein grayscale of the liver in the experimental group (PNAC infants with the *Mdr3* mutations c.2793_2794insA, c.1031G>A, c.3347G>A, and c.485T>A) was significantly lighter than that of the normal liver donor group (259 ± 121 vs. 2617 ± 139, respectively, $t = 12.186$, $p < 0.01$). The protein grayscale ratio of *Mdr3 P-gp/β-Actin* in the experimental liver biopsy group was significantly lower than that of the normal liver donor group (0.059 ± 0.027 vs. 1.114 ± 0.098, respectively, $t = 20.686$, $p < 0.01$). Western blot analysis showed that in the four infants with the *Mdr3* gene mutations c.2793_2794insA, c.1031G>A, c.3347G>A, or c.485T>A, the expression of *Mdr3 P-gp* in hepatocytes was significantly lower than in the normal liver donor group (Figure 4). This is a representative print based on the experiment we did three times.

5 | DISCUSSION

TPN provides nutrition for preterm infants and improves survival (Klein et al., 2010). However, long-term use of PN may result in PNAC. Due to the complications of PNAC, it is important to identify its pathogenic factors. The domestic and foreign literature suggest that mutation of the *Mdr3* gene is closely related to intrahepatic cholestasis. The *Mdr3* gene, also known as the B4 gene of the ATP-binding cassette transporter family, is

TABLE 2 Comparison of frequency of genotypes and alleles in *Mdr3* gene mutation between PNAC and non-PNAC group

SNPs	Genotype frequency and alleles	PNAC group	Non-PNAC group	χ^2	*p*
c.1031G>A	GG	30 (39.47%)	47 (58.75%)	9.516	<0.05
	GA	5 (6.58%)	0		
	AA	41 (53.94%)	33 (41.25%)		
	G	65 (49.34%)	94 (58.75%)	7.769	<0.05
	A	87 (50.65%)	66 (41.25%)		
c.3347G>A	GG	39 (52.63)	52 (65%)	6.084	<0.05
	GA	4 (3.95%)	0		
	AA	33 (43.42%)	28 (35%)		
	G	82 (53.95%)	104 (65%)	4.992	<0.05
	A	70 (46.05%)	56 (35%)		
c.485T>A	TT	40 (52.63%)	58 (72.5%)	7.956	<0.05
	TA	5 (6.58%)	1 (1.25%)		
	AA	31 (40.79%)	21 (26.25%)		
	T	85 (55.92%)	117 (73.13%)	9.984	<0.05
	A	67 (44.08%)	43 (26.87%)		

TABLE 3 Comparison of liver biochemistry on the 30th day after birth between PNAC groups with and without *Mdr3*c.485T>A, c.2793_2794insA, c.1031G>A, and c.3347G>A gene mutations

Group	Cases	ALT (U/L)	T-BIL (μmol/L)	D-BIL (μmol/L)	TBA (μmol/L)	γ-GT (U/L)
A group	16	189 ± 25	205 ± 33	183 ± 29	156 ± 27	151 ± 32
B group	60	110 ± 19	158 ± 27	86 ± 17	105 ± 24	127 ± 19
t value		13.458	5.763	16.811	7.183	3.738
p		<.05	<.05	<.05	<.05	<.05

Note: A group: PNAC group with *Mdr3* c.485T>A, c.2793_2794insA, c.1031G>A, c.3347G>A mutation. B group: PNAC group without *Mdr3* c.485T>A, c.2793_2794insA, c.1031G>A, c.3347G>A mutation.

a member of the ATP-binding cassette transporter family of the supergene glycoprotein gene family. The *Mdr3* gene is located at 7q21.1 and contains 28 exons, including 27 exons of coding sequences. The *Mdr3 P-gp* is encoded by the *Mdr3* gene and is one type of hepatic capillary membrane transporter (Davit-Spraul et al., 2010; Groen et al., 2011; Lang et al., 2006) involved in the secretion of bile lecithin. As the main phospholipid in bile, phosphatidylcholine can prevent cholesterol precipitation and bile salt damage to the bile duct epithelium after emulsification with cholesterol and bile salts. This phospholipid carrier is involved in the transport and secretion of normal bile acid, and this play an important role in the process of cholestasis. *Mdr3* gene expression may affect the content of lecithin in bile. Decreased expression of *Mdr3 P-gp* results in decreased lecithin secretion and increased vesicular cholesterol content, and this may also lead to biliary tract inflammation, gallstone deposition, bile duct injury, and further bile and liver damage (Trauner & Boyer, 2003).

Twenty-eight exons of the *Mdr3* gene were detected in the PNAC preterm infants in order to study the relationship between PNAC and *Mdr3* gene mutations. The *Mdr3* missense mutations c.1031G>A, c.3347G>A, and c.485T>A, and the frameshift mutation c.2793_2794insA may be high-risk factors for PNAC. Low expression of *Mdr3 P-gp* in hepatocytes was observed in infants with one of these four types of *Mdr3* gene mutation. There are few reports on the relationship between *Mdr3* gene mutations and PNAC in preterm infants. The results of this research shows that these four *Mdr3* gene mutations may be closely related to the development of PNAC, and the relationship between PNAC and *Mdr3* gene mutations in preterm infants may be a research hotspot in the future.

In conclusion, the *Mdr3* gene mutations c.2793_2794insA, c.1031G>A, c.3347G>A, and c.485T>A may be hereditary factors in the development of neonatal PNAC though further research is required for verification.

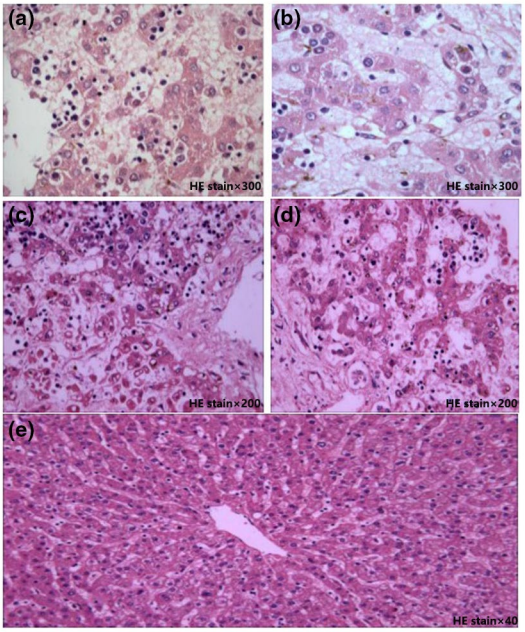

FIGURE 3 (a) The pathological changes of the liver of PNAC infants with the *Mdr3* c.2793_2794insA gene mutation (HE stain ×300). (b) The pathological changes of the liver of PNAC infants with the *Mdr3* c.485T>A gene mutation (HE stain ×300). (c) The pathological changes of the liver of PNAC infants with the *Mdr3* c.3347G>A gene mutation (HE stain ×200). (d) The pathological changes of the liver of PNAC infants with the *Mdr3* c.1031G>A gene mutation (HE stain ×200). (e) The changes of normal donor liver (HE stain ×40)

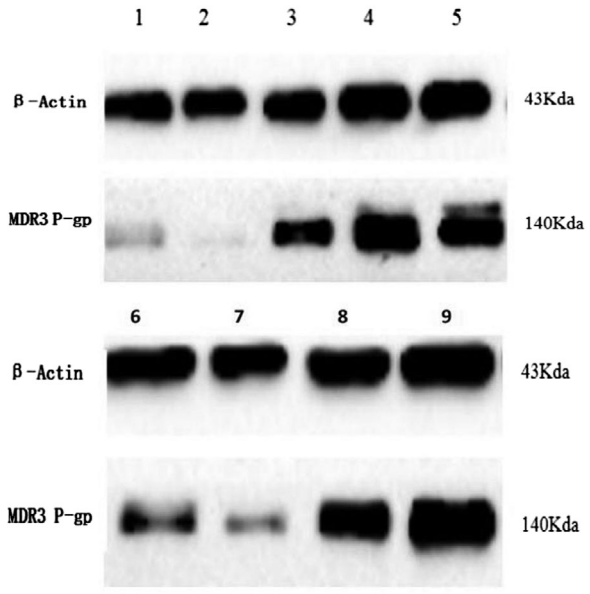

FIGURE 4 The results of Western blot analysis of the *Mdr3 pg-P* in liver tissue of PNAC infants with the *Mdr3* gene mutations and normal liver donors. (lane 1: The liver tissue of an infant with c.485T>a; lane 2: The liver tissue of an infant with c.2793_2794insA; lane 6: The liver tissue of an infant with c.1031G>A; lane 7: The liver tissue of an infant with c.3347G>A; lanes 3, 4, 5, 8, and 9: The liver tissue of normal liver donors)

ETHICS STATEMENT

This study was conducted with approval from the Ethics Committee of Zhongshan Hospital Affiliated to Sun Yat-Sen University (B20110891). This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

AUTHOR CONTRIBUTIONS

XFY designed the study, took part in coordination and helped to draft the paper. GSL is responsible for the clinical diagnosis and management of the cases. BY carried out molecular genetics research, took part in sequence alignment and drafted paper. All the authors read and approved the final paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Xiufang Yang https://orcid.org/0000-0002-6721-9811

REFERENCES

Barnett, R., & Larson, G. (2012). A phenol-chloroform protocol for extracting DNA from ancient samples. *Methods in Molecular Biology*, *840*, 13–19.

BioEdit protein contrast software. 2011. http://www.piaodown.com

Blau, J., Sridhar, S., Mathieson, S., & Chawla, A. (2007). Effects of protein/nonprotein caloric intake on parenteral nutrition associated cholestasis in premature infants weighing 600-1000 grams. *Journal of Parenteral and Enteral Nutrition*, *31*, 487–490.

Carter, B. A., & Shulman, R. J. (2007). Mechanisms of disease: Update on the molecular etiology and fundamentals of parenteral nutrition associated cholestasis. *Nature Clinical Practice Gastroenterology & Hepatology*, *4*, 277–287.

Cholestatic Liver Disease Diagnosis and Treatment Expert Committee. (2009). Experts consensus on diagnosis and therapy of cholestasis. *Chinese Journal of Experimental and Clinical Infectious Diseases (Electronic Edition)*, *3*, 474–487 (In Chinese).

Davit-Spraul, A., Gonzales, E., Baussan, C., & Jacquemin, E. (2010). The spectrum of liver diseases related to ABCB4 gene mutations: Pathophysiology and clinical aspects. *Seminars in Liver Disease*, *30*, 134–146.

Denk, G., Bikker, H., Dit, L., Deprez, R. H., Terpstra, V., Loos, C. D., Beuers, U., Rust, C., & Pusl, T. (2010). ABCB4 deficiency: A family saga of early onset cholelithiasis, sclerosing cholangitis and cirrhosis and a novel mutation in the ABCB4 gene. *Hepatology Research*, *40*, 937–941.

Duro, D., Mitchell, P. D., Kalish, L. A., Martin, C., McCarthy, M., Jaksic, T., Dunn, J., Brandt, M. L., Nobuhara, K. K., Sylvester, K. G., Moss, R. L., & Duggan, C. (2011). Risk factors for parenteral nutrition-associated liver disease following surgical therapy for necrotizing enterocolitis. *Journal of Pediatric Gastroenterology and Nutrition*, *52*, 595–600.

GenBank. http://www.ncbi.nlm.nih.gov/web/Genbank. Accessed June 01, 2011.

Gotthardt, D., Runz, H., Keitel, V., Fischer, C., Flechtenmacher, C., Wirtenberger, M., Weiss, K. H., Imparato, S., Braun, A., Hemminki, K., Stremmel, W., Rüschendorf, F., Stiehl, A., Kubitz, R., Bl, B., Schirmacher, P., Knisely, A. S., Zschocke, J., & Sauer, P. (2008). A mutation in the canalicular phospholipid transporter gene, ABCB4, is associated with cholestasis, ductopenia, and cirrhosis in adults. *Hepatology*, *48*, 1157–1166.

Groen, A., Romero, M. R., Kunne, C., Hoosdally, S. J., Dixon, P. H., Wooding, C., Williamson, C., Seppen, J., Oever, K. V., Mok, K. S., Paulusma, C. C., Linton, K. J., & Oude Elferink, R. P. J. (2011). Complementary functions of the flippase ATP8B1 and the floppase ABCB4 in maintaining canalicular membrane integrity. *Gastroenterology*, *141*, 1927–1937.

Harikar, W., Kansal, S., Oude Elferink, R. P., & Angus, P. (2009). Intrahepatic cholestasis of pregnancy: When should you look further? *World Journal of Gastroenterology*, *15*, 1126–1129.

Kaufman, S. S., Gondolesi, G. E., & Fishbein, T. M. (2003). Parenteral nutrition associated live disease. *Semin Neonato*, *l8*, 375–381.

Klein, C. J., Revenis, M., Kusenda, C., & Scavo, L. (2010). Parenteral nutrition associated conjugated hyperbilirubinemia in hospitalized infants. *Journal of the American Dietetic Association*, *110*, 1684–1695.

Lang, T., Haberl, M., Jung, D., Drescher, A., Schlagenhaufer, R., Keil, A., Mornhinweg, E., Stieger, B., Kullak-Ublick, G. A., & Kerb, R. (2006). Genetic variability, haplotype structures and ethnic diversity of hepatic transporters MDR3 (ABCB4) and bile salt export pump (ABCB11). *Drug Metabolism and Disposition*, *34*, 1582–1599.

Oude Elferink, R. P. J., & Paulusma, C. C. (2007). Function and pathophysiological importance of ABCB4 (MDR3 p-glycoprotein). *Pflügers Archiv*, *453*, 601–610.

Schneider, G., Paus, T. C., Kullak, U. G. A., Meier, P. J., Wienker, T. F., Lang, T., Vondel, P. D., Sauerbruch, T., & Reichel, C. (2007). Linkage between a new splicing site mutation in the MDR3 alias ABCB4 gene and intrahepatic cholestasis of pregnancy. *Heptology*, *45*, 150–158.

Suchy, F. J. (2001). Approach to the infant with cholestasis. In J. Suchy, R. J. Sokol, & W. F. Balistreri (Eds.), *Liver disease in children* (2nd ed., pp. 187–194). Lippincott, Williams & Wilkins.

Trauner, M., & Boyer, J. L. (2003). Bile salt transporters: Molecular characterization, function, and regulation. *Physiological Reviews*, *83*, 633–671.

Wasmuth, H. E., Glantz, A., Keppeler, H., Simon, E., Bartz, C., Rath, W., Mattsson, L.-A., Marschall, H.-U., & Lammert, F. (2007). Intrahepatic cholestasis of pregnancy: The severe form is associated with common variants of the hepatobiliary phospholipid transporter ABCB4 gene. *Gut*, *56*, 265–270.

Ziol, M., Barbu, V., Rosmorduc, O., Frassati-Biaggi, A., Barget, N., Hermelin, B., Scheffer, G. L., Bennouna, S., Trinchet, J. C., Beaugrand, M., & Ganne-Carrié, N. (2008). ABCB4 heterozygous gene mutations associated with fibrosing cholestatic liver disease in adults. *Gastroenterology*, *135*, 131–141.

How to cite this article: Yang, X., Liu, G., & Yi, B. (2022). *Mdr3* gene mutation in preterm infants with parenteral nutrition-associated cholestasis. *Molecular Genetics & Genomic Medicine*, 10, e1875. https://doi.org/10.1002/mgg3.1875