นิพนธ์ต้นฉบับ

การวิเคราะห์ทารกที่มีภาวะน้ำดีคั่งตับที่มีระดับแกมมา-กลูตามิลทรานสเฟอเรสต่ำถึงปกติ

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บทคัดย่อ

ความเป็นมา: ภาวะน้ำดีคั่งในทารกแรกเกิดที่มีระดับเอนไซม์แกมมา-กลูตามิลทรานสเฟอเรส (GGT) ต่ำถึงปกติพบได้น้อยแต่มีความสำคัญ เนื่องจากสัมพันธ์กับโรคทางพันธุกรรมหลายชนิด โดยเฉพาะกลุ่ม progressive familial intrahepatic cholestasis (PFIC) ซึ่งหนึ่งในสาเหตุหลักคือการกลายพันธุ์ของยีน *ABCB11* ซึ่งทำหน้าที่สร้างโปรตีน bile salt export pump (BSEP) ที่มีบทบาทสำคัญในการขับกรดน้ำดีออกจากเซลล์ตับ

วัตถุประสงค์: เพื่อศึกษาการกลายพันธุ์ของยีน *ABCB11* ในภาวะน้ำดีคั่งในทารกแรกเกิดที่มีระดับ GGT ต่ำถึงปกติ

วิธีการศึกษา: การศึกษาเชิงพยากรณ์ (prospective study) ดำเนินการระหว่างปี พ.ศ. 2560 ถึง 2564 ที่โรงพยาบาลศรีนครินทร์ จังหวัดขอนแก่น ประเทศไทย โดยศึกษาในทารกแรกเกิดจำนวน 6 รายที่มีภาวะน้ำดีคั่งและระดับ GGT ต่ำถึงปกติ ที่ไม่ได้มีสาเหตุจากการติดเชื้อ ผ่าตัด หรือกลุ่มอาการทางพันธุกรรม โดยทำการตรวจวิเคราะห์กรดน้ำดีในปัสสาวะเพื่อคัดกรองภาวะความผิดปกติในการสังเคราะห์กรดน้ำดี และตรวจหาการกลายพันธุ์ของยีน *ABCB11* ทั้ง 28 เอ็กซอน (exons) ด้วยเทคนิค next-generation sequencing (NGS)

ผลการศึกษา: ผู้ป่วยทั้ง 6 รายมีผลตรวจทางชีวเคมีที่บ่งชี้ภาวะน้ำดีคั่งและพยาธิสภาพทางเนื้อเยื่อตับที่สอดคล้องกับการบาดเจ็บของเซลล์ตับ อย่างไรก็ตาม ไม่พบการกลายพันธุ์ที่ก่อโรคในยีน *ABCB11* โดยตรวจพบเพียงการเปลี่ยนแปลงของรหัสพันธุกรรมที่จัดอยู่ในกลุ่ม benign variants และ variants of uncertain significance (VUS) เท่านั้น

สรุป: การไม่พบการกลายพันธุ์ที่ก่อโรคในยีน *ABCB11* ในผู้ป่วยกลุ่มนี้ ชี้ให้เห็นถึงความจำเป็นในการเพิ่มศักยภาพของการตรวจทางพันธุกรรม และการวิเคราะห์การทำงานของโปรตีน เพื่อช่วยในการวินิจฉัยโรคภาวะน้ำดีคั่งในทารกแรกเกิดที่มีระดับ GGT ต่ำอย่างแม่นยำยิ่งขึ้น

คำสำคัญ: ภาวะน้ำดีคั่งในทารกแรกเกิด, ระดับ GGT ต่ำถึงปกติ

Analysis of cholestasis infants with low to normal gamma-glutamyl transferase level

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Abstract

Background: Neonatal cholestatic jaundice with low to normal gamma-glutamyl transferase (GGT) is infrequent but clinically significant and associated with a wide spectrum of genetic diseases, including progressive familial intrahepatic cholestasis (PFIC). Among these, mutations in the *ABCB11* gene are a prominent etiology, as encodes the bile salt export pump (BSEP) are a prominent etiology.

Objective: To investigate the *ABCB11* mutations in neonatal cholestatic jaundice with low to normal GGT levels.

Methods: A prospective study was conducted from 2017 to 2021 at Srinagarind Hospital, Khon Kaen, Thailand. Six neonates with cholestasis and low to normal GGT were enrolled. After exclusion of infectious, surgical, and syndromic causes. Urine bile acid analysis was performed to exclude bile acid synthesis defects. DNA was extracted and sequenced in all 28 exons of the *ABCB1*1 gene using next-generation sequencing.

Results: All 6 patients revealed biochemical evidence of cholestasis and liver histological features of hepatocellular injury. No pathogenic mutations were identified in the *ABCB11* gene; only benign variants and variants of uncertain significance (VUS) were detected.

Conclusion: The undetectable of pathogenic *ABCB11* mutations highlights the need for expanded genomic analysis and functional testing.

Keywords: Neonatal cholestatic jaundice, Low to normal gamma-glutamyl transferase (GGT) level

Introduction

Neonatal cholestatic jaundice with low to normal gamma-glutamyl transferase (GGT) levels is a rare but clinically significant condition. It represents a broad spectrum of diseases, varying in severity from mild, recurrent cholestatic jaundice to severe chronic liver disease, and may increase the risk of hepatocellular carcinoma.[1] A low GGT level is a key biochemical hallmark that helps differentiate this disease spectrum from more common neonatal cholestatic disorders such as biliary atresia or neonatal hepatitis, where GGT is typically elevated. The associated disorders can be either familial or sporadic. The major disease group is progressive familial intrahepatic cholestasis (PFIC), a group of autosomal recessive disorders. Subtypes include PFIC 1 (severe *ATP8B1* disease), benign recurrent intrahepatic cholestasis type 1 (BRIC 1, mild *ATP8B1* disease), PFIC 2 (severe *ABCB11* disease), BRIC 2 (mild *ABCB11* disease), PFIC 4 (*TJP2* deficiency), PFIC 5 (*NR1H4* mutation), and PFIC 6 (*MYO5B*-associated cholestasis).[2,3]

Severe mutations in the *ATP8B1* and *ABCB11* genes result in significant disruption of the proteins responsible for intrinsic bile acid transport, leading to the development of severe liver disease. In contrast, the benign recurrent intrahepatic cholestasis (BRIC) phenotype, which represents a milder form of the disorder, retains partial residual protein activity in bile acid transport. Consequently, episodes of cholestatic jaundice in BRIC may resolve spontaneously without causing permanent hepatocellular injury.[4]

Other relevant disorders include Arthrogryposis-Renal Dysfunction-Cholestasis (ARC) syndrome and bile acid synthesis defects. Given the wide disease spectrum, definitive diagnosis requires specific investigations, especially genetic testing, beyond conventional liver function tests, histology, or immunohistochemistry. Although our laboratory cannot analyze serum bile acids, we collaborated with the Junshin Clinic Bile Acid Institute in Tokyo, Japan, to evaluate urine bile acids for our patients. Due to limited resources, our study focused on *ABCB11* mutations, which are commonly present during infancy, rather than *ATP8B1* mutations, which often present in the neonatal period.[4]

Objective

To investigate the *ABCB11* mutations in neonatal cholestatic jaundice with low to normal GGT levels.

Patients

A prospective study was conducted from 2017 to 2021 on neonatal cholestatic jaundice with low to normal GGT levels at Srinagarind Hospital, Khon Kaen, Thailand. The reference ranges for pediatric serum GGT levels were 12–122 U/L in male and 15–133 U/L in female neonates aged 1–182 days, while in infants aged 183–365 days, the reference range was 1–39 U/L for both genders.[5]

A total of six patients were enrolled after informed consent was obtained. Other causes of cholestatic jaundice, such as Alagille syndrome, infectious diseases, and ARC syndrome, were excluded. Demographic data including age of onset, gender, personal and family history, clinical presentation, and physical examination were recorded. Laboratory parameters included complete blood count, total and direct bilirubin (TB/DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), GGT, albumin, prothrombin time, and international normalized ratio (INR). Urine bile acid analysis was performed by the Junshin Clinic Bile Acid Institute, Tokyo, Japan. Hepatobiliary scanning and abdominal ultrasonography were used to exclude surgical conditions such as biliary atresia and choledochal cysts. Liver histology was interpreted by the Division of Pathology, Khon Kaen University, Thailand.

The study was approved by the Institutional Review Board, Center for Ethics in Human Research, Khon Kaen University, Human Research Ethics Committee #HE 611592).

Methods

DNA Extraction

Genomic DNA was extracted from six peripheral blood samples using the isopropanol-fractionation method with concentrated NaI and SDS, as described by Wang L.H., 1994.[6]

Polymerase Chain Reaction (PCR) Amplification

PCR was performed using primers for the 28 exons, adopted from the study by Strautnieks S.S. et al., 2008[7] PCR amplification used 100 ng of genomic DNA in a 30 µL reaction containing 10 mM Tris-HCl (pH 8.5), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 µM forward primer, 5 µM reverse primer, and Taq DNA polymerase (GoTaq® Colorless Master Mix, Promega® USA). PCR conditions were as follows: initial denaturation at 94°C for 5 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes (Nexus GSX1 Mastercycler®, Eppendorf®).

DNA Sequencing

PCR products were submitted to U2Bio Co., Ltd. for nucleotide sequencing using Next-Generation Sequencing (NGS) based on Illumina® technology.

Exon 1–28 Sequencing

Raw sequencing data from all six samples were analyzed using Galaxy, Franklin, and IGV software to identify variants across the 28 exons. Despite regular updates of the GeneBank database, we also cross-validated the gene variants using established genetic databases such as ClinVar (in calendar year 2024).

Statistical Methods

Continuous and categorical variables are described using ranges and frequencies (%). Data analysis was performed using the Stata software package, version 10.1 (StataCorp LP) program (Texas, USA).

Results

Six neonates with cholestatic jaundice and low to normal GGT levels were enrolled. Age at onset ranged from 1.5 to 2.5 months. Four patients were male (66.6%) and two were female (33.3%). All presented with cholestasis and hypo- to acholic stools. Hepatomegaly was observed in four patients; the remaining two had hepatosplenomegaly. Laboratory findings were consistent with cholestasis, showing elevated total and direct bilirubin (TB/DB), high levels of transaminases (AST, ALT), and normal urine bile acids (Table 1). Hepatobiliary obstruction was excluded by abdominal ultrasonography (and, in some cases, hepatobiliary scanning). All liver histology revealed ballooning and giant cell transformation of hepatocytes, without evidence of biliary obstruction or ductal hypoplasia.

Table 1 Patients characteristic and laboratory at disease's onset

Patients	Age of onset (months)	Clinical presentation	TB/DB (mg/ dL)	AST (U/L)	ALT (U/L)	GGT(U/L)	Urine bile acid
1 (male)	2.5	cholestatic jaundice	14.5/ 11.6	562	345	117	normal
2 (male)	2.4	cholestatic jaundice	6.5/ 5.5	239	139	113	normal
3 (female)	1.5	cholestatic jaundice	13/ 10.1	398	302	119	normal
4 (female)	2.5	cholestatic jaundice	13.6/9.3	556	259	50	normal
5 (male)	2	cholestatic jaundice	9.7/ 8.8	108	56	109	normal
6 (male)	1.9	cholestatic jaundice	9.5/ 6.9	105	129	95	normal

Exons sequencing

Analysis of the base sequences from all six samples across the 28 exons revealed no pathogenic variants in the *ABCB11* gene. All six patients showed either benign variant or variants of uncertain significance (VUS)**.** The table 2 presents data exclusively for cases in which genetic variants were detected. (Table 2).

Among the six infants included in this study, all patients received ursodeoxycholic acid (UDCA). Two patients from the Lao People's Democratic Republic (one male and one female) were lost to follow-up. Two infants (one male and one female) demonstrated spontaneous resolution of cholestatic jaundice, with no clinical relapse observed at 12 and 18 months of age, respectively. The remaining two male infants survived with their native livers, experienced occasional relapses, and continued to be followed in our clinic.

Table 2 The results of variant analysis for the *ABCB11* gene in six samples.

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
1/1	485	chr2-169887613 T>A	ABCB11:c.-28+122A>T	heterozygote	benign
		chr2-169887719 A>G	ABCB11:c.-28+16T>C	heterozygote	VUS
		chr2-169887968 A>G	ABCB11:c.-261T>C	heterozygote	benign
1/2	264	chr2-169874712 C>T	ABCB11:c.-27-50G>A	heterozygote	benign
1/4	201	chr2-169870855A>G	ABCB11:c.108 T>C	heterozygote	benign
		chr2-169870882 A>G	ABCB11:c.99-18T>C	heterozygote	benign
1/5	401	chr2-169869901 A>G	ABCB11:c.270 T>C	heterozygote	benign
1/9	388	chr2-169847412 A>G	ABCB11:c.807 T>C	heterozygote	benign
		ABCB11:c.784-87T>A	ABCB11:c.784 -87T>A	heterozygote	benign
1/10	453	chr2-169842809 T>C	ABCB11:c.909 -15A>G	homozygote	benign
1/13	280	chr2-169830328 A>G	ABCB11:c.133 1T>C	homozygote	benign

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
1/14	493	chr2-169828277 G>A	ABCB11:c.1638+80C>T	homozygote	benign
		chr2-169828325 A>G	ABCB11:c.1638+32T>C	homozygote	benign
1/15	466	chr2-169826877 C>T	ABCB11:c.1639-152G>A	homozygote	benign
1/19	363	chr2-169814655 G>T	ABCB11:c.2179-17C>A	homozygote	benign
1/20	359	chr2-169801488 A>G	ABCB11:c.2344-17T>C	homozygote	benign
1/24	434	chr2-169788825 C>A	ABCB11:c.3213+62G>T	heterozygote	VUS
		chr2-169788858 T>C	ABCB11:c.3213+29A>G	heterozygote	VUS
		chr2-169789016 T>C	ABCB11:c.3084A>G	homozygote	benign
1/28	580	chr2-169779896 T>C	ABCB11:c.*236A>G	homozygote	benign
		chr2-169780366 T>C	ABCB11:c.3766-34A>G	homozygote	benign
1/28	580	chr2-169779896 T>C	ABCB11:c.*236A>G	homozygote	benign
		chr2-169780366 T>C	ABCB11:c.3766-34A>G	homozygote	benign

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
2/1	485	chr2-169887719 A>G	ABCB11:c.-28+16T>C	heterozygote	VUS
		chr2-169887968 A>G	ABCB11:c.-261T>C	heterozygote	benign
2/3	168	chr2-169873316 GA>G	ABCB11:c.77-8del	heterozygote	likely benign
2/4	201	chr2-169870855 A>G	ABCB11:c.108 T>C	heterozygote	benign
2/9	388	chr2-169847412 A>G	ABCB11:c.807 T>C	homozygote	benign
		chr2-169847522 A>T	ABCB11:c.784 -87T>A	homozygote	benign
2/10	453	chr2-169842809 T>C	ABCB11:c.909 -15A>G	homozygote	benign
2/13	280	chr2-169830182 T>C	ABCB11:c.143 4+43A>G	heterozygote	VUS
		chr2-169830328 A>G	ABCB11:c.133 1T>C	homozygote	benign
2/14	493	chr2-169828277 G>A	ABCB11:c.163 8+80C>T	homozygote	benign
		chr2-169828325 A>G	ABCB11:c.163 8+32T>C	homozygote	benign
2/15	466	chr2-169826877 C>T	ABCB11:c.163 9-152G>A	homozygote	benign

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
2/18	298	chr2-169820618 A>G	ABCB11:c.2178+98T>C	heterozygote	benign
		chr2-169820619 T>C	ABCB11:c.2178+97A>G	heterozygote	benign
2/19	363	chr2-169814655 G>T	ABCB11:c.2179-17C>A	homozygote	benign
2/20	359	chr2-169801488 A>G	ABCB11:c.2344-17T>C	homozygote	benign
2/24	434	chr2-169788825 C>A	ABCB11:c.3213+62G>T	heterozygote	VUS
		chr2-169789016 T>C	ABCB11:c.3084A>G	homozygote	benign
2/28	580	chr2-169779896 T>C	ABCB11:c.*236A>G	homozygote	benign
		chr2-169780366 T>C	ABCB11:c.3766-34A>G	homozygote	benign
3/10	453	chr2-169842809 T>C	ABCB11:c.909-15A>G	heterozygote	benign
3/13	280	chr2-169830328 A>G	ABCB11:c.1331T>C	heterozygote	benign
3/14	493	chr2-169828277 G>A	ABCB11:c.1638+80C>T	heterozygote	benign
		chr2-169828325 A>G	ABCB11:c.1638+32T>C	heterozygote	benign

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
3/15	466	chr2-169826877 C>T	ABCB11:c.1639-152G>A	heterozygote	benign
3/18	298	chr2-169820618 A>G	ABCB11:c.2178+98T>C	heterozygote	benign
		chr2-169820619 T>C	ABCB11:c.2178+97A>G	heterozygote	benign
3/19	363	chr2-169814655 G>T	ABCB11:c.2179-17C>A	heterozygote	benign
3/20	359	chr2-169801488 A>G	ABCB11:c.2344-17T>C	homozygote	benign
3/24	434	chr2-169789016 T>C	ABCB11:c.3084A>G	heterozygote	benign
3/28	580	chr2-169779896 T>C	ABCB11:c.*236A>G	heterozygote	benign
		chr2-169780366 T>C	ABCB11:c.3766-34A>G	heterozygote	benign
4/1	516	chr2-169887613 T>A	ABCB11:c.-28+122A>T	heterozygote	benign
4/4	201	chr2-169870882 A>G	ABCB11:c.99-18T>C	heterozygote	benign
4/5	401	chr2-169869901 A>G	ABCB11:c.270T>C	heterozygote	benign
4/10	453	chr2-169842809 T>C	ABCB11:c.909-15A>G	homozygote	benign

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
4/14	493	chr2-169828277 G>A chr2-169828325 A>G	ABCB11:c.1638+80C>T ABCB11:c.1638+32T>C	heterozygote homozygote	benign benign
4/15	466	chr2-169826877 C>T	ABCB11:c.1639-152G>A	heterozygote	benign
4/19	363	chr2-169814655 G>T	ABCB11:c.2179-17C>A	heterozygote	benign
4/20	359	chr2-169801488 A>G	ABCB11:c.2344-17T>C	heterozygote	benign
4/28	580	chr2-169779896 T>C	ABCB11:c.*236A>G	homozygote	benign
		chr2-169780366 T>C	ABCB11:c.3766-34A>G	homozygote	benign
5/1	516	chr2-169887613 T>A	ABCB11:c.-28+122A>T	heterozygote	benign
5/2	264	chr2-169874712 C>T	ABCB11:c.-27-50G>A	heterozygote	benign
5/4	201	chr2-169870882 A>G	ABCB11:c.99-18T>C	heterozygote	benign
5/5	401	chr2-169869901 A>G	ABCB11:c.270 T>C	heterozygote	benign
5/10	453	chr2-169842809 T>C	ABCB11:c.909-15A>G	homozygote	benign

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
5/13	280	chr2-169830328 A>G	ABCB11:c.1331T>C	homozygote	benign
5/14	493	chr2-169828277 G>A	ABCB11:c.1638+80C>T	homozygote	benign
		chr2-169828325 A>G	ABCB11:c.1638+32T>C	homozygote	benign
5/15	466	chr2-169826877 C>T	ABCB11:c.1639-152G>A	homozygote	benign
5/18	298	chr2-169820618 A>G	ABCB11:c.2178+98T>C	heterozygote	benign
		chr2-169820619 T>C	ABCB11:c.2178+97A>G	heterozygote	benign
5/19	363	chr2-169814655 G>T	ABCB11:c.2179-17C>A	heterozygote	benign
5/20	359	chr2-169801488 A>G	ABCB11:c.2344-17T>C	homozygote	benign
5/28	580	chr2-169779896 T>C	ABCB11:c.*236A>G	homozygote	benign
		chr2-169780366 T>C	ABCB11:c.3766-34A>G	heterozygote	benign
6/13	280	chr2-169830328 A>G	ABCB11:c.1331T>C	homozygote	benign

Sample no./ Exon	Reference (bp)	Variant chromosome	Variant gene	Zygosity	Classification
6/14	493	chr2-169828277 G>A	ABCB11:c.1638+80C>T	homozygote	benign
		chr2-169828325 A>G	ABCB11:c.1638+32T>C	homozygote	benign
6/15	466	chr2-169826877 C>T	ABCB11:c.1639-152G>A	homozygote	benign
6/20	359	chr2- 169801488 A>G	ABCB11:c.2344-17T>C	homozygote	benign
6/24	434	chr2-169788784 T>C	ABCB11:c.3213+103A>G	heterozygote	VUS
		chr2-169788825 C>A chr2-169788852 C>T	ABCB11:c.3213+62G>T ABCB11:c.3213+35G>A	heterozygote heterozygote	VUS VUS

The alphabets A, T, C, and G are the four nucleotide bases (adenine, thymine, cytosine, guanine) that make up the DNA code, The notation A>G signifies a genetic variant where adenine (A) is the reference allele, and guanine (G) is the variant allele at a specific position in a gene.

Discussion

The genetic variants associated with neonatal cholestatic jaundice with low to normal GGT are highly diverse. They include a broad spectrum of diseases, primarily involving hepatobiliary transport defects or the PFIC group, with severity ranging from benign (BRIC1 and BRIC2) to severe liver diseases (severe *ATP8B1* disease, severe *ABCB11* disease, *TJP2* deficiency, *NR1H4* mutation, and *MYO5B*-associated cholestasis), as well as other conditions such as ARC syndrome and bile acid synthesis defects. Our study objected to investigate the etiology of neonatal cholestatic jaundice with low to normal GGT. After ruling out other causes of cholestasis with low to normal GGT through urine bile acid analysis for bile acid synthesis defects,

the decision to analyze *ABCB11* mutations was based on their known higher prevalence than other causes of neonatal cholestatic jaundice with low to normal GGT in Asia and globally.[1-4, 6-10] Even this study performed all 28 exons of *ABCB11* gene but revealed only benign or VUS condition, on the other hand, the study from Egypt found pathogenic mutation in exons 14, 15, and 24 of the *ABCB11* gene[9] and the study from Japan also found 59 genetic variations, including novel nonsynonymous and synonymous variations.[1] In a cohort of Thai infants, novel mutations in the *ABCB11* gene were identified, including a four-nucleotide deletion in exon 3 (c.90_93delGAAA) and a single-nucleotide insertion in exon 5 (c.249_250insT).[11] These might be the genetic differences between the terrain and the number of populations.

Due to several limitations, particularly financial constraints that restricted the use of whole exome sequencing, the detection of genetic mutations other than *ABCB11* was limited. In addition, access to advanced diagnostic modalities such as mass spectrometry-based plasma bile acid profiling or serum biliary bile acid measurement was unavailable. Although urine bile acid analysis could be performed, its diagnostic utility is limited by relatively low specificity, especially in early disease stages or in cases involving subtle defects in bile acid transporters.[7] Furthermore, assessment of BSEP expression by liver immunohistochemistry could not be performed. Despite these limitations, our findings of only benign or VUS variants in all 28 exons of the *ABCB11* gene highlight the need for future biobanking and re-analysis of undiagnosed cases as gene discovery evolves. Long-term follow-up is essential, as progressive liver disease may occur even in genetically ambiguous cases, and a stepwise diagnostic algorithm for neonatal cholestasis with low GGT should be implemented.

Conclusions

In this prospective study, no pathogenic variants were identified across all 28 exons of the *ABCB11* gene. Although only benign variants and VUS were detected, our findings highlight the diagnostic challenges in resource-limited settings, where advanced molecular and functional assays are partially covered due to the budget. Comprehensive genetic testing, functional studies, and long-term follow-up remain essential for accurate diagnosis and management of patients with suspected bile salt transport disorders. Future directions incorporating biobanking and expanded genomic technologies are warranted in unresolved cases and develop a stepwise diagnostic approach.

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