Genetic Analysis of Progressive Familial Intrahepatic Cholestasis:
A Novel Insight into Hepatorenal Ciliopathy

PhD thesis

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1. Introduction

Progressive Familial Intrahepatic Cholestasis (PFIC) represents a heterogeneous spectrum of rare, autosomal-recessive genetic disorders that lead to impaired bile flow, chronic cholestasis, and progressive liver failure during childhood. Despite significant advancements in the field of genetic diagnostics, a notable proportion of pediatric cholestatic liver disease cases remain without a precise molecular diagnosis. This gap in diagnostic capability underscores the critical need for continued genetic discovery and the comprehensive profiling of pediatric populations, particularly in regions with high rates of consanguinity such as Saudi Arabia.

Bile acids are central to the enterohepatic circulation, a process that maintains bile-acid homeostasis and facilitates fat absorption.

Approximately 95% of bile acids are actively reabsorbed in the terminal ileum via the apical sodium-dependent bile acid transporter (ASBT) and returned to the liver via the portal vein, completing a cycle that occurs 4 to 12 times per day. Bile acids that escape reuptake enter the colon, where microbial enzymes deconjugate them and convert

them into secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA).

Disruption of bile-acid transport is the central pathophysiological mechanism of PFIC. Mutations in key transporters cause the primary or secondary impairment of bile acid flow. For example, PFIC type 2 is caused by variants in the *ABCB11* gene, resulting in deficient BSEP transport activity, severe cholestasis, and a markedly increased risk of hepatocellular carcinoma (HCC). Similarly, Multi Drug Resistance protein type 3 (MDR3) deficiency, caused by variants in the *ABCB4* gene (PFIC type 3), leads to impaired biliary secretion of phospholipids. This leaves biliary bile acids unshielded, causing detergent-mediated damage to the biliary tree, potential gallstone formation, and secondary bile acid accumulation in hepatocytes. Other genetic subtypes of PFIC include PFIC1 (FIC1 deficiency due to *ATP8B1* mutations), which affects aminophospholipid translocation and causes systemic manifestations like watery diarrhea and hearing loss; Tight Junction Protein 2 (*TJP2*) deficiency, which leads to rapidly progressive liver disease; and FXR deficiency, causing neonatal liver failure and severe coagulopathy.

Current **therapeutic strategies** for PFIC aim to restore or modulate this enterohepatic circulation. Ursodeoxycholic acid (UDCA) is used to enhance bile flow and reduce detergent toxicity, showing particular efficacy in MDR3 deficiency. Surgical interventions, such as partial external biliary diversion (PEBD), and medical therapies utilizing ileal bile-acid transporter (IBAT) inhibitors, interrupt bile acid recycling to lower serum levels and relieve severe pruritus. However, because therapeutic efficacy varies significantly between PFIC subtypes, obtaining a precise molecular diagnosis is paramount for guiding genotype-specific clinical management.

2. Objectives

The primary purpose of this thesis is to investigate the genetic landscape of Progressive Familial Intrahepatic Cholestasis, with a specific focus on uncovering novel etiologies. The research leverages a unique pediatric cohort from Saudi Arabia, a population characterized by high consanguinity rates, providing an unparalleled opportunity to study autosomal-recessive genetic disorders.

The specific aims of the research were:

1. **To investigate the clinical utility of next-generation sequencing (NGS)** in diagnosing advanced cholestatic liver diseases within a highly consanguineous population.

2. **To identify and thoroughly characterize novel genetic contributors to PFIC**, with a dedicated focus on discovering the *PSKH1* (Protein Serine Kinase H1) gene as a novel etiology associated with a newly proposed hepato-renal ciliopathy.

3. **To Understand the Genotype-Phenotype correlation** in patient with loss of function (LOS) mutation in BSEP deficiency

3. Methods

The methodology of this research was divided into comprehensive human subject profiling and the subsequent validation of findings using a genetically engineered animal model. The study was conducted following ethical approvals from the King Faisal Specialist Hospital and Research Centre (KFSHRC) and the Walter and Eliza Hall Institute.

Human Subjects - Part 1: Targeted Gene Panels The initial phase of the study included 98 children (aged 0–18 years, mean age 5.6 years) referred to the center between 2013 and 2016 for suspected advanced cholestatic liver disease. Inclusion criteria required elevated liver enzymes (ALT > 25 U/L, AST > 45 U/L), elevated gamma-glutamyl transferase (GGT > 32 U/L), high total bilirubin (> 5 μmol/L), or high serum bile acids (> 10 μmol/L). Patients with identifiable metabolic or anatomical causes of liver disease were excluded. A targeted next-generation sequencing gastrointestinal (GI) panel was utilized, encompassing 189 genes with established links to Mendelian diseases presenting with gastrointestinal and cholestatic phenotypes.

Human Subjects - Part 2: Large-Scale Genomic Investigation (Exome Sequencing) To expand diagnostic capabilities, a second, larger cohort was evaluated between March 2019 and March 2023. This cohort comprised 299 pediatric patients from 279 families diagnosed with cholestasis. The mean age of disease onset was 2.75 years. The population exhibited a positive family history in 71.3% of cases and a high consanguinity rate of 72.4%. To map the genetic variants, patients and their

relatives were genotyped on the Axiom SNP Array platform. Researchers analyzed genome-wide genotypes for runs of homozygosity to serve as surrogates for autozygosity. Linkage analysis was conducted using EasyLINKAGE under an assumption of a fully penetrant autosomal-recessive model. Variants were then filtered within candidate autozygomes and classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Pathogenicity predictions relied on public databases, minor allele frequencies, and *in silico* scores (PolyPhen, SIFT, CADD), with final confirmations achieved via Sanger sequencing.

Animal Model of *PSKH1* Following the genomic identification of *PSKH1* variants in human subjects, researchers characterized an N-ethyl-N-nitrosourea (ENU)-induced *Pskh1* mutant mouse model to study the physiological and functional impacts of the variants on the liver and kidneys. Specifically, the *Pskh1*mpc232H mutant mice were bred using a multi-generational scheme. First-generation heterozygous male mice were mated with wild-type females, and subsequent generations were genotyped and bred to produce homozygous mutant mice for phenotypic screening.

Phenotypic assessments included comprehensive histopathology, serum liver and kidney function tests, and detailed morphological examinations of primary cilia via immunofluorescence.

A subset of twenty-five patients who were homozygous for (LOF) ABCB11 (c.379del; p.Thr127HisfsTer6) All patients came from consanguineous relationship with age at first presentation 3.1 (0.2-5.9 years) We observed that elevated AFP is predominantly associated with HCC with a median level of 4586.5 µg/L, with 16% of patients developing HCC , 84% of the patients (21/25), with the median age at transplantation being 4 years (range: 0.9-13.5 years). Four patients (16%) died before transplantation; because of pulmonary hemorrhage with HCC (n=2) and HCC with metastasis (n=2) within 2 years of life before transplantation.

4. Results

Part 1 Using targeted gene panels

A likely causal mutation was identified in the majority (n = 60, 61%) of the enrollees for a total of 37 mutations including 15 that are novel. The most commonly mutated

genes were ABCB11 (16 cases, 25%) followed by ABCB4 (12 cases, 20%)

Part I1 Using Exome sequencing

Building on expanding genetic diagnostics and a growing pediatric hepatology/transplant program, genetic analyses were re-evaluated in 2024. The Part II cohort comprised 279 families (299 patients) with pediatric cholestasis. Positive family history occurred in 199 (71.3%) and consanguinity in 202 (72.4%). Solved cases were significantly more likely to show family history (90% vs 59%) and consanguinity (83% vs 47%) compared to unsolved cases. A likely causal variant was identified in 135 families (151 patients, 48.6%), spanning 37 genes (118 variants) with established disease links

Identification of PSKH1 as a Novel Candidate Gene in Pediatric Cholestasis

Among 156 families without variants in known or tentative cholestasis genes, two PSKH1 variants were identified in three families sharing a distinctive phenotype. Collaboration via GeneMatcher (30) revealed

a fourth family carrying another unique homozygous variant.

Understanding the genotype-phenotype correlation in patients with loss of function mutation is BSEP deficiency

Pruritus was reported at presentation in 60% (15 patients), reflecting the chronic and severe nature of bile acid accumulation. In the natural history of the disease, AFP attains to the normal level from high at the early stage. We observed that elevated AFP is predominantly associated with HCC with a median level of 4586.5 μg/L, with 16% of patients developing HCC. Fat-soluble vitamin levels were assessed in most patients, revealing notable deficiencies. Liver transplantation was undertaken in 84% of the patients (21/25), with the median age at transplantation being 4 years (range: 0.9-13.5 years). Amongst those who underwent transplantation, 61.9% received a living donor liver, 23.8% from cadaveric sources, and 14.3% via domino transplantation. Post-transplant survival was good overall, with 2 deaths occurring after transplantation (9.5%) due to chronic rejection with sepsis, and HCC with metastasis. Four

patients (16%) died before transplantation; because of pulmonary hemorrhage with HCC (n=2) and HCC with metastasis (n=2) within 2 years of life before transplantation.

5. Conclusions

The comprehensive genetic analyses detailed in this thesis underscore the transformative power of next-generation sequencing as a first-tier diagnostic tool for pediatric patients with advanced cholestatic liver disease. The high rate of consanguinity within the studied Saudi Arabian cohort profoundly facilitated the identification of homozygous pathogenic variants, highlighting the heavy regional burden of autosomal-recessive diseases.

The application of targeted gene panels (Part 1) successfully identified likely causal mutations in 61% of the enrolled patients, establishing a minimum disease burden of 1:7246 for cholestatic liver disease in Saudi Arabia. The expanded whole-exome sequencing approach (Part 2) represented the largest WES-based cohort study of pediatric cholestasis in a high-consanguinity population to date, achieving a diagnostic resolution for 48.6% of families across 37 established disease genes.

A landmark contribution of this research was the discovery and functional validation of the *PSKH1* gene as a novel genetic etiology responsible for a previously uncharacterized, early-onset hepatorenal ciliopathy (provisionally termed PFIC13). *PSKH1* founder mutations were identified in multiple unrelated consanguineous families exhibiting combined liver and renal failure. The causality of *PSKH1* was rigorously supported by cellular findings and the *Pskh1*mpc232H mouse model, which successfully replicated the human phenotype of biliary cirrhosis, renal dysplasia, and abnormally elongated, worm-like primary cilia in bile ducts and renal tubules. Mechanistically, this demonstrates that *PSKH1* is a critical serine kinase for ciliary regulation, linking bile-acid homeostasis to hepatocellular ciliary integrity.

The cohort data underscores the critical nature of early intervention in BSEP deficiency, particularly for those with LOF variants who experience rapid disease progression and high rates of HCC without having a reliable tool to anticipate except the genotype

From the cohort observation, preemptive transplantation is strongly advised for patients with the specific homozygous (ABCB11: c.379del; p.Thr127HisfsTer6) mutation rather than depending on AFP

Ultimately, this thesis establishes a vital framework for **precision hepatology**. By integrating detailed molecular insights into routine clinical practice, healthcare providers can drastically reduce the need for invasive liver biopsies, more accurately predict disease progression, and tailor highly specific treatment strategies. For instance, patients identified with loss-of-function variants in *ABCB11* face rapid disease progression and a markedly increased risk of hepatocellular carcinoma; thus, preemptive genotype-driven liver transplantation is strongly advised for this subset of patients. Future research directions must focus on elucidating the precise intracellular mechanisms of *PSKH1*-related ciliopathies, translating animal model findings into viable human therapies, and utilizing

multi-omics to resolve cases that currently remain genetically inconclusive.

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