Genetic Insights Into PFIC-Associated Genes in Unexplained Chronic Cholestasis and Liver Disease: Frequency and Implications of Variant Combinations

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Brett J. Hoskins,[1] Tiziano Pramparo,[2] Ethan Gough,[3] Wikrom Karnsakul[4]

[1]Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, Indiana; [2]Mirum Pharmaceuticals, Inc., Foster City, California; [3]Department of Pediatrics, Biostatistics, Epidemiology, and Data Management (BEAD) Core, The Johns Hopkins University School of Medicine, Baltimore, Maryland; [4]Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Introduction

- Cholestasis results from impairment of bile flow due to various hepatobiliary disorders, including progressive familial intrahepatic cholestasis (PFIC), Alagille syndrome, cerebrotendinous xanthomatosis, other bile acid synthesis disorders, alpha-1-antitrypsin deficiency, cystic fibrosis, and polycystic kidney disease.[1-4]
- Genetic causes account for ~25% of cases of neonatal cholestasis.[5-8]
- For patients with cholestasis, diagnosis can be challenging due to variable presentations and overlapping genetic conditions.[2,3]
- Next-generation sequencing using a cholestasis gene panel enables efficient detection of genetic causes and supports targeted management.[2,9-13]
- PFIC is a group of genetic disorders causing chronic cholestasis due to disrupted bile composition, most often due to variants in *ATP8B1*, *ABCB11*, *ABCB4*, *TJP2*, *NR1H4*, and *MYO5B*.[14,15]
- Heterozygous pathogenic variants in PFIC-associated genes may contribute to unexplained cholestasis and chronic liver disease.

Objective

- To report the frequency and potential implications of compound heterozygous states involving pathogenic variants and variants of uncertain significance (VUS) in PFIC-associated genes.

Methods

Patient Eligibility: Current or previous history of cholestasis without an identifiable cause or unexplained chronic liver disease

Genetic Testing Period: February 2016 to February 2022 (N=10,894)

DNA Sequencing: Using custom-designed, 77-gene cholestasis panel and optimized capture libraries[a,b]

Data Analysis and Variant Classification: Aggregate data reviewed and variants classified

Analysis of PFIC-Associated Genes: Data reviewed for *ABCB11*, *ABCB4*, *ATP8B1*, *MYO5B*, *NR1H4*, and *TJP2*

Variant Classification According to Diagnostic Laboratory Guidelines and ACMG/AMP Criteria

Benign | Likely benign | Pathogenic | Likely pathogenic | Variant of uncertain significance

Definitive diagnoses
- Pathogenic or likely pathogenic variants consistent with the mode of inheritance of the disorder

Potential diagnoses
- Recessive disorders: genes with 1 pathogenic or likely pathogenic allele and 1 VUS
- Autosomal dominant disorders: 1 VUS

[a]DNA sequencing was performed using custom-designed and optimized capture libraries (SureSelect by Eurofins/Emory Genetics Laboratory [2016-2021] and PGxome® by PreventionGenetics [2021-2024]). [b]Cholestasis panel was initially composed of 57 genes, with 9 and 11 genes added in 2017 and 2022, respectively.

Results

Most Patients (51.1%) Were <1 Year of Age at the Time of Genetic Testing

Table 1. Key Demographics and Baseline Characteristics

Parameter[a]	Total (N=10,894)
Age, range, y	0-88
Age category	
<1 y	5570 (51.1)
1-10 y	2850 (26.2)
11-17 y	1477 (13.6)
>18 y	997 (9.2)
Sex[b]	
Female	1281/3275 (39.1)
Male	1994/3275 (60.9)
Ethnicity	
Caucasian/Northwestern European	3681 (33.8)
Hispanic	2010 (18.5)
African American	1621 (14.9)
Asian	658 (6.0)
Native American	104 (1.0)
Other	122 (1.1)
Unknown	2080 (19.1)
Mixed/multiple	618 (5.7)

[a]Values are n (%) except where otherwise indicated. [b]Sex data were only available from PreventionGenetics.

88% of Genes in the Panel Had ≥1 Pathogenic or Likely Pathogenic Variant

Figure 1. Frequency of Genes With Pathogenic and Likely Pathogenic Variants

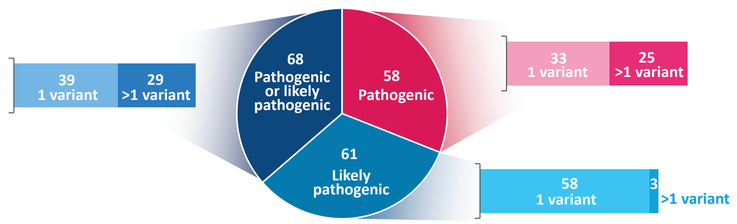


Figure 2. Patients With ≥1 Variant

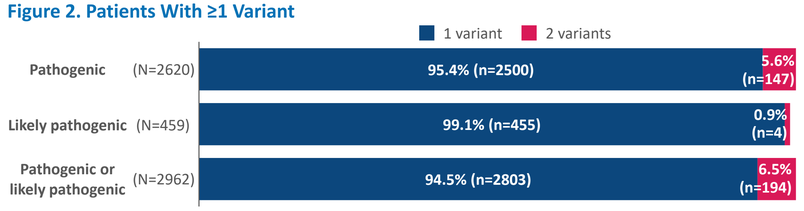
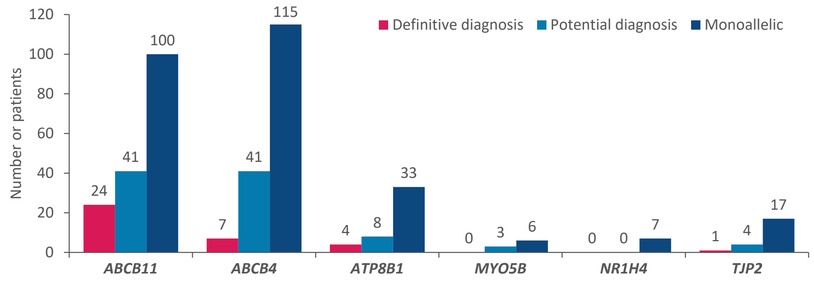


Figure 3. Diagnostic Classification of Patients With >1 Pathogenic and/or Likely Pathogenic Variant



- Monoallelic pathogenic variants were identified in PFIC genes: *ABCB11* (n=100), *ABCB4* (n=115), *ATP8B1* (n=33), *MYO5B* (n=6), *NR1H4* (n=7), and *TJP2* (n=17).
- Potential compound heterozygous states with VUS were also identified in PFIC genes: *ABCB11* (n=41), *ABCB4* (n=41), *ATP8B1* (n=8), *MYO5B* (n=3), and *TJP2* (n=4).
- No patients exhibited concurrent monoallelic pathogenic variants across multiple PFIC genes, although VUS in other PFIC genes were observed.

Table 2. Combinations of Variants in PFIC Genes

Gene	Homozygous pathogenic/likely pathogenic, n	2 pathogenic/ likely pathogenic alleles, n	2 alleles: 1 VUS + 1 pathogenic/ likely pathogenic, n	1 pathogenic/ likely pathogenic allele, n	Homozygous VUS, n	1 or 2 heterozygous alleles VUS, n
ABCB11[a]	17	7	41	100	3	466
ABCB4[a]	5	2	41	115	1	506
ATP8B1	3	1	8	33		331
MYO5B			3	6		143
NR1H4				7		108
TJP2	1		4	17		395

[a]Gene with >2 VUS.

- Of 33 patients with *ATP8B1* monoallelic pathogenic variants, 1 had an *ACBC4* VUS.
- Of 100 patients with *ABCB11* monoallelic pathogenic variants, concurrent VUS were identified in *ATP8B1* (n=1), *ABCB4* (n=1), *TJP2* (n=3), *NR1H4* (n=1), and *MYO5B* (n=2).
- Of 115 patients with *ABCB4* monoallelic pathogenic variants, concurrent VUS were identified in *ATP8B1* (n=4), *ABCB11* (n=2), *TJP2* (n=1), *NR1H4* (n=2), and *MYO5B* (n=2).
- Of 17 patients with *TJP2* monoallelic pathogenic variants, concurrent VUS were identified in *ATP8B1* (n=1) and *ABCB4* (n=3).
- Of 7 patients with *NR1H4* monoallelic pathogenic variants, 2 VUS were identified in *ABCB11* (n=2), and 1 VUS was identified *TJP2* (n=1).

Conclusions

- The frequent occurrence of monoallelic pathogenic variants with VUS in the same gene highlights the need for urgent reclassification of VUS, especially when clinically significant.
- These findings may aid in diagnosing PFIC-related conditions and refining the interpretation of genetic results.
- Further studies and data-sharing initiatives are warranted to pool data and collaboratively reclassify VUS based on combined genetic, clinical, and functional data to improve diagnostic accuracy and explore the potential modifying effects of variants in other PFIC-associated genes (eg, *ABCB4* VUS in patients with *ABCB11* monoallelic pathogenic variants).

Abbreviations

ABCB11, adenosine triphosphate binding cassette subfamily B member 11; *ABCB4*, adenosine triphosphate binding cassette subfamily B member 4; ACMG, American College of Medical Genetics and Genomics; AMP, Association for Molecular Pathology; *ATP8B1*, ATPase phospholipid transporting 8B1; *MYO5B*, myosin Vb; *NR1H4*, nuclear receptor subfamily 1 group H member 4; PFIC, progressive familial intrahepatic cholestasis; *TJP2*, tight junction protein 2; VUS, variant of uncertain significance.

Disclosures

BJH is a consultant for Mirum Pharmaceuticals, Inc. TP is an employee of Mirum Pharmaceuticals, Inc., and previous employee of Retrophin/Travere Therapeutics. EG has nothing to disclose. WK is a consultant for Mirum Pharmaceuticals, Inc., Albireo Pharmaceuticals, Travere Therapeutics, and Gilead Sciences.

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