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Clinical Diversity and Outcomes of Progressive Familial Intrahepatic Cholestasis Diagnosed by Whole Genome Sequencing in Pakistani Children

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Abstract
Progressive familial intrahepatic cholestasis (PFIC) is a rare group of genetic disorders that typically presents in infants and children, often progressing to end-stage liver disease. We used whole genome sequencing (WGS) for diagnosis to assess phenotypic features and outcomes in Pakistani children with different types of PFIC. The study included 116 pediatric participants with five PFIC types: PFIC1, *ATP8B1* gene (n = 19); PFIC2, *ABCB11* (n = 28); PFIC3, *ABCB4* (n = 52); PFIC4 *TJP2* (n = 15); and PFIC5 *NR1H4* (n = 2). Seventy unique variants were identified across the five genes. The age at genetic diagnosis was higher in patients with PFIC3. Clinical and laboratory findings showed significant overlap among all PFIC types. PFIC3 had a less aggressive course and better survival outcomes than PFIC1, PFIC2, and PFIC4. The cumulative survival rate was significantly higher at 89% (95% CI 43–98%) for patients who underwent liver transplantation, compared to 9% (95% CI 1–29%) for those who did not (p = 0.016). The study provided the first comprehensive analysis of PFIC in Pakistani children, highlighting significant clinical overlap and the critical need for early genetic diagnosis using WGS. The findings underscore the importance of personalized treatment approaches, including early consideration for liver transplantation, to improve patient outcomes.

Keywords:
clinical outcomes; genotype-phenotype correlation; progressive familial intrahepatic cholestasis (PFIC); whole genome sequencing (WGS)

1. Introduction

Genetic factors are implicated in a significant proportion of pediatric cholestatic liver diseases, with estimates suggesting that at least 45% of cases have a genetic basis [1]. Progressive familial intrahepatic cholestasis (PFIC) is a rare group of autosomal recessive liver disorders that cause intrahepatic cholestasis in children. It typically presents in infancy with jaundice, pruritus, failure to thrive, and often progresses to end-stage liver disease. The disease is genetically heterogeneous, with multiple subtypes caused by mutations in different genes affecting bile formation and secretion. These subtypes show overlapping clinical features but may vary in severity, age of onset, and prognosis [1–3]. In the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/), bi-allelic (homozygous or compound heterozygous) variants in 12 genes have been associated with PFIC [4].

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Worldwide, PFIC has an estimated incidence of 1 per 50,000–100,000 live births [5]; however, the exact incidence in Pakistan is unknown.

Clinical presentations and laboratory findings in PFIC patients often show overlapping features among various PFIC types. While it is possible to suspect and differentiate between different PFIC types based on clinical presentation and basic diagnostic tests, confirmatory diagnosis usually relies on genetic testing. Pakistani hospitals have implemented whole genome sequencing (WGS) as part of routine diagnostics for patients with cholestatic liver disease. This study aims to assess the clinical and laboratory diversity, as well as the overlap in these diagnostic findings, among various types of PFIC in a large cohort of Pakistani children at the time of their genetic confirmation. The study also evaluates the clinical outcomes of these patients.

2. Methods

2.1. Study Participants

This observational study analyzed pediatric patients from hospitals in Islamabad, Karachi, Lahore, Multan, and Peshawar, Pakistan. Patients presenting with clinical and laboratory features of cholestatic liver disease from January 2019 to January 2023 were offered WGS as part of their diagnostic work-up, without pre-selection based on severity or etiology. For the current analysis, we included only patients with genetically confirmed PFIC, defined by bi-allelic pathogenic or likely pathogenic variants in PFIC-associated genes. We specifically included individuals possessing homozygous or compound heterozygous variants in a set of genes known to be associated with PFICs: *ATP8B1*, *ABCB11*, *ABCB4*, *TJP2*, *NR1H4*, *SLC51A*, *USP53*, *KIF12*, *ZFYVE19*, *MYO5B*, *SEMA7A*, and *VPS33B*. Individuals with a single heterozygous variant were excluded, as such findings are considered insufficient for a definitive diagnosis of PFIC. Informed consent was obtained from all participants (or their parents/legal guardians) involved in the study. DNA samples were collected in Pakistan and sent to Germany, where WGS and primary data analysis were performed. WGS reports were shared with the treating physicians in Pakistan to support clinical decision-making. Ethical approvals for the study were obtained from the Institutional Review Board of the Children's Hospital and the Institute of Child Health, Lahore (Pakistan), 2019-54-CHICH, 31 August 2019, and the Ethics Committee of Rostock University (Germany), A2022-0072, 25 April 2022. Demographic, clinical, and laboratory features at the time of PFIC genetic diagnosis were recorded and further analyzed. The overall prognosis and outcomes were also collected, and patients' survival was analyzed. Patients with inconclusive genetic results, loss to follow-up, or insufficient clinical data were excluded from the analysis.

2.2. Sequencing and Data Analysis

Whole Genome Sequencing (WGS). For our study, DNA samples were prepared using the TruSeq DNA Nano Library Prep Kit from Illumina. Sequencing was performed on an Illumina platform utilizing the 150 bp paired-end protocol, achieving an average coverage depth of 30× for the nuclear genome. The alignment of raw reads to the reference genome GRCH38 and the calling of variants, including single nucleotide substitutions (SNVs), small insertions/deletions (Indels), and structural variants (SVs), were conducted using DRAGEN (version 3.10.4, Illumina). Annotation of SNVs and indels was carried out by Varvis (Limbus Medical Technologies GmbH; https://www.limbus-medtec.com/, Accessed 11 June 2025), while structural variants were annotated using ANNOTSV3.1. All genetic variants were described according to the Human Genome Variation Society (HGVS) recommendations (www.hgvs.org).

2.3. Variant Evaluation and Interpretation

We considered only high-quality variants with a minimum of 9 reads and an alternate allele frequency of at least 0.3%. Candidate variants underwent evaluation for their pathogenicity and causality using a 5-tier classification: pathogenic (P), likely pathogenic (LP), variants of uncertain significance (VUS), likely benign (LB), and benign (B). Our analysis was restricted to genes with clear associations with the participants' phenotypes, using Human Phenotype Ontology nomenclature (HPO) (https://hpo.jax.org/app/, Accessed 11 June 2025). Factors considered included allele frequency in control databases (gnomAD), in silico pathogenicity predictions, potential protein impacts, variant type-disease mechanisms, familial segregation, and external evidence from OMIM (https://www.omim.org/, 11 June 2025), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/, Accessed 11 June 2025), and MasterMind (https://mastermind.genomenon.com/, 11 June 2025), along with genotype-phenotype correlations.

2.4. Statistics

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Data are presented as median (range) and median (25–75 percentile) for continuous variables, and as frequencies and percentages

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for categorical variables. Comparisons between groups were conducted using the Chi-square test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Post-hoc pairwise comparisons were performed using Dunn's test with Bonferroni correction for multiple comparisons. Survival rates were calculated using the Kaplan-Meier method, and differences between survival curves were assessed using the log-rank test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Participants

Of all children with clinical cholestasis tested with WGS in Pakistani hospitals during the period from January 2019 to January 2023, 116 patients with genetically confirmed PFIC were included in our study. There were patients with five different types of PFIC: PFIC1 (*ATP8B1*)—19 (16.4%), PFIC2 (*ABCB11*)—28 (24.1%), PFIC3 (*ABCB4*)—52 (44.8%), PFIC4 (*TJP2*)—15 (12.9%), PFIC5 (*NR1H4*)—2 (1.7%).

3.2. PFIC Variants

Seventy unique PFIC variants were identified in the study (Table S1). Some of these variants were found in multiple patients, with the most common variant occurring in up to 10 patients. In each PFIC type, cases were marked by homozygous variants in their respective genes (*ATP8B1*, *ABCB11*, *ABCB4*, *TJP2*, and *NR1H4*), all classified as either pathogenic or likely pathogenic (P/LP). One rare occurrence of PFIC2 involved a compound heterozygous mutation in the *ABCB11* gene.

Additionally, one case of PFIC3, associated with the *ABCB4* gene, exhibited a variant of uncertain significance (VUS). In terms of impact on the protein, our study detected the following types of variants: frameshift—15 variants in 25 patients; nonsense—15 variants in 30 patients; stop gain—2 variants in 3 patients; splice—4 variants in 5 patients; start loss—1 variant in 1 patient; missense—30 variants in 50 patients; and substitution—2 variants in 2 patients. In 64 patients, 37 unique variants affecting protein function were classified as truncated.

3.3. Clinical and Laboratory Findings

Tables 1–3 outline detailed clinical and laboratory findings of these cases. The study included only two male participants diagnosed with PFIC5. Given the small number of PFIC5 patients, comparisons with other groups were omitted.

When comparing the PFIC1, 2, 3, and 4 groups, at the time of genetic diagnosis, both genders were equally represented across all groups (Table 1). Patients diagnosed with PFIC3 were older than those in other groups (p-value < 0.001). As a result, PFIC3 patients had significantly higher weight and height measurements compared to other groups (p-value < 0.001). However, when assessing growth relative to age and gender norms using height and weight z-scores (relative growth measures using WHO standards) [6], there were no significant differences between the groups. The follow-up period ranged from 2 to 43 months, with clinical manifestations varying from mild/moderate to end-stage liver disease. A significant proportion (52.6–73.1%) of patients in each group reported a family history of liver issues. Common clinical findings included hepatomegaly and jaundice (including scleral icterus), with increased direct and total bilirubin levels observed in all participants. Interestingly (Table 2), the most pronounced increase in conjugated bilirubin was observed in patients with PFIC4 (Table 3). Acholic stool was more common in PFIC2 patients (46.4%) and less frequent in PFIC3 patients (23.1%), though not statistically significant. Pruritus occurred less frequently in PFIC2 (60.7%) compared to other groups (66.7%–79.0%), although this difference was not statistically significant. The diagnosis of pruritus in preverbal children was based on clinical judgment, taking into account several key factors: observable physical signs such as scratches, lichenification, and secondary skin infections; behavioral indicators including excessive rubbing, agitation or irritability, and disturbed sleep patterns; and the child's response to antipruritic treatment. Importantly, the absence of pruritus was not limited to preverbal patients; therefore, the absence of this symptom in some cases reflects true inter-individual and subtype-related variation. Splenomegaly was less common in PFIC1 patients (47.4%) compared to others (75.0–86.7%), though this difference was not statistically significant. Portal hypertension, the most consistent clinical marker of decompensated chronic liver disease (DCLD), is characterized by features such as esophageal varices, ascites, encephalopathy, and leg swelling. It was observed in 37 of 114 patients (32.5%) with PFIC1–4. The subtype distribution was as follows: PFIC1—5.3%, PFIC2—35.7%, PFIC3—36.5%, and PFIC4—46.7% (p-value = 0.040). The growth of the participants was evaluated using the height-for-age and weight-to-age metric—Z-scores. Stunting was identified when the Z-score value fell below two standard deviations (SD) from the median of the World Health Organization (WHO) Child Growth Standards (https://www.who.int/tools/child-growth-standards, Accessed 11 June 2025) [6]. The stunted growth was observed in more than half of the participants in all groups (57.1–66.7%). Early

Table 1: PFIC patients' clinical data.

	PFIC1 *(ATP8B1)*	PFIC2 *(ABCB11)*	PFIC3 *(ABCB4)*	PFIC4 *(TJP2)*	*p*-Value
n (%)	19 (16.4%)	28 (24.1%)	52 (44.8%)	15 (12.9%)	n/a
m/f	11/8	14/14	27/25	11/4	0.461 *
Age (month), median (range)	47 (20–227)	40.5 (15–107)	98 (23–160)	47 (18–203)	<0.001 #
Weight (kg), median (range)	12 (8–28)	11.5 (6–22)	18.5 (9–30)	12 (6–48)	<0.001 #
Height (cm), median (range)	96 (74–146)	85 (74–120)	117 (76–145)	94 (76–160)	<0.001 #
Weight Z-score ^	−2.3 (−3.7 to 0.2) n = 15	−1.8 (−4.2 to 1.1) n = 23	−2.3 (−4.1 to 0.0) n = 9	−2.2 (−3.9 to 0.0) n = 13	0.342 #
Height Z-score ^	−0.3 (−3.7 to 1.7) n = 15	−0.3 (−3.6 to 1.4) n = 23	−0.4 (−3.4 to 1.8) n = 9	−0.2 (−3.7 to 2.1) n = 13	0.624 #
Follow-up time (month), median (range)	18 (3–41)	24.5 (2–43)	18 (2–41)	27 (3–41)	0.150 #
History [1], n (%)	10 (52.6)	18 (64.3)	38 (73.1)	10 (66.7)	0.437 *
Jaundice, n (%)	19 (100)	28 (100)	52 (100)	15 (100)	1.00
Acholic stools, n (%)	7 (36.8)	13 (46.4)	12 (23.1)	5 (33.3)	0.191 *
Pruritus, n (%)	15 (79.0)	17 (60.7)	38 (73.1)	10 (66.7)	0.533 *
Hepatomegaly, n (%)	19 (100)	28 (100)	52 (100)	15 (100)	1.00
Splenomegaly, n (%)	9 (47.4)	21 (75.0)	39 (75.0)	13 (86.7)	0.053 *
Portal hypertension, n (%)	1 (5.3)	10 (35.7)	19 (36.5)	7 (46.7)	0.040 *
Diarrhea, n (%)	11 (57.9)	4 (14.3)	2 (3.9)	1 (6.7)	<0.001 *
Stunted growth, n (%)	12 (63.2)	16 (57.1)	32 (61.5)	10 (66.7)	0.937*
Hearing loss, n (%)	3 (15.8)	0 (0)	1 (1.9)	2 (13.3)	0.031 *

[1]—presence of one or more first-degree relatives (parents, siblings) or second-degree relatives (grandparents, aunts, uncles, nieces, nephews, or half-siblings) with known or suspected chronic liver conditions, including but not limited to jaundice, hepatomegaly, abnormal liver function tests (LFTs); ^—only participants <60 months were included (https://www.who.int/tools/child-growth-standards, Accessed 11 June 2025); *—Chi-square test; #—Analysis of variance (ANOVA).

Table 2: PFIC patients' laboratory findings: number of patients with laboratory values higher than the upper limit of normal (ULN) or lower than the lower limit of normal (LLN).

Test	PFIC1 n = 19	PFIC2 n = 28	PFIC3 n = 52	PFIC4 n = 15	*p*-Value *
Bil. tot. > 1.0 mg/dL	19 (100%)	28 (100%)	51 (98.1%)	15 (100%)	1.00
Bil. con. > 0.3 mg/dL	19 (100%)	28 (100%)	52 (100%)	15 (100%)	1.00
GGT > 55 IU	13 (68.4%)	12 (42.9%)	50 (96.2%)	6 (40%)	<0.001
ALP > 350 IU	2 (10.5%)	5 (17.9%)	22 (42.3%)	6 (40%)	0.001
ALT > 40 IU	18 (94.7%)	27 (96.4%)	52 (100%)	15 (100%)	0.378
AST > 40 IU	19 (100%)	27 (96.4%)	51 (98.1%)	15 (100%)	0.765
aPTT(R) > 1.2	19 (100%)	28 (100%)	49 (94.2%)	15 (100%)	0.565
INR > 1.2	18 (94.7%)	24 (85.7%)	44 (84.6%)	13 (86.75)	0.731
PLT < 150*10^9/L	1 (5.3%)	2 (7.1%)	9 (17.3%)	3 (20.0%)	0.345
Hg < 110 g/L	16 (84.2%)	25 (89.3%)	44 (84.6%)	13 (86.7%)	0.943

Bil tot—bilirubin total; Bil. con.—bilirubin conjugated; GGT—Gamma-glutamyl transferase; ALP—alkaline phosphatase; AST—aspartate aminotransferase; ALT—alanine aminotransferase; aPTT(R)—activated partial thromboplastin time (ratio); INR—international normalized ratio; PLT—blood platelet count; Hb—hemoglobin; AFT—alpha-fetoprotein; n/a—not available. *—Chi-square test.

diagnosis of hearing loss is essential, as it significantly influences language development, social skills, and overall learning outcomes. In our study, the otoacoustic emissions (OAE) test [7] was employed to identify hearing loss, with six patients (PFIC1—3; PFIC3— 1, and PFIC4—2) subsequently diagnosed with the condition. The PFIC1 and PFIC4 patients were more prone (p-value = 0.031) to develop hearing loss. Almost all patients (84.6–100%) ex-

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Table 3: PFIC patients' laboratory values.

Test	PFIC1 n = 19	PFIC2 n = 28	PFIC3 n = 52	PFIC4 n = 15	*p*-Value *
Bil. tot. mg/dL #	120 (103–127)	130 (104–144)	109 (77–139)	134 (112–164)	0.083
Bil. con. mg/dL #	102 (86–110)	110 (84–123)	86 (60–118)	131 (110–156)	<0.001 a,b
GGT, IU #	61 (50–64)	55 (18–58)	188 (176–198)	123 (59–167)	<0.001 a,c
ALP, IU #	263 (236–287)	281 (237–314)	766 (760–788)	287 (243–310)	<0.001 a,d
ALT, IU #	96 (78–122)	121 (90–144)	201 (122–456)	134 (98–176)	<0.001 a,e
AST, IU #	68 (56–87)	104 (75–198)	420 (234–644)	108 (89–123)	<0.001 a,f
aPTT(R) #	1.28 (1.24–1.32)	1.28 (1.24–1.32)	1.28 (1.19–1.28)	1.29 (1.24–1.34)	0.167
INR #	1.60 (1.40–1.70)	1.24 (1.11–1.45)	1.65 (1.23–1.98)	1.70 (1.50–2.00)	<0.001 a,g
PLT, 10^9/L #	220 (176–234)	211 (178–274)	176 (99–210)	210 (152–234)	0.051
Hg, 110 g/dL #	10.6 (9.8–11.1)	10.1 (9.1–10.8)	10.0 (8.3–10.8)	10.2 (9.0–11.2)	0.054

Bil tot—bilirubin total; Bil. con.—bilirubin conjugated; GGT—Gamma-glutamyl transferase; ALP—alkaline phosphatase; AST—aspartate aminotransferase; ALT—alanine aminotransferase; aPTT(R)—activated partial thromboplastin time (ratio); INR—international normalized ratio; PLT—platelet count; Hb—hemoglobin; AFT—alpha-fetoprotein; n/a—not available. #—Median (25–75 Percentiles); *—Kruskal-Wallis Test; a—Dunn's Test with Bonferroni Correction: b—PFIC 4 vs. 1 (adjusted *p*-value = 0.005), 4 vs. 2 (<0.001), 4 vs. 3 (0.028); c—3 vs. 1 (<0.001), 3 vs. 2 (<0.001), 3 vs. 4 (<0.001), 4 vs. 1 (<0.001), 4 vs. 2 (<0.001); d—3 vs. 1 (<0.001), 3 vs. 2 (<0.001), 3 vs. 4 (<0.001); e—3 vs. 1 (<0.001), 3 vs. 2 (<0.001), 3 vs. 4 (<0.001), 4 vs. 1 (0.014), 4 vs. 2 (<0.026); f—3 vs. 1 (<0.001), 3 vs. 2 (<0.001), 3 vs. 4 (<0.001), 4 vs. 1 (<0.001); g—2 vs. 1 (<0.001), 2 vs. 3 (<0.001), 2 vs. 4 (<0.001).

hibited abnormal blood coagulation tests (activated Partial Thromboplastin Time/Ratio [aPTT/R] > 1.2 and/or International Normalized Ratio [INR] > 1.2) (Table 2), the INR was least elevated in PFIC2 patients (Table 3). These abnormalities indicate decreased production of clotting factors by the liver, and support that many patients were diagnosed in a decompensated stage of the disease. Diarrhea was reported in the majority (59.4%) of PFIC1 patients (*p*-value < 0.001), with lower frequencies in other groups (3.9–14.3%).

Gamma-glutamyl transferase (GGT) levels were elevated in one-third of PFIC1 (68.4%), about half of PFIC2 (42.9%) and PFIC4 (40.0%) patients, and in almost all (96.2%) of PFIC3 patients (*p*-value < 0.001) (Table 2). The increase in GGT was most pronounced in patients with PFIC3 (*p*-value < 0.001), where levels were more than three times the upper limit of normal (3ULN); for patients with PFIC4, GGT levels were higher than 2ULN. In contrast, the increase in GGT levels for patients with PFIC1 and PFIC2 did not exceed 2ULN (Table 3). In PFIC1 patients, GGT showed a strong negative correlation with ALT (r = −0.58), AST (r = −0.82), and total bilirubin (r = −0.47), suggesting an inverse relationship with liver injury markers. PFIC2 patients exhibited a strong positive correlation between GGT and total bilirubin (r = 0.74), while correlations with ALT (r = −0.32) and AST (r = −0.44) were weak or negative (Table S2, Supplementary Materials). GGT elevations were more frequently observed in PFIC1 and PFIC2 patients treated with rifampicin than those not; however, the difference was not statistically significant. Almost all patients in each group showed elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (Table 2), with the most pronounced increase in PFIC3 patients (Table 3). The elevation (Table 3) and prevalence (Table 2) of alkaline phosphatase (ALP) were also highest in PFIC3 patients. Alpha-fetoprotein (AFP) levels were tested in 7 (2—PFIC2, 3—PFIC3, and 1—PFIC4) patients during the period of treatment and follow-up. Moderate elevations (<400 mcg/L) were revealed in 2/2 PFIC2, 2/3 in PFIC3 and 1/1 PFIC4 patients. Only a small proportion of patients (5.3–20.0%) exhibited low platelet counts, with no differences between the groups. Anemia was typical for the vast majority (84–89%) of patients in all groups. Since the study participants underwent WGS, other common genetic causes of anemia, such as sideroblastic anemia, Fanconi anemia, and sickle cell disease, were effectively ruled out.

The two male participants, diagnosed with PFIC5 within the age range of 0–5 years (not included in the Tables), consistently exhibited jaundice, scleral icterus, hepatomegaly, splenomegaly, and stunted growth at the time of genetic diagnosis. However, neither showed signs of acholic stools or diarrhea. One patient displayed symptoms of pruritus. They demonstrated elevated levels of both total and conjugated bilirubin. Their GGT and ALP levels remained within normal ranges. However, there were elevations in ALT and AST, 121 IU, 123 IU, and 86 IU, 87 IU, respectively. Blood coagulation was also affected: aPTT/R—1.32 and 1.34, and INR—1.54 and 1.62, accordingly. Both patients maintained normal hemoglobin

levels and platelet counts. Their follow-up periods were 18 and 26 months, respectively. Both participants died.

3.4. Management and Outcomes

In our cohort, the treatment strategy for the patients primarily included oral administration of ursodeoxycholic acid (UDCA) and fat-soluble vitamins (A, D, E, K), supplemented by nutritional interventions. For symptomatic relief of pruritus, agents such as cholestyramine and rifampicin were prescribed. Surgical interventions were also part of the treatment regimen, with nine patients undergoing Biliary Diversion (BD) surgery and ten patients receiving Liver Transplant (LT). Recognizing the hereditary nature of PFICs and the frequent delays in generic diagnosis and treatment initiation, we focused on assessing cumulative survival rather than post-diagnostic (on-treatment) survival. Over 20 years (240 months), the cumulative survival rate for all PFIC patients was 20% (95% CI 5–41%) (Figure 1A). Of the 10 PFIC patients who underwent LT, only 1 patient died, in contrast to 44 of the 106 patients (42%) who were treated with standard methods. Despite LT being performed in cases with severe disease and/or at an advanced stage, the cumulative survival rate of patients with LT was significantly higher at 89% (95% CI 43–98%), compared to 9% (95% CI 1–29%) for those not undergoing LT, p-value = 0.016 (Figure 1B). Among the four PFIC types, PFIC3 exhibited a less aggressive course, with a better survival outcome compared to PFIC1, PFIC2, and PFIC4, p-value = 0.023 (Figure 1C). The cumulative survival of patients with non-truncated genetic variants was higher, 32% (95% CI 8–60%) (not significantly), than in patients with truncated variants, 14% (95% CI 1–41%) (Figure 1D).

Specifically, for PFIC1 (*ATP8B1*), alongside medical treatments, 3 of 19 patients underwent BD, and 5 received LT. The 12-year survival rate was 27% (95% CI 2–66%); without LT, this rate decreased to 18% (95% CI 1–53%), p-value = 0.194. In the PFIC2 (*ABCB11*) group, five underwent BD and one received LT. The 20-year survival was 25% (95% CI 2–63%) (Figure 1C). Notably, none of the six patients who underwent BD or LT died, in contrast to 9 of 22 patients (41%) on standard medical treatment. For PFIC3 (*ABCB4*), three patients received LT. None of the PFIC3 LT patients died, while 16 (31%) of the other PFIC3 patients did. In the PFIC4 (*TJP2*) patients, the cumulative survival rate was 19% (95% CI 1–52%) (Figure 1C). One patient who underwent LT is currently alive. In contrast, 9 out of the remaining 14 patients (60%) have died. Both patients with PFIC5 (*NR1H4*) experienced severe progressive disease and passed away, having not undergone any surgical interventions.

4. Discussion

In our study, we aimed to determine the distribution, disease characteristics, and clinical outcomes of patients with PFICs in Pakistan. In our cohort, PFIC3 emerged as the predominant type, accounting for nearly half of the cases (44.8%). This is followed by PFIC2, PFIC1, PFIC4, and PFIC5, which comprise 24.1%, 16.4%, 12.9%, and 1.7% of the cases, respectively. Earlier studies included diverse patient populations from France [8], Germany [9], India [10], without specifying the ethnicity [11], and the United States, encompassing Caucasian, African-American, Japanese, and Korean origins [12]. In these studies, PFIC1 and PFIC2 were the most frequent types, followed by PFIC3; PFIC4 and PFIC5 were much rarer. However, PFIC3 was the most common type (59.5%) in the study from Saudi Arabia, followed by PFIC2 (34.2%), PFIC1 (5.1%), and PFIC4 (1.3%) [5].

The clinical and laboratory profiles of PFIC types have been extensively documented in case reports and systematic reviews collating data from numerous publications on PFICs. The clinical presentations and laboratory findings in our study largely align with those reported previously. However, several features in our cohort diverge from previous reports and may reflect population-specific characteristics.

Generally, PFIC1 and PFIC2 manifest within the first year of life, while PFIC3 presents over a broader age range from infancy to early adulthood. PFIC4 and PFIC5 typically show symptoms in early childhood, although the exact age of onset can vary due to their rarity and clinical variability [2,5,13,14]. In our study, at the time of genetic diagnosis, PFIC3 patients were the oldest (median age—98 months) compared to other PFIC types diagnosed within 40–48 months of age (p-value < 0.001), and this finding aligns with previous studies. However, our results suggest that PFIC genetic diagnosis in Pakistan may be delayed by several months, highlighting the need for increased awareness and timely diagnostic protocols to improve early detection and management.

In our study, the majority of patients in all PFIC groups reported a family history of liver diseases. Almost all PFIC variants, with only one exception, were homozygous rather than compound heterozygous. This suggests a highly consanguineous population within our cohort. Generally, consanguineous marriages in Pakistan are reported to range from 46–98%, depending on the region, which correlates with high rates of genetically inherited diseases and infant mortality [15].

Jaundice, due to elevated conjugated bilirubin, is present in all PFIC patients. Conjugated bilirubin was most elevated in PFIC4 and less elevated in PFIC3, with

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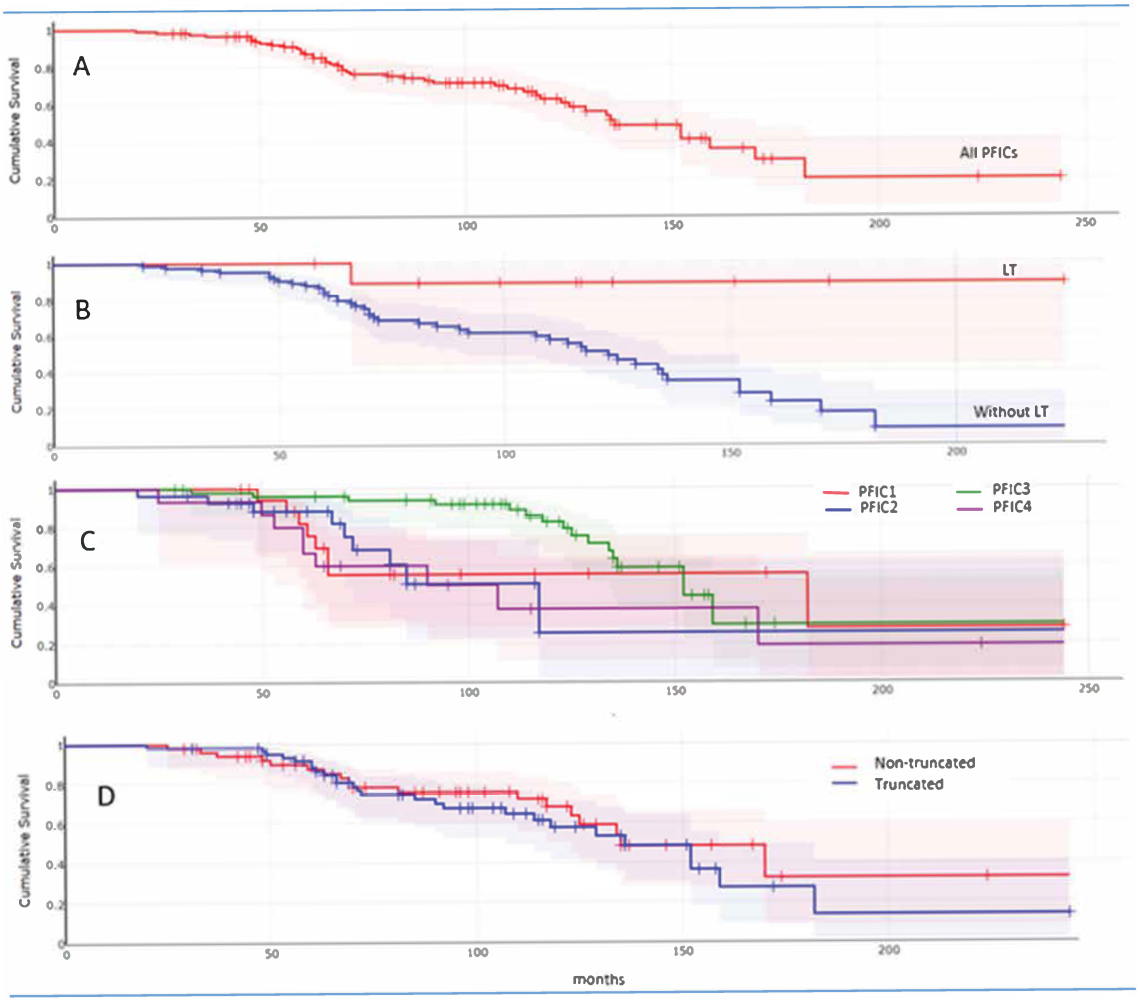


Figure 1: PFIC patients' cumulative survival (95% Confidence Interval [CI]). (**A**): all PFIC patients' cumulative survival—20% (95% CI 5–41%); (**B**): cumulative survival with liver transplant (LT) 89%—(95% CI 43–98%) vs. without liver transplant—9% (95% CI 1–29%), *p*-value = 0.016; (**C**): cumulative survival of different PFICs: PFIC1—27% (95% CI 2–66%), PFIC2—25% (95% CI 2–63%); PFIC3—29% (95% CI 7–57%); PFIC4—19% (95% CI 1–52%). PFIC3 showed better survival outcome, *p*-value = 0.023; (**D**): cumulative survival of patients with truncated variants—14% (95% CI 1–41%) vs. non-truncated variants—32% (95% CI 8–60%), *p*-value = 0.547.

a significant difference. This disparity is likely due to the underlying genetic mutations and their impact on bile secretion pathways. TJP2 is essential for maintaining the integrity of tight junctions in hepatocytes. Loss of TJP2 function leads to impaired tight junction sealing in the bile canaliculi, resulting in severe bile leakage and paracellular reflux of bile constituents, including conjugated bilirubin, back into the bloodstream. This disruption of bile flow at the cellular junctional level leads to marked cholestasis and high levels of conjugated bilirubin, even early in the disease course [16–18]. In contrast, PFIC3 results from mutations in the ABCB4 gene, which encodes the MDR3 protein responsible for transporting phosphatidylcholine into bile. While this defect leads to toxic bile composition and progressive cholangiopathy, the onset is typically later and progression is more gradual, resulting in less dramatic elevations in conjugated bilirubin at the time of diagnosis [16,19,20].

Acholic stool is a common cholestatic symptom. The proportion of patients with PFICs affected and the severity of this symptom can vary depending on bile secretion and flow [2,21]. In our study, acholic stool was observed in 23.1% to 46.4% of patients across different PFIC types, without a statistically significant difference between the groups.

Pruritus is commonly regarded as a hallmark symptom of cholestasis, including in patients with PFICs [3, 22,23]. In this study, we observed that around a quarter of PFIC1 and PFIC3 patients, and a third of PFIC2 and PFIC4 patients, did not report pruritus. Previous studies indicate that pruritus remains a significant and distressing symptom in PFICs, particularly in PFIC1 and PFIC2,

where it is often severe and nearly universal; PFIC3 patients tend to experience milder pruritus, while PFIC4 and PFIC5 patients have fewer comprehensive data but include pruritus as a symptom for most patients [13,24,25]. However, pruritus may be underreported in preverbal children, leading to potential underestimation of its prevalence [26]. This underscores the need for increased clinician awareness of pruritus to ensure timely and accurate diagnosis and management.

Splenomegaly and portal hypertension were less frequent in PFIC1 patients in our study compared to other PFIC types. This observation aligns with previous findings on the distinct nature of genetic mutations and their impact on liver pathology. PFIC1, caused by mutations in the *ATP8B1* gene, leads to a unique progression of liver disease characterized primarily by issues with bile acid transport and liver cell damage, rather than extensive bile duct proliferation and fibrosis. This results in a less frequent progression to cirrhosis and portal hypertension in the early stages of the disease course. In PFIC2 and PFIC3 types, mutations in the *ABCB11* and *ABCB4* genes, respectively, often lead to more severe bile secretion defects, increased bile duct proliferation, and quicker progression to liver fibrosis and portal hypertension [22,27].

The higher prevalence of diarrhea in PFIC1 patients compared to other PFIC types can be attributed to the unique pathophysiology associated with mutations in the *ATP8B1* gene. PFIC1 involves defects in the FIC1/ATP8b1 protein, which is crucial for maintaining the balance of bile acids and other lipids in the liver and intestines. This defect leads to steatorrhea and diarrhea due to impaired bile acid reabsorption in the intestines. Additionally, mutations in the *ATP8B1* gene can affect pancreatic function, resulting in exocrine pancreatic insufficiency and subsequent diarrhea. These extrahepatic manifestations are distinct in PFIC1. In contrast, the lower rates of diarrhea observed in PFIC2, PFIC3, and PFIC4 align with their pathophysiological mechanisms, which primarily involve isolated hepatic involvement without significant extrahepatic effects [13,14,16,22].

In our study, 15.8% of PFIC1 and 13.3% of PFIC4 patients developed hearing loss. Previous studies have documented instances of hearing loss in patients with PFIC1 and PFIC4, linking these symptoms to specific genetic mutations. In PFIC1, the *ATP8B1* gene product, the FIC1/Atp8b1 protein, is specifically localized in the stereocilia of cochlear hair cells. The mechanosensory function and integrity of these hair cells critically depend on ATP8B1 activity, which maintains lipid asymmetry in the cellular membranes of the stereocilia. Disruptions in this function due to *ATP8B1* mutations can lead to hearing loss [22,28]. Similarly, mutations in the *TJP2* gene, which encodes tight junction protein 2 (TJP2), can be associated with PFIC4. The TJP2 protein is crucial for maintaining cell-cell adhesion and the integrity of tight junctions in the inner ear, and mutations in *TJP2* can disrupt these structures, leading to hearing loss [29]. Hearing loss in one out of 52 patients with PFIC3 is likely not related to the PFIC disease.

Previous studies have shown that among PFIC types, PFIC3 is characterized by high levels of GGT; in contrast, PFIC1 and PFIC2 typically present with normal or low GGT levels [2,13,14,16,22]. PFIC4 can also exhibit elevated GGT levels, but this is not as consistent as in PFIC3 [17]. However, in our study, about half of the PFIC1 and PFIC2 patients exhibited elevated GGT levels, though these increases were mild to moderate (<2 ULN). The observed correlations suggest that in PFIC1, elevated GGT does not reflect more severe liver injury. In PFIC2, the strong positive correlation between GGT and bilirubin confirms its association with cholestatic injury. Additionally, other factors, such as comorbidities (e.g., viral hepatitis, pancreatitis, nonalcoholic fatty liver disease, diabetes mellitus) [30] or medications such as rifampicin can contribute to GGT elevation [31,32]. Therefore, GGT elevation in PFIC1 and PFIC2 may reflect multifactorial influences beyond the primary genetic defect.

Increases in ALT, AST, and ALP, as well as impaired blood coagulation tests, have been well-documented in all types of PFIC. The predominant increase in one or more of these markers among patients with different types of PFIC in our cohort can likely be attributed to the specific set of patients presenting at various stages and severities of the disease at the time of genetic diagnosis across the PFIC groups. This variation reflects the heterogeneous nature of PFIC progression and the liver's response to ongoing cholestasis and hepatocellular damage.

The literature suggests that PFIC2, PFIC3, and PFIC4 carry an elevated risk for the development of liver tumors [33]. However, we did not observe any liver tumors in our cohort, including patients beyond their second decade of life. AFP testing was performed in only seven patients, with only one demonstrating a normal level. While AFP > 400 mcg/L is considered suggestive of HCC in the appropriate context [34], moderate elevations (<400 mcg/L) may occur in other liver conditions, including PFIC [22,23,35]. Although not specific for HCC, such findings warrant further evaluation, particularly as HCC often remains undiagnosed until advanced stages, and is sometimes only detected post-mortem [36]. This highlights the need for systematic tumor surveillance in PFIC patients.

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Anemia in PFIC patients can result from various factors related to liver dysfunction and disease impact, and usually develops at the advanced stage of the disease. Anemia in PFIC patients arises from several factors: chronic liver disease disrupts iron metabolism and protein production; splenomegaly can lead to hypersplenism, causing excessive red blood cell destruction; nutritional deficiencies due to malabsorption further contribute to anemia; increased bleeding risk through reducing clotting factor synthesis can lead to chronic blood loss; chronic inflammation can suppress bone marrow function, reducing red blood cell production [2,3,14,22,23,37]. Additionally, frequent blood draws during hospital stays exacerbate the condition, particularly in children [38]. A significant majority of PFIC patients in our cohort (84.2–89.3%) presented with anemia, indicating that most patients received their PFIC genetic diagnosis at the advanced stage of the disease. This delay is likely due to limited awareness of genetic cholestasis among general pediatricians and restricted access to WGS, which is mostly available in tertiary centers. As a result, many children face prolonged diagnostic delays. To improve early detection, we recommend national guidelines with clear referral criteria and standardized use of genetic testing, along with targeted training for healthcare providers.

A common feature among all PFIC types in our cohort was a poor prognosis, with a low cumulative survival rate of 20% (95% CI: 5–41%) over 20 years. Consistent with previous reports, untreated PFIC1 and PFIC3 typically progress to end-stage liver disease and death within 10–20 years, with an even worse prognosis in cases of PFIC2 [2,16,39]. A review of 10 studies [14] showed mortality rates as high as 87%, with the median age at death being 4 years; survival rates for patients who did not undergo biliary surgery or LT dropped to 50% by age 10, with virtually no survivors by age 20. This high mortality rate underscores the frequent need for LT in PFIC patients, with transplantation rates as high as 65% [5].

The literature indicates that PFIC1, PFIC2, PFIC4, and PFIC5 are particularly aggressive in their disease course, often rapidly progressing to end-stage liver disease [23]. This aligns with our observations: PFIC3 exhibited a less aggressive course compared to PFIC1, PFIC2, and PFIC4 in the first decade.

Truncated variants, such as frameshift, nonsense, stop gain, splice, and start loss mutations, lead to the production of dysfunctional or absent proteins, exacerbating the pathophysiological mechanisms of PFIC. Consequently, patients with these variants often present with more severe symptoms, faster disease progression, and poorer outcomes compared to those with non-truncated variants, such as missense and substitution mutations [13,24,40–42]. In our cohort, patients with non-truncated variants showed better, though not statistically significant, cumulative survival (Figure 1D). The increased aggressiveness of the disease in patients with truncated variants is also associated with poorer responses to conventional treatments and a higher necessity for LT. These findings underscore the importance of early genetic diagnosis and personalized treatment approaches to improve outcomes for PFIC patients.

This study acknowledges certain limitations. There is a possibility that patients with a late onset of symptoms, particularly in their second decade, were not captured in our study. The study did not include patients with cholestasis and negative genetic results, preventing the analysis and comparison of genetic and idiopathic forms of PFIC. An important limitation is that the analysis of mutation type (truncated vs. non-truncated) concerning survival outcomes was performed across the full PFIC cohort and not stratified by individual PFIC types due to limited sample sizes. This may have obscured subtype-specific genotype-phenotype correlations, particularly in PFIC2, which warrants focused analysis in future studies. While WGS offers broader and more complete variant detection than WES or targeted panels, it may still miss certain types of genetic variation (e.g., complex structural rearrangements), and its diagnostic accuracy depends heavily on the robustness of bioinformatic pipelines and expert interpretation. Another limitation of this study is the absence of data on age at symptom onset, which relied solely on parental recall and was often inconsistently documented. Given the lack of standardized criteria for defining PFIC onset, ranging from mild pruritus to overt liver symptoms, we excluded this variable to avoid introducing bias. Instead, we reported age at genetic diagnosis, which was uniformly available and reflects diagnostic delays. Future studies should aim to capture onset data prospectively using clear clinical definitions. While our cohort was not based on a pre-defined sample size, it includes all consecutively diagnosed PFIC cases over four years, providing a real-world snapshot; nonetheless, future studies with larger samples and standardized inclusion criteria are warranted to validate these findings.

The clinical applicability of our findings lies in their potential to inform earlier diagnosis and management strategies in pediatric patients with cholestatic liver disease. WGS not only enables precise diagnosis but also guides clinical decision-making, including timely referral for liver transplantation in severe cases. In regions with high consanguinity and limited access to advanced diagnostics, such as Pakistan, our data underscore the importance of integrating genetic testing into routine hepatology care. Implementation of early genetic screening, particu-

larly for high-risk families, may significantly improve outcomes and reduce mortality in PFIC patients.

5. Conclusions

This study represents the first comprehensive analysis of PFIC in Pakistani children, providing valuable insights into the genetic and phenotypic diversity of this condition. All PFIC types demonstrated significant clinical and laboratory overlap, making differentiation based on these results challenging. WGS proved to be a simple diagnostic tool, crucial for accurate diagnosis.

The clinical presentations, laboratory findings, and outcomes in our study largely align with those reported previously. However, we identified some distinguishing features. In our cohort, PFIC3 emerged as the most prevalent type, followed by PFIC2, PFIC1, PFIC4, and PFIC5. The increased GGT in half of the PFIC1 and PFIC2 patients may reflect multifactorial influences beyond the primary genetic defect. Anemia and abnormal coagulation profiles in nearly all patients with all PFIC types at the time of genetic diagnosis can be attributed to delayed PFIC genetic diagnosis in Pakistani children. This highlights the need for the implementation of genetic testing protocols.

The decrease in the proportion of patients with pruritus and the absence of liver tumors in the large cohort may indicate the need for the implementation of additional protocols for pruritus diagnosis in preverbal children, as well as the implementation of a screening and monitoring system for liver tumors in these patients in Pakistan.

Patients with PFIC1, PFIC2, PFIC4, and/or truncated variants exhibited poorer outcomes, underscoring the importance of early genetic diagnosis and personalized treatment approaches, early consideration for LT in particular, to improve outcomes.

List of Abbreviations

AFP	Alpha-Fetoprotein
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
aPTT(R)	Activated Partial Thromboplastin Time (Ratio)
AST	Aspartate Aminotransferase
BD	Biliary Diversion
CI	Confidence Interval
DCLD	Decompensated Chronic Liver Disease
DNA	Deoxyribonucleic Acid
GGT	Gamma-Glutamyl Transferase
Hb	Hemoglobin
HPO	Human Phenotype Ontology
INR	International Normalized Ratio
LT	Liver Transplantation
OMIM	Online Mendelian Inheritance in Man
OAE	Otoacoustic Emissions
PFIC	Progressive Familial Intrahepatic Cholestasis
PLT	Platelet Count
SD	Standard Deviation
ULN	Upper Limit of Normal
VUS	Variant of Uncertain Significance
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing
WHO	World Health Organization
Z-score	Standard Deviation Score (relative to age/gender norms)

Author Contributions

Idea conception, drafting, and revision: H.A.C., A.S. (Aliaksandr Skrahin), Z.F., and A.R.; Participants' enrolment, clinical evaluation, and sampling: M.A.A., M.N.A., N.W., K.U.R., A.S. (Anjum Saeed), and V.S.; Genomic data analysis: A.S. (Aliaksandr Skrahin), V.S., and A.M.; Manuscript writing: A.S. (Aliaksandr Skrahin), A.R., V.S., and N.W. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

The genetic variants from this study have been submitted to the NCBI ClinVar database (http://www.clinvar.com/) under accession numbers SCV004232458-SCV004232527.

Consent for Publication

The study was approved by the Institutional Review Board of the Children's Hospital and the Institute of Child Health, Lahore (Pakistan), 2019-54-CHICH, 31 August 2019, and the Ethics Committee of Rostock University (Germany), A2022-0072, 25 April 2022. Written informed consent for participation in the study and publication of this paper was obtained from all participants or their parents/legal guardians.

Conflicts of Interest

No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article. A.S. (Aliaksandr Skrahin), A.R., A.M. and V.S. declared employment in a Rare Disease Consulting Company.

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Supplementary Materials

The supplementary materials are available at https://doi.org/10.69709/GenomC.2025.165225.

Figures Originality

All figures included in the manuscript are original and have not been previously published or reproduced from other sources.

Ethics Committee

This study was approved by the Institutional Review Board of the Children's Hospital and the Institute of Child Health, Lahore (Pakistan), 2019-54-CHICH, 31.08.2019, and the Ethics Committee of Rostock University (Germany), A2022-0072, 25.04.2022. Informed consent was obtained from all participants or their legal guardians.

Human Rights Statement—Declaration of Helsinki

The study was conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments.

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