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.

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

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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
including familial intrahepatic cholestasis, pregnancy cholestasis, and cholelithiasis (Denk et al., 2010; Harikar et al., 2009; Schneider et al., 2007; Wasmuth et al., 2007; Zioli 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 Al) 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...
| 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′-GCAGGTTGCAGGTAGATGAG-3′ | 65 | 404 |
|        | Reverse primer | 5′-AACCGGATGCAAGACCCCTTC-3′ | | |
| Exon 3 | Forward primer | 5′-CTTTTCTGTGTATGAGCTCTG-3′ | 65 | 453 |
|        | Reverse primer | 5′-TCCAGGCTGTCTCAAACCTC-3′ | | |
| Exon 4 | Forward primer | 5′-GATTAGTTTCCTCAGAATAAAAAA-3′ | 62 | 416 |
|        | Reverse primer | 5′-TCTTGAGTCAAACCAGATATCCA-3′ | | |
| Exon 5 | Forward primer | 5′-CCTAAACCATGACGAAACCAA-3′ | 62 | 432 |
|        | Reverse primer | 5′-AAATGGGATTTGAGGGAAC-3′ | | |
| Exon 6 | Forward primer | 5′-TTGAGTGAGCGTAGACATGTA-3′ | 62 | 720 |
|        | Reverse primer | 5′-TACAGATGCCATGCTGG-3′ | | |
| Exon 7 | Forward primer | 5′-GGCTTGCGAGTGAAGA-3′ | 62 | 488 |
|        | Reverse primer | 5′-CCAGGCTGTACATTGGA-3′ | | |
| Exon 8 | Forward primer | 5′-TGCACTGTGATTTTGAA-3′ | 58 | 415 |
|        | Reverse primer | 5′-GCCATCATGAAAGGGTGCTT-3′ | | |
| Exon 9 | Forward primer | 5′-GGCTGCTATCCTGGATTA-3′ | 62 | 309 |
|        | Reverse primer | 5′-TCTTAATTTTACTAAGGTGGTCAC-3′ | | |
| Exon 10 | Forward primer | 5′-ACCCCAAGGAGGAAGGCACATA-3′ | 62 | 322 |
|        | Reverse primer | 5′-CCAGTCCTATTTTTTGGATTA-3′ | | |
| Exon 11 | Forward primer | 5′-TCCTCGTTTGTGGAGGTA-3′ | 5′-TTGCAGGTTTCCTGCTTGGCAG-3′ | 62 | 432 |
|        | Reverse primer | 5′-AAAGGAAAGGATAAACCCTAAACCTAAT-3′ | | |
| Exon 12 | Forward primer | 5′-TGAGCTTGGTCTGAGAGGG-3′ | 62 | 403 |
|        | Reverse primer | 5′-TGAAACGTCACAGGAGGGTG-3′ | | |
| Exon 13 | Forward primer | 5′-TTTTCGCTTTCTGAGCTG-3′ | 62 | 450 |
|        | Reverse primer | 5′-AACCTGATCGTTAGGAGGACT-3′ | | |
| Exon 14 | Forward primer | 5′-TTCACTACAGATGTCTGGATTTT-3′ | 62 | 500 |
|        | Reverse primer | 5′-GAAATCAATACAGCTCCCCATGAG-3′ | | |
| Exon 15 | Forward primer | 5′-TGAGTGCCATGGGCTGAT-3′ | 62 | 450 |
|        | Reverse primer | 5′-TCCCCCTATTTTCTCAGCTT-3′ | | |
| Exon 16 | Forward primer | 5′-AAATTACATGTTTTGTAGTTG-3′ | 62 | 322 |
|        | Reverse primer | 5′-TGGCTCTATAGACGACTCTG-3′ | | |
| Exon 17 | Forward primer | 5′-GCTTGTATTCTCTGACCTT-3′ | 62 | 423 |
|        | Reverse primer | 5′-TTAAAGGATCTGGTCTGTTAT-3′ | | |
| Exon 18 | Forward primer | 5′-CCACAAATACCAAAACCCCTAC-3′ | 62 | 403 |
|        | Reverse primer | 5′-TACCCCTCAGACAGCCTTA-3′ | | |
| Exon 19 | Forward primer | 5′-CTGCCATGTTGGTCTCGC-3′ | 62 | 500 |
|        | Reverse primer | 5′-GCTCTCTAAGGACCAGGACA-3′ | | |
| Exon 20 | Forward primer | 5′-AGAGATGCCTCCTGCTAC-3′ | 62 | 450 |
|        | Reverse primer | 5′-GCAGTGTTGCTAATCACCCT-3′ | | |
| Exon 21 | Forward primer | 5′-GGAATGGAGAGTGTAAGAGGCAA-3′ | 65 | 352 |
|        | Reverse primer | 5′-CAAATATATTGTGTAGGGGCACAAA-3′ | | |
| Exon 22 | Forward primer | 5′-TCAGCCTTTCGAGCATAC-3′ | 62 | 329 |
|        | Reverse primer | 5′-TTTCAGTGACAGAAATTGTTGAAAA-3′ | | |

(Continues)
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).

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 \( \gamma \)-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
lymphocytes and monocytes, degeneration of hepatocytes, dilation of hepatic sinuses, and brown pigmentation. Hepatocyte degeneration, bile accumulation, eosinophil formation, and brownish pigmentation were found in the PNAC infants with the \textit{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 \textit{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 \textit{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 \textit{Mdr3} P-\textit{gp} protein grayscale of the liver in the experimental group (PNAC infants with the \textit{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 \textit{Mdr3} P-\textit{gp}/\textit{β}-\textit{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 \textit{Mdr3} gene mutations c.2793_2794insA, c.1031G>A, c.3347G>A, or c.485T>A, the expression of \textit{Mdr3} P-\textit{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 \textit{Mdr3} gene is closely related to intrahepatic cholestasis. The \textit{Mdr3} gene, also known as the B4 gene of the ATP-binding cassette transporter family, is
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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.

| SNPs      | Genotype and alleles | PNAC group | Non-PNAC group | \( \chi^2 \) | \( p \) |
|-----------|----------------------|------------|----------------|-------------|--------|
| 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 (26.5%)     |             |        |
|           | T                    | 85 (55.92%)| 117 (73.13%)   | 9.984       | <0.05  |
|           | A                    | 67 (44.08%)| 43 (26.87%)    |             |        |

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

| Genotype and alleles | PNAC group | Non-PNAC group | \( \chi^2 \) | \( p \) |
|----------------------|------------|----------------|-------------|--------|
| 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%)    |             |        |
| 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%)       |             |        |
| 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%)       |             |        |

### 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) | \( \gamma \)-GT (U/L) |
|-------|-------|-----------|----------------|---------------|--------------|---------------------|
| A     | 16    | 189 ± 25  | 205 ± 33       | 183 ± 29      | 156 ± 27     | 151 ± 32            |
| B     | 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.
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.

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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)
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**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](https://doi.org/10.1002/mgg3.1875)