SEMMELWEIS EGYETEM

DOKTORI ISKOLA

# Ph.D. értekezések

## 3517.

# SHAGRANI MOHAMMAD

**Gyermekkori betegségek klinikuma, élettana és prevenciója**
című program

Programvezető: Dr. Szabó Attila, egyetemi tanár

Témavezető: Dr. Dezsőfi-Gottl Antal, habilitált egyetemi docens

# Genetic Analysis of Progressive Familial Intrahepatic Cholestasis:

# A Novel Insight into Hepatorenal Ciliopathy

**PhD thesis**

**Mohammad Shagrani**

Semmelweis University Doctoral College

Károly Rácz Conservative Medicine Division

Clinical aspects, physiology and prevention of diseases in childhood

Supervisor: Antal Dezsőfi-Gottl MD, PhD.

Official reviewers: András Arató MD, DSc., Dániel Szűcs MD, PhD.

Head of the Complex Examination Committee: György Reusz MD, PhD.

Members of the Complex Examination Committee: Nóra Béres MD, PhD., László Szabó MD, PhD.

Budapest

2026

## TABLE OF CONTENTS

**Content**

**List of Abbreviations**

1. **Introduction**
2. **Objectives**
3. **Methods**
    - Patients
    - Genetic methodologies
    - Animal model of *PSKH1*
4. **Results**
    - Genetic profiling and diagnostic yield
    - Identification of *PSKH1* as a novel candidate gene in pediatric cholestasis
    - Phenotypic recapitulation in mouse model
    - Understanding the genotype-phenotype correlation in patients with loss of function mutation is BSEP deficiency
5. **Discussion**
    - The utility of next-generation sequencing in diagnosing advanced cholestatic liver diseases in a high-consanguinity population
    - Identification and characterization of novel genetic contributors to PFIC, with a focus on *PSKH1*
    - Characterization of animal model to study disease mechanisms and therapeutic targets
    - Limitations and future research direction
6. **Conclusions**
7. **Summary**
8. **References**
9. **Bibliography of the candidate**
10. **Acknowledgements**

**List of Abbreviations**

| Abbreviation | Full Term / Definition |
|---|---|
| *ABCB4* | ATP-binding cassette sub-family B member 4 (MDR3) |
| *ABCB11* | ATP-binding cassette sub-family B member 11 (Bile Salt Export Pump, BSEP) |
| ACMG | American College of Medical Genetics and Genomics |
| ALT | Alanine Aminotransferase |
| AMP | Association for Molecular Pathology |
| ARC | Arthrogryposis–Renal–Cholestasis Syndrome |
| ASBT | Apical Sodium-Dependent Bile Acid Transporter (Ileal Bile Acid Transporter, IBAT) |
| AST | Aspartate Aminotransferase |
| *ATP8B1* | Aminophospholipid Transporting ATPase 8B1 |
| BSEP | Bile Salt Export Pump (ABCB11) |
| CA | Cholic Acid |
| CDCA | Chenodeoxycholic Acid |
| CNS | Crigler–Najjar Syndrome |
| *CYP7A1* | Cholesterol 7α-Hydroxylase |
| DCA | Deoxycholic Acid |
| DNA | Deoxyribonucleic Acid |
| EDTA | Ethylenediaminetetraacetic Acid |
| EASL | European Association for the Study of the Liver |
| FGF19 | Fibroblast Growth Factor 19 |
| FXR | Farnesoid X Receptor |
| GGT | Gamma-Glutamyl Transferase |
| GGT/γ-GT | Gamma-Glutamyl Transferase (used interchangeably) |
| GNOMAD | Genome Aggregation Database |
| GST | Glutathione S-Transferase |
| HCC | Hepatocellular Carcinoma |

| | |
|---|---|
| HGNC | HUGO Nomenclature Committee |
| IBAT | Ileal Bile Acid Transporter (ASBT) |
| IRB | Institutional Review Board |
| LCA | Lithocholic Acid |
| LOF | Loss of Function |
| MAPK | Mitogen-Activated Protein Kinase |
| MAF | Minor Allele Frequency |
| MDR3 | Multidrug Resistance Protein 3 (ABCB4) |
| mRNA | Messenger RNA |
| NGS | Next-Generation Sequencing |
| NLS | Native liver survival |
| *NR1H4* | Nuclear Receptor Subfamily 1, Group H, Member 4 (FXR) |
| OCA | Obeticholic Acid |
| PCR | Polymerase Chain Reaction |
| PFIC | Progressive Familial Intrahepatic Cholestasis |
| PFIC1 | Progressive Familial Intrahepatic Cholestasis Type 1 (*ATP8B1* Deficiency) |
| PFIC2 | Progressive Familial Intrahepatic Cholestasis Type 2 (BSEP Deficiency, *ABCB11*) |
| PFIC3 | Progressive Familial Intrahepatic Cholestasis Type 3 (MDR3 Deficiency, *ABCB4*) |
| PFIC5 | Progressive Familial Intrahepatic Cholestasis Type 5 (*NR1H4/FXR* Deficiency) |
| PFIC13 | Newly Proposed PFIC Subtype (*PSKH1*-related Hepato-Renal Ciliopathy) |
| PMID | PubMed Identifier |
| *PSKH1* | Protein Serine/Threonine Kinase H1 |
| RNA | Ribonucleic Acid |
| SNP | Single Nucleotide Polymorphism |

| | |
|---|---|
| *TJP2* | Tight Junction Protein 2 |
| UDCA | Ursodeoxycholic Acid |
| VUS | Variant of Uncertain Significance |
| WES | Whole-Exome Sequencing |
| γ-GT | Gamma-Glutamyl Transferase |

## 1. Introduction

Progressive Familial Intrahepatic Cholestasis (PFIC) encompasses a spectrum of rare genetic disorders leading to liver failure in childhood. Despite advances in genetic diagnostics, a significant fraction of cases remains unexplained. Large cohort studies, including Saudi pediatric populations, have demonstrated that even with comprehensive next-generation sequencing, a notable proportion of cholestatic liver disease cases remain without molecular diagnosis, underscoring the need for continued gene discovery. To better understand the pathomechanism of cholestatic liver disease in children, especially the pathophysiology of PFIC, a short overview is given on bile acid synthesis and metabolism and a summary of the genetic background of PFIC.

*Bile acid synthesis and metabolism*

The human liver synthesizes about 200–600 mg of bile acids per day and excretes the same amount in feces. The net turnover of bile acids per enterohepatic cycle is ≈ 5 % of a total bile acid pool of about 3g. Conversion of cholesterol to bile acids involves 17 distinct enzymes located in the cytosol, endoplasmic reticulum, mitochondria, and peroxisomes (1).

There are two major bile acid biosynthetic pathways: the neutral ("classic") and the acidic ("alternative") pathway. The acidic pathway contributes ≈ 9 % of total bile acid synthesis in humans but may be more important in neonates and in patients with liver disease (1). Two primary bile acids are synthesized in the liver cholic acid (CA) and chenodeoxycholic acid (CDCA). In humans, bile acids are conjugated with glycine or taurine in a ratio of about 3:1. If conjugated bile acids escape reuptake via the apical sodium-dependent bile acid transporter (ASBT, also called ileal bile acid transporter or IBAT) in the distal intestine, they enter the colon. Microbial bile-salt hydrolases deconjugate CA and CDCA, and bacterial 7α-dehydroxylase converts them to the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA). Unconjugated bile acids are hydrophobic and may be reabsorbed passively in the colon. Unconjugated LCA is hardly reabsorbed compared with DCA. A bile-acid pool of ≈ 3 g (≈ 40 % CA, 40 % CDCA, 20 %

DCA, traces LCA) is recycled 4–12 times per day. Bile acids lost in feces (≈ 0.5 g/day) are replenished by de novo hepatic synthesis to maintain pool size (1).

Small amounts of bile acids in portal blood may escape first-pass hepatic uptake and spill into systemic circulation. Bile acids in the systemic circulation may be taken up by the liver by secondary hepatic passage (either via the hepatic artery or the portal vein). Bile acids in the systemic circulation can also be secreted to some extent by the kidney into the primary urine, but subsequently they will be taken up again when passing through the renal tubules in the kidney, via renal expression of ASBT, and are then circulated back to the liver through systemic circulation. Under physiological conditions, accordingly, the urinary excretion of bile acids is very low and does not quantitatively contribute to whole body bile acid turnover. A minor fraction of bile acids secreted into bile is reabsorbed via ASBT in cholangiocytes and recycled to hepatocytes (the cholangiohepatic shunt).

*Enterohepatic circulation of bile acids*

The enterohepatic circulation maintains bile-acid homeostasis and fat absorption. Once secreted into bile, bile acids form micelles with phospholipids and cholesterol, stored in the gallbladder and released into the intestine upon cholecystokinin stimulation. About 95% of bile acids are reabsorbed in the terminal ileum via ASBT and return to the liver through the portal vein (1). Within the liver, bile acids undergo re-conjugation and are re-secreted into bile, completing the cycle. The process is tightly regulated by nuclear receptor feedback, particularly the farnesoid X receptor (FXR) (2).

*Role of Farnesoid X Receptor in PFIC pathophysiology and therapeutics*

FXR is a nuclear receptor expressed in the liver, intestine, and kidney that regulates bile-acid homeostasis. It suppresses bile-acid synthesis by down-regulating cholesterol 7α-hydroxylase (*CYP7A1*) through fibroblast growth factor 19 (*FGF19*) (2). In PFIC, dysregulated FXR signaling leads to hepatocellular injury and bile-acid accumulation (3). Impaired FXR activity reduces bile-acid transporter expression such as the bile-salt export

pump (BSEP, *ABCB11*), worsening cholestasis (4). FXR also influences lipid and glucose metabolism, intestinal barrier integrity, and inflammation (2). FXR agonists, such as obeticholic acid (OCA), have demonstrated efficacy in cholestatic diseases including primary biliary cholangitis and subsets of PFIC, promoting bile-acid efflux and reducing hepatic synthesis (5). Preclinical data suggest they also relieve pruritus by lowering circulating bile-acid concentrations (6). Despite their promise, inter-patient variability and long-term safety concerns remain (7).

*Pathophysiological implications in PFIC*

Disruption of bile-acid transport is central to PFIC. Mutations in key bile acid transporters, such as Bile Salt Export Pump (BSEP; *ABCB11*) and Multi Drug Resistance protein type 3 (MDR3; *ABCB4*), can cause primarily or secondarily impair bile acid flow, respectively, leading to their toxic accumulation in hepatocytes (8-10). For instance, PFIC type 2, caused by variants in *ABCB11*, results in deficient BSEP transport activity of bile acids into the bile, leading to severe cholestasis and an increased risk of hepatocellular carcinoma (9). MDR3 deficiency results in impaired biliary secretion of phospholipids into the bile, leaving the biliary bile acids unshielded and resulting in detergent bile acid damage of the biliary tree. Upon progressive damage of the biliary tree, the drainage of bile and of biliary bile acids will decrease, causing the secondary accumulation of bile acids in hepatocytes and in the systemic circulation (11). Dysregulation of hepatobiliary bile acid transport impacts other organs. Secondary bile acids like LCA can damage intestinal epithelia, and loss of bile-acid recycling causes malabsorption and fat-soluble vitamin deficiencies (A, D, E, K) (12).

*Therapeutic strategies targeting enterohepatic circulation*

Given its central role in PFIC, therapeutic strategies often aim to restore or modulate enterohepatic circulation. Ursodeoxycholic acid (UDCA), a hydrophilic bile acid, enhances

bile flow and reduces detergent toxicity, particularly in MDR3 deficiency (13), what subsequently protects cholangiocytes and the biliary tree from detergent bile acid injury. Partial external biliary diversion (PEBD) and ileal bile-acid transporter inhibitors interrupt recycling to lower serum bile-acid levels and relieve pruritus (7, 11, 14). However, therapeutic efficacy varies between PFIC subtypes, requiring genotype-specific approaches.

*Genetic etiologies of PFIC*

Neonatal cholestasis is potentially life-threatening and requires prompt diagnosis. Causes include biliary atresia, infections, and inherited disorders such as PFIC (15). The prognosis of neonatal cholestasis can depend on the age at which treatment or surgery is provided, such as performance of Kasai hepato-portoenterostomy in biliary atresia. If not rapidly recognized, neonatal cholestasis in early life can also cause life-threatening vitamin K deficiency bleedings, including intracranial hemorrhages (16). PFIC often presents during infancy and comprises hereditary diseases caused by mutations in specific genes. Advances in genetics have improved understanding of PFIC phenotypes and enabled tailored therapies. Homozygous truncating *ABCB11* mutations frequently cause severe phenotypes of PFIC2, that are resistant to surgical or medical interruption of the enterohepatic circulation (van Wessel et al, 2020).

PFIC was first described in 1969 and is classified by serum γ-glutamyl-transferase (GGT) activity. Most PFIC patients exhibited normal or near-normal GGT. Genetic variants underlying PFIC include *ATP8B1* (PFIC1 or FIC1 deficiency), *ABCB11* (PFIC2 or BSEP deficiency), and *ABCB4* (PFIC3 or MDR3 deficiency), each affecting the process of bile formation differently. *ABCB4* variants produce high-GGT cholestasis and related disorders such as intrahepatic cholestasis of pregnancy (17). PFIC2 is associated with increased hepatocellular carcinoma (HCC) risk in severe cases (18). PFIC is categorized into subtypes based on the affected genes (Table 1) (14, 19). The first three types of PFIC disease include:

- **FIC deficiency (PFIC1, *ATP8B1*):** Affects aminophospholipid translocation, leading to systemic manifestations including chronic diarrhea and failure to thrive.

- **BSEP deficiency (PFIC2, *ABCB11*)**: Impairs bile salt export, increasing risk of hepatocellular carcinoma and severe pruritus.
- **MDR3 deficiency (PFIC3, *ABCB4*)**: Involves defective phospholipid secretion, causing bile salt toxicity in cholangiocytes and potential gallstone formation.

Genetic variations within each PFIC subtype may not only determine disease severity but also influence response to therapeutic interventions, underscoring the importance of precise molecular diagnoses (8).

Patients carrying predicted protein truncating variants (loss of function) on both copies of the gene represent a particularly severe subset. These variants either lead to the production of non-functional proteins or result in a complete absence in BSEP protein. Patients harboring such variants exhibit the poorest clinical outcomes, with significantly reduced native liver survival (NLS) and an increased risk of hepatocellular carcinoma.

**Table 1.** Current classification of progressive familial intrahepatic cholestasis

| **Disease** | **Protein Function** | **Clinical Manifestations** | **Key Lab Abnormalities** |
|---|---|---|---|
| FIC1 Deficiency (PFIC1) | Maintains cell membrane lipid asymmetry (aminophospholipid flippase). | Severe pruritus (itching), watery diarrhea, short stature, hearing loss, pancreatitis. | Low/Normal GGT, high bile acids, high bilirubin. |
| BSEP Deficiency (PFIC2) | Main transporter for pumping bile salts out of the liver into the bile duct. | Early-onset severe pruritus, rapid progression to liver failure, high risk of liver cancer (HCC). | Low/Normal GGT, very high bile acids, high bilirubin. |
| MDR3 Deficiency (PFIC3) | Transports phospholipids (lecithin) into bile to protect bile ducts from bile acid damage. | Pruritus, gallstones, portal hypertension, cirrhosis later in childhood/adulthood. | High GGT, high bile acids, high bilirubin. |

| | | | |
|---|---|---|---|
| TJP2 Deficiency | Tight junction protein; maintains the seal between liver cells. | Rapidly progressive liver disease, severe cholestasis, risk of HCC. | Low/Normal GGT, high bile acids. |
| FXR Deficiency | Master nuclear receptor that regulates bile acid synthesis and transport. | Neonatal liver failure, severe coagulopathy, rapid progression. | Low/Normal GGT, high bile acids, low AFP. |
| SLC51A Deficiency | Transporter involved in the recycling of bile acids from the gut back to the liver. | Congenital diarrhea, malabsorption, cholestasis. | Low/Normal GGT, high bile acids. |
| USP53 Deficiency | Interacts with tight junction proteins to maintain biliary barrier. | Pruritus, hearing loss (sometimes), cholestasis. | Low/Normal GGT, high bile acids. |
| KIF12 / ZFYVE19 / MYO5B / VPS33B | Involved in intracellular trafficking and "parking" of transporters at the cell membrane. | Cholestasis, varying degrees of pruritus. VPS33B and MYO5B often involve multi-organ issues (renal, intestinal, or skin). | Low/Normal GGT , high bile acids, High GGT in KIF12 |
| SEMA7A / PSKH1 | Proteins involved in signaling and cell structure/maintenance. | Generally rare; characterized by neonatal cholestasis and potential fibrosis. | Variable GGT, elevated bile acids. |

Adapted from (20, 21). Abbreviations in the Table: FIC1, familial intrahepatic cholestasis protein 1; BSEP, bile salt export pump; MDR3, multidrug resistance protein 3; *TJP2*, tight junction protein 2; FXR, farnesoid X receptor; *SLC51A*, solute carrier family 51, subunit alpha; *USP53*, ubiquitin specific peptidase 53; *KIF12*, kinesin family member 12; *ZFYVE19*, zinc finger fyve domain-containing protein 19; *MYO5B*, myosin 5B; *SEMA7A*, semaphorin 7A; *VPS33B*, vacuolar protein sorting 33, yeast, homolog of, B; *ATP8B1*, ATPase phospholipid transporting 8B1; ABCB11, ATP binding cassette subfamily B member 11; *ABCB4*, ATP binding cassette subfamily B member 4; *NR1H4*, nuclear receptor subfamily 1 group H member 4.

## 2. Objectives

This thesis investigates the genetic landscape of Progressive Familial Intrahepatic Cholestasis with a focus on novel etiologies, leveraging a cohort from Saudi Arabia, where high consanguinity rates provide a unique opportunity to study recessive genetic disorders. Previous regional genomic studies have demonstrated that consanguineous populations, such as the Saudi population, are particularly informative for identifying novel autosomal-recessive liver diseases (22). The thesis also explores bile-acid metabolism with a focus on its role in enterohepatic circulation, synthesis, and the genetic factors influencing progressive familial intrahepatic cholestasis.

Our aims were:

1. Investigate the utility of next-generation sequencing in diagnosing advanced cholestatic liver diseases in a high-consanguinity population.
2. Identify and characterize novel genetic contributors to PFIC, with a focus on the discovery of *PSKH1* (Protein Serine Kinase H1) as a novel gene associated with hepato-renal ciliopathy.
3. Understanding the genotype-phenotype correlation in patients with loss of function mutation in BSEP deficiency

## 3. Methods

### Human Subjects

*Part 1. Genetic profiling of children with advanced cholestatic liver disease by using targeted gene panels*

Children (aged 0–18 years, n=98; 57 male, 41 female) with suspected cholestatic liver disease including those with elevated liver enzymes, bilirubin, or serum bile acids - who were referred to our center for evaluation between 2013 and 2016 were included. The mean age was 5.6 years (SD 4.8 years; median age 4.0 years, IQR 2.0-10.0 years), The following cutoff values were used: Alanine Aminotransferase (ALT) > 25 U/L, Aspartate Aminotransferase (AST) > 45 U/L, GGT > 32 U/L, total bilirubin > 5 μmol/L, serum bile acids > 10 μmol/L. Venous blood was collected in EDTA tubes from index cases and available family members. Exclusion criteria included identifiable metabolic or anatomical causes of liver disease. Ninety eight patients were recruited for this study.

*Part 2. Large Scale Genomic Investigation of Pediatric Cholestasis by exome sequencing*

All pediatric patients (aged 18 and younger, n=299 patients, from 279 families) diagnosed with cholestasis were included, referred to our center between March 2019 and March 2023. The mean age of onset of disease was 2.75 years (median age 0.7 years, IQR 0.06-3.6 years). Positive family history was noted in 199 (71.3%) and consanguinity was noted in 202 (72.4%). Case definition of cholestasis followed the guidelines of The European Association for the Study of the Liver (EASL) (20) and NAtural course and Prognosis of PFIC and Effect of biliary Diversion (NAPPED) consortium (23). A liver biopsy suggestive of extrahepatic biliary atresia was only used as an exclusion criterion if it was associated with high GGT and extrahepatic biliary atresia was confirmed by laparoscopic intraoperative cholangiogram.

A subset of twenty-five patients who were homozygous for (LOV) ABCB11 (c.379del; p.Thr127HisfsTer6) variant out of these 12 patients (48%) were male, age at first

presentation in referral centre was 3.1 (0.2-5.9) years. All patients came from consanguineous relationships.

*Biological samples collection (Blood and tissues), ethical approvals*

Human subjects are consented for biological sample collection and ethical approvals for patient studies and review of clinical cases were obtained (KFSHRC RAC #2121053, #2130029 and #2181266), with additional approval for variant functional studies on animal models from the Walter and Eliza Hall Institute (Project 23/41) in accordance with national ethics standards.

**Genetic methodologies**

*Targeted amplification and next-generation sequencing*

The design of the GI panel has been described elsewhere (24). Briefly, 189 genes were selected based on established links in the literature with Mendelian diseases that present with significant gastrointestinal phenotypes, including those linked to cholestatic liver disease. Multiplex amplification was performed using the AmpliSeq assay with proprietary primer design. Sequencing was performed on the Ion Proton platform following standard library preparation.

*Exome sequencing and variant curation*

Exomes were sequenced on an Illumina NovaSeq 6000 using 150-bp paired-end reads. Libraries were captured with the Illumina DNA Prep Nextera Enrichment Kit and processed via Illumina Dragen (v3) using the UCSC hg19 reference as described in (25).

*Autozygome analysis (positional mapping)*

Patients and relatives were genotyped on the Axiom SNP Array platform (Affymetrix). Genome-wide genotypes were analyzed for runs of homozygosity as surrogates of autozygosity[37]. Linkage analysis was performed using EasyLINKAGE, assuming a fully penetrant autosomal-recessive model.

*Variant prioritization and interpretation*

Variants were filtered within the candidate autozygome (26) and classified per ACMG guidelines (27). The known disease genes were prioritized first; novel candidate genes were investigated in negative cases. Known disease genes were pursued first and, where negative, novel candidate genes were pursued. Causal variants were considered likely when:

I. zygosity matched inheritance pattern (heterozygous for dominant and homozygous or compound heterozygous for recessive);
II. allele frequency was novel or extremely rare using public and in-house databases; and
III. predicted pathogenicity was high (truncating or missense with high PolyPhen, SIFT, and CADD scores). Variants were confirmed by Sanger sequencing.

**Animal model of *PSKH1***

Following identification of *PSKH1* variants as a likely cause of a novel form of PFIC (described in Results), we characterized a Pskh1 mutant mouse model to test liver-specific effects. Phenotypic assessments included histopathology, serum liver/kidney function, and ciliary morphology. The timeline of disease onset in mice was studied to correlate with human presentations.

***Production of Pskh1 Mutant (Pskh1$^{mpc232H}$) Mice***

N-ethyl-N-nitrosourea (ENU) treated C57BL/6J males are mated to C3H/HeH WT females to produce the first generation (G1) progeny (Figure 1, MRCP). G1 heterozygous (HET) male mice are mated to WT females, and the HET female progeny (G2) are then mated back to their G1 father. G3 mice are used to screen for recessive mutations that cause abnormal phenotypes. Frozen embryos of the third generation (G3) were imported to TCP from MRC Harwell. Embryos were thawed and transplanted to foster mothers (Figure 1, TCP). The HET mice of G3 were bred to C3H/HeJ WT mice to generate sufficient HET

mice for further breeding. Male and female homozygous (HOM) mice at 4-5 weeks of age (G5) were generated by breeding HET mice of G4 for further characterization of phenotypes. For a developmental study in embryos and postnatal mice, male and female HOM mice of G6 were generated by breeding HET mice of G5 and were used at embryonic day (E) 15.5, E18.5, postnatal day (PN) 4, PN7, and PN11.

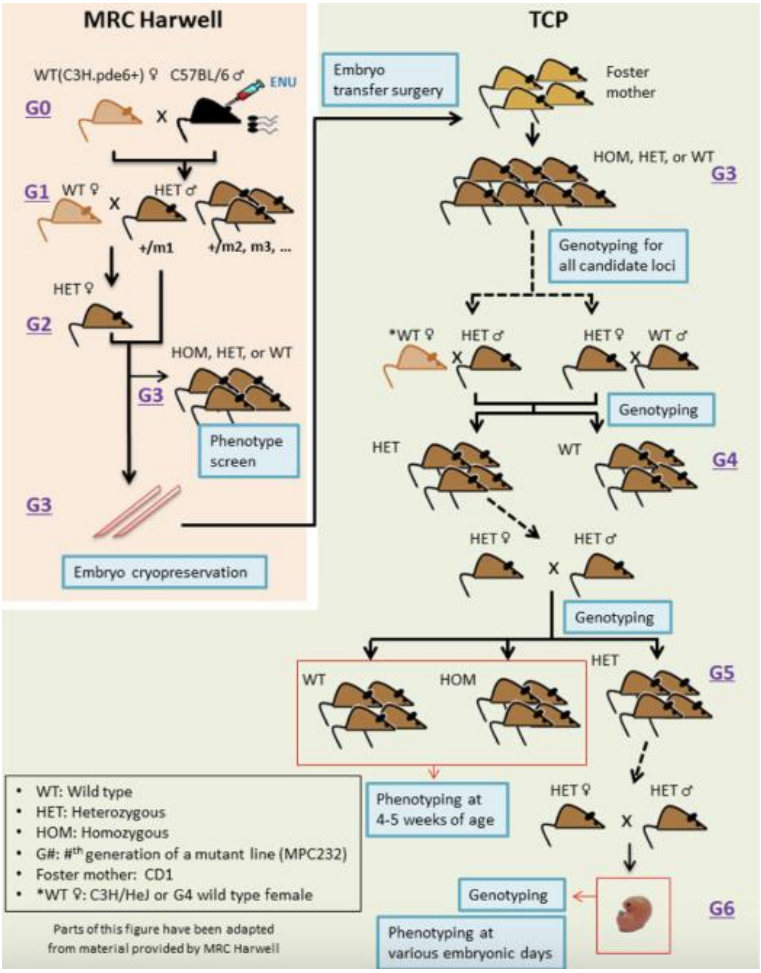



**Figure 1**. Breeding scheme for production of *Pskh1* mutant (***Pskh1***$^{mpc232H}$) mice at MRC Harwell (left panel) and TCP (right panel). Author's original artwork.

## 4. Results

Genetic Profiling and Diagnostic Yield

*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%) and *UGT1A1* (7 cases 12%). *TJP2* mutations (which have only been reported by (28), accounted for seven mutation-positive cases (Figure 2). Other genetic diagnosis made include PFIC1, Alagille syndrome, bile acid synthesis defect, tyrosinemia, Wilson disease, *ABCC2*-related mutation (Dubin–Johnson syndrome), *G6PC*-related glycogen storage disease, *SLC10A2*-related bile acid malabsorption, *MYO5B*-related microvillous inclusion disease and *VIPAS39*-related cholestasis, renal impairment and arthrogryposis syndrome. Nearly half of our mutation-positive patients (29, 50%) belonged to this group of autosomal recessive cholestatic disorders that present as intrahepatic cholestasis in children or early adulthood and often requiring liver transplant early in life. In our cohort, half of PFIC cases (16/29, 55%) had *ABCB11* mutations. MDR3 deficiency caused by *ABCB4* mutations accounted for most of the remaining PFIC cases (12/29, 41%). Interestingly, three patients with *ABCB4* variants had been referred to us with the diagnosis of Wilson disease.

*High disease burden of autosomal recessive cholestatic liver disease*

Consistent with our observation of other autosomal recessive diseases, most of the mutations were private young mutations that were rendered homozygous through the consanguinity loop (28, 68%) (22). However, the remaining 32% were found based on their detection in apparently unrelated individuals and the cumulative carrier frequency was 0.0115 (1 in 87). This translates into a minimum disease burden of cholestatic liver disease in Saudi Arabia of 1:7246, a very high estimate even when compared with countries with high burden of cholestatic liver disease in children like Japan (29).

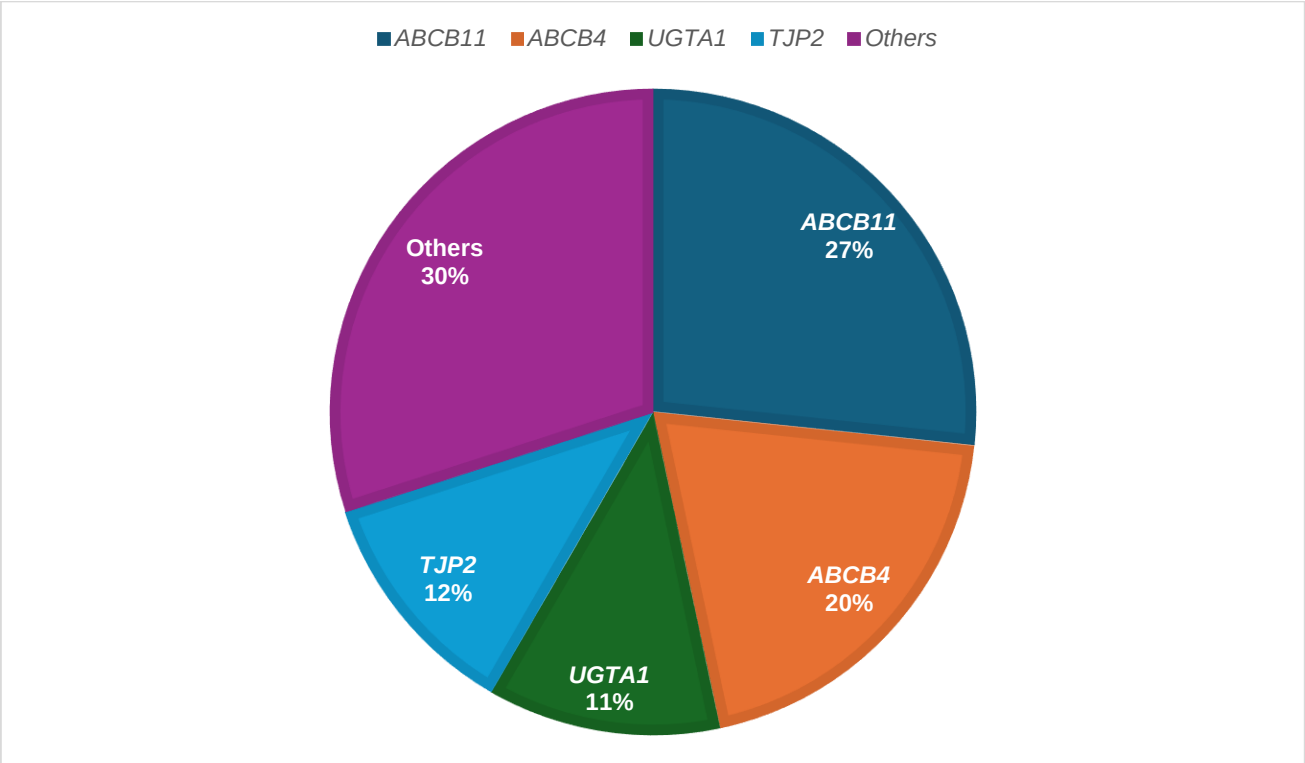



**Figure 2.** Relative contribution of individual genes to the overall mutation burden in the Part 1 cohort. The data includes *ABCB11* (ATP Binding Cassette Subfamily B Member 11) at 27%, *ABCB4* (ATP Binding Cassette Subfamily B Member 4) at 20%, *TJP2* (Tight Junction Protein 2) at 12%, and *UGTA1** (UDP Glucuronosyltransferase Family 1 Member A1) at 11%, with various other genes accounting for the remaining 30%.

*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 (Figure 3.). Of the 144 unsolved cases, 25 had variants of uncertain significance (VUS) across 18 genes including *ABCB4, CCDC115, CEP290, NR1H4, TJP2,* and *USP53* and were classified as inconclusive (15, 21).




**Figure 3.** Schematic presentation of the Part 2 / 2024 cohort and diagnostic outcome, with yield breakdown by inheritance, family history, and consanguinity. Author's original artwork.

The majority of the variants identified in our cohort are autosomal recessive (124 homozygous and 4 compound heterozygous). Dominant heterozygous variants (n = 7), all involving *JAG1* (HGNC:6188), were identified in 7 families (with no family history). The most frequently encountered genes were *ABCB4* (HGNC:45), *ABCB11* (HGNC:42), *ATP7B* (HGNC:870), *DCDC2* (HGNC:18141), and *KIF12*, which accounted for nearly half (54%, n

= 73) of the solved cases. In addition, we identified families with variants of uncertain significance (VUS) in 24 families spanning 18 genes (*ABCB4*, *AGL* [HGNC:321], *CARKD* [HGNC:25576], *CCDC115* [HGNC:28178], *CEP290* [HGNC:29021], *COG6* [HGNC:18621], *LSR, NEK8* [HGNC:13387], *NR1H4* [HGNC:7967], *NOTCH2* [HGNC:7882], *ONECUT1* [HGNC:8138], *PKD1* [HGNC:9008], *PKHD1* [HGNC:9016], *PTPRC* [HGNC:9666], *TALDO1* [HGNC:11559], *TRMU* [HGNC:25481], and *TJP2* [HGNC:11828], *USP53*) and these were considered inconclusive (Figure 4).

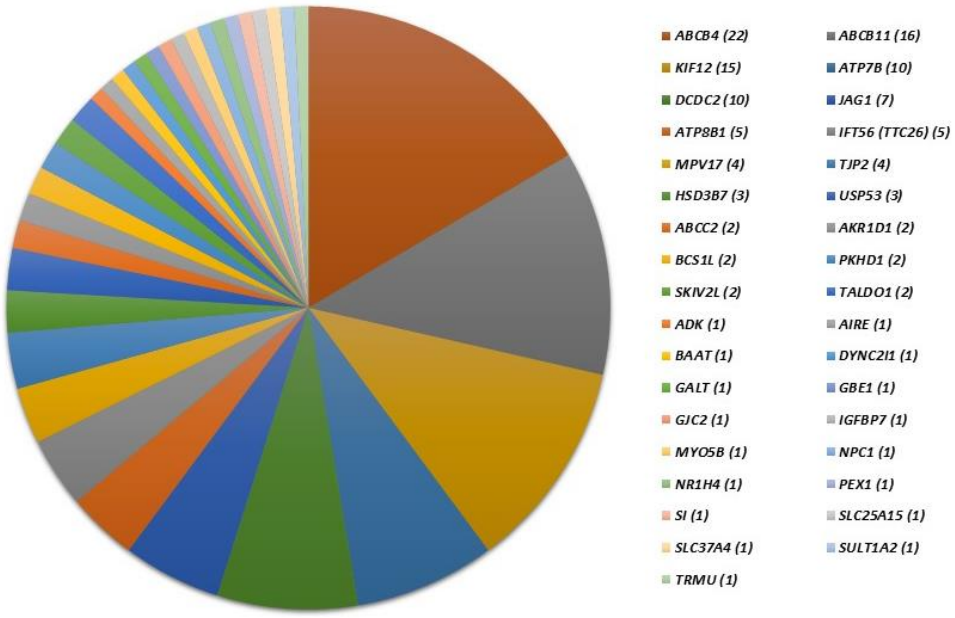



**Figure 4.** Diagnostic yield of exome sequencing in Part 2 (2024) cohort and breakdown of the number of families per gene reveals marked genetic heterogeneity and uneven distribution. *ABCB4* (ATP Binding Cassette Subfamily B Member 4), *ABCB11* (ATP Binding Cassette Subfamily B Member 11), *KIF12* (Kinesin Family Member 12), *ATP7B* (ATPase Copper Transporting Beta), *DCDC2* (Doublecortin Domain Containing 2), *JAG1* (Jagged Canonical Notch Ligand 1), *ATP8B1* (ATPase Phospholipid Transporting 8B1),

*IFT56/TTC26* (Intraflagellar Transport 56/Tetratricopeptide Repeat Domain 26), *MPV17* (Mitochondrial Inner Membrane Protein MPV17), *TJP2* (Tight Junction Protein 2), *HSD3B7* (Hydroxy-Delta-5-Steroid Dehydrogenase, 3 Beta- And Steroid Delta-Isomerase 7), *USP53* (Ubiquitin Specific Peptidase 53), *ABCC2* (ATP Binding Cassette Subfamily C Member 2), *AKR1D1* (Aldo-Keto Reductase Family 1 Member D1), *BCS1L* (BCS1 Homolog, Ubiquinol-Cytochrome C Reductase Complex Chaperone), *PKHD1* (PKHD1 Ciliary GDP-GTP Exchange Factor), *SKIV2L* (SKI2 Like RNA Helicase), *TALDO1* (Transaldolase 1), *ADK* (Adenosine Kinase), *AIRE* (Autoimmune Regulator), BAAT (Bile Acid-CoA:Amino Acid N-Acyltransferase), *DYNC2I1* (Dynein Cytoplasmic 2 Intermediate Chain 1), *GALT* (Galactose-1-Phosphate Uridylyltransferase), GBE1 (Glycogen Branching Enzyme 1), *GJC2* (Gap Junction Protein Gamma 2), *IGFBP7* (Insulin Like Growth Factor Binding Protein 7), *MYO5B* (Myosin VB), *NPC1* (NPC Intracellular Cholesterol Transporter 1), *NR1H4* (Nuclear Receptor Subfamily 1 Group H Member 4), *PEX1* (Peroxisomal Biogenesis Factor 1), *SI* (Sucrase-Isomaltase), *SLC25A15* (Solute Carrier Family 25 Member 15), *SLC37A4* (Solute Carrier Family 37 Member 4), *SULT1A2* (Sulfotransferase Family 1A Member 2), and *TRMU* (tRNA Methyltransferase 5-Methylaminomethyl-2-Thiouridylate Methyltransferase). Author's original artwork.

**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 (Table 2).

**Table 2.** Clinical features of the 8 patients with homozygous variants in *PSKH1*.

| Family ID | Family 1 | Family 2 | | | Family 3 | | Family 4 | |
|---|---|---|---|---|---|---|---|---|
| Case ID | 16DG0991 | 21DG0840 | 21DG0844 | 24DG0114 | 24DG0307 | 24DG0324 | 23DG1306 | 23DG1307 |
| Gene | *PSKH1* | *PSKH1* | *PSKH1* | *PSKH1* | *PSKH1* | *PSKH1* | *PSKH1* | *PSKH1* |
| Transcript (NM_006742.3) | c.361C>T | c.361C>T | c.361C>T | c.361C>T | c.376A>G | c.376A>G | c.547C>T | c.547C>T |
| Predicted Protein (NP_006733.1) | p.(Arg121Trp) | p.(Arg121Trp) | p.(Arg121Trp) | p.(Arg121Trp) | p.(Ile126Val) | p.(Ile126Val) | p.(Arg183Cys) | p.(Arg183Cys) |
| Chr position (hg19) (NC_000016.9) | g.67943013C>T | g.67943013C>T | g.67943013C>T | g.67943013C>T | g.67943028A>G | g.67943028A>G | g.67943199C>T | g.67943199C>T |
| Zygosity | Homozygous | Homozygous | Homozygous | Homozygous | Homozygous | Homozygous | Homozygous | Homozygous |
| Age (last visit) | 10 months | 23 months | 22 months | 7 months | 22 years | 38 years | 4.5 years | 2.5 years |
| Gender | Female | Female | Female | Female | Male | Female | Female | Male |
| Living Status | Deceased (10 month of age) | Alive | Alive | Alive | Alive | Alive | Alive | Alive |
| Consanguinity | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Family history | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Liver phenotype | | | | | | | | |
| Liver impairment | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Ultrasound | Normal in size and shape | Increased liver parenchymal echogenicity with irregular outlines compatible with liver cirrhosis with no focal lesion. Reversed flow in the main portal vein and right and left portal veins. Moderate amount of ascites. | Thrombosis of the main, right and left portal veins. Mild to moderate amount of ascites. | Mild to moderate diffuse-increased liver parenchymal and periportal triads echogenicity, mild hepatomegaly with no focal lesion. Small gallbladder volume with no sludge debris, wall thickening or biliary ducts dilations. | Hepatic cirrhosis and diffuse moderate hepatic steatosis. Sluggish portal venous flow. Marked splenomegaly. | Hepatic cirrhosis | Liver cirrhosis with hepatoportal flow in the portal vein and normal flow velocities. Increased liver stiffness (9.8 kPa). Enlarged liver and spleen. No focal lesions. | Progressive inhomogeneous liver parenchyma. Persistent periportal echogenicity. Normal liver size. Increased gracile aspect of the vena porta, probably still some hepatoportal flow. Collateral formation at the right liver point. umbilical vein not recanalized. Enlarged spleen. No ascites. |
| Biopsy | Mild focal bile duct damage and no evidence of biliary atresia, neonatal hepatitis or hemochromatasis | Severe cholestasis with severe bile duct dilatation and destruction | Biliary cirrhosis with cholestasis | NA | NA | NA | Biliary cirrhosis. Substantial ductular reaction, increased bile ducts. Around librosis feathery degeneration and canalicular cholestasis. CK7 accentuates the ductular profileration and ductular metaplasia. Periportal copper deposition. | Fibrosis and severe canalicular cholestasis with giant call transformation. The severe cholestasis is similar to sister. Increased connective tissue and hint of porto-portal connections. Some ductular reaction in some portal fields. |
| Liver transplant | No | Yes | Yes | Yes | No | Yes | No | Yes |
| Kidney phenotype | | | | | | | | |
| Ultrasound | Absent right kidny. Increase in echogenicity with mild hydronephrosis of left kidney. | Increase in echogenicity and diffuse scattered calcifications bilaterally | Moderate to large left perinephric hematoma. , small collection along the right kidney lower pole. | Mild to moderate increased bilateral renal parenchymal echogenicity with no focal lesions, stones or hydronephrosis. | Solitary right kidney | Solitary right kidney | Normal | Normal |

Detailed genetic analysis revealed two autozygous intervals exclusive to affected members of families 1 and 2 (Figure 5). Both shared an identical haplotype on chromosome 16 (ch16: 65.9-68.7 Mb, GRCh37) indicating a founder mutation, despite the apparent lack of relatedness between the 2 Saudi families.

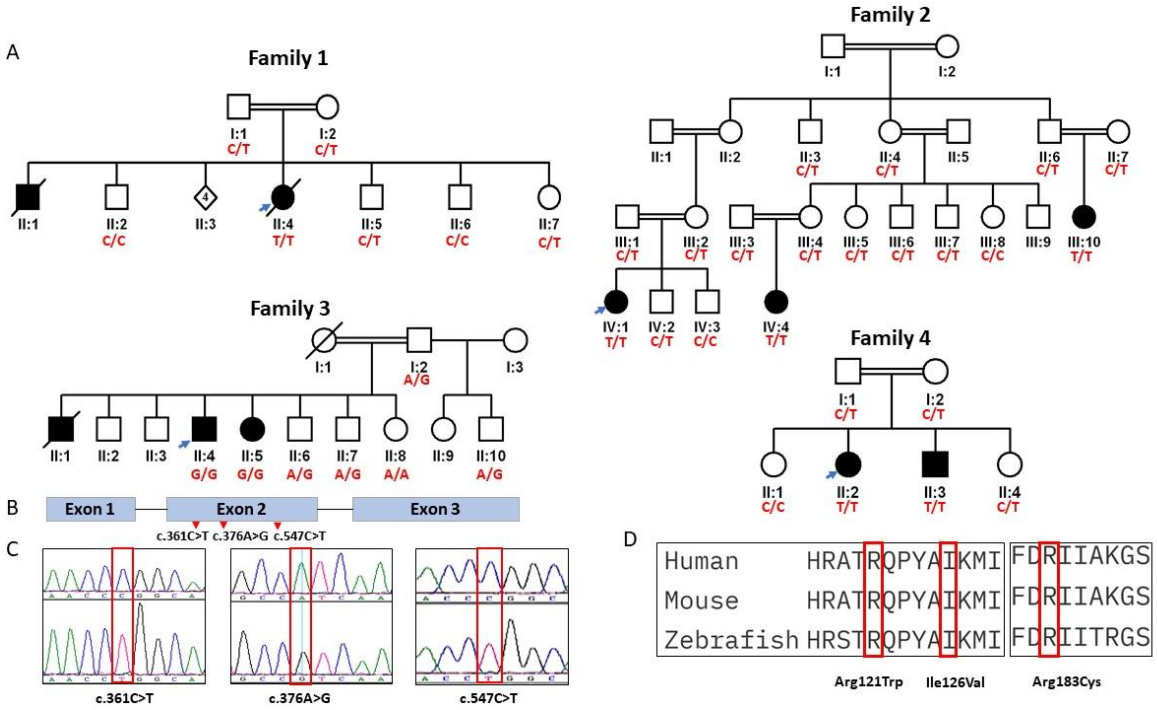



**Figure 5.** Identification of families with candidate *PSKH1* variants. A. Pedigrees of the families with homozygous PSKH1 variants and their genotypes. B. Cartoon of *PSKH1* and the identified variants. C. Familial segregation and sequence chromatograms of three identified variants. D. Conservation of p.(Arg121Trp), p.(Ile126Val) and p.(Arg183Cys) in human, mouse, and zebrafish. Author's original artwork.

Linkage analysis confirmed a significant LOD peak of 5.3 within this interval. Absence of known ciliopathy genes suggested a novel hepato-renal ciliopathy locus. By restricting analysis to novel homozygous coding/splicing variants in this locus, exome sequencing of families 1 and 2 identified a single founder variant in *PSKH1*

(NM_006742.2:c.361C>T, p.(Arg121Trp)). Independent exome analysis in family 3 found *PSKH1* p.(Ile126Val) as the most relevant variant. GeneMatcher facilitated identifying a fourth family carrying p.(Arg183Cys). All three variants were ultra-rare (minor allele frequency < $4\times10^{-6}$ in gnomAD v4.1.0) and predicted deleterious *in silico*. The Arg121, Ile126, and Arg183 residues in *PSKH1* are highly conserved from humans to zebrafish (Figure 5), consistent with the importance of these residues toward PSKH1 function. Importantly, the variants fully segregated with the disease in the 4 families (28 individuals including 8 affected individuals) under a fully penetrant autosomal recessive model.

**Phenotypic Analysis of PSKH1 Variant in Mouse Model**

Given the ciliopathy-like phenotype observed in these 4 families, we aimed to investigate a potential functional impact of a *PSKH1* variant using an animal model. An ENU-induced *Pskh1*$^{b2b1230Clo}$ variant (p.(Leu219Pro)) was previously reported to cause situs inversus totalis, a known ciliopathy feature (31). In addition, a large screen for mouse lines with late-onset phenotypes described another autosomal recessive ENU- induced Pskh1 mutant (*Pskh1*$^{mpc232H}$) with a nucleotide substitution (NM_173432:c.23T>A) that results in the amino acid substitution of aspartic acid for valine at position 8 (p.(Val8Asp)). The phenotype was “renal developmental abnormalities,” which may represent a renal ciliopathy (31).

We characterized the latter model: homozygous *Pskh1*$^{mpc232H}$ mice appeared lethargic at 3–4 weeks and exhibited hepatic and renal abnormalities. Histology showed periportal biliary hyperplasia, biliary cirrhosis, and glomerular sclerosis, mirroring human PSKH1-associated disease (Figure 6).

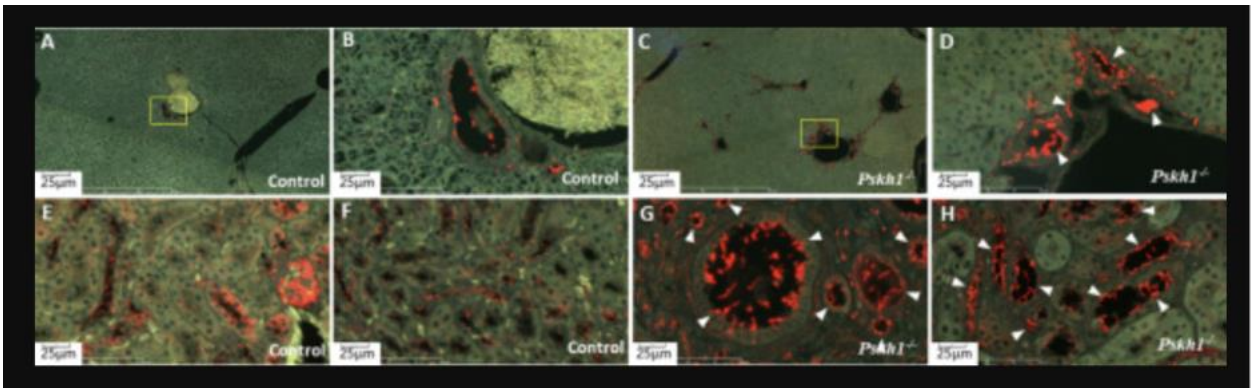



**Figure** 6. Histopathology demonstrating biliary and renal lesions in *Pskh1*$^{mpc232H}$ mice. Homozygous *Pskh1* variants are associated with abnormal cilia. Representative immunofluorescence for acetylated α-tubulin from homozygous *Pskh1*$^{mpc232H}$ mice and wild type controls. (A and B) Wildtype bile duct (10x and 63x, respectively); (C and D) Cilia (arrowheads) are longer, convoluted, and worm-like in bile duct of *Pskh1*$^{mpc232H}$ mice (10x and 63x, respectively); (E and F) Wildtype renal tubules in the cortex and medulla (63x and 63x, respectively); (G and H) Cilia (arrowheads) are longer, convoluted, and worm-like in renal tubules of the cortex and medulla of *Pskh1*$^{mpc232H}$ mice (63x and 63x, respectively). Author's original artwork.

Immunofluorescence for acetylated α-tubulin revealed elongated, worm-like cilia in bile ducts and renal tubules of mutant mice, consistent with ciliary dysregulation. Serum biochemistry confirmed elevated liver enzymes and renal parameters, reproducing patient phenotypes. In summary, homozygous *Pskh1mpc232H* mutant mice exhibited the core human disease features biliary cirrhosis, renal dysplasia, and abnormal ciliary morphology supporting *PSKH1* as a novel PFIC gene.

**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 (Table 3). 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.

**Table 3**. General and molecular characteristics of the BSEP truncated cohort.

| | |
|---|---|
| Year of birth, y<br><br>Available n (%) | 2018 (2010-2023)<br><br>25 (100) |
| Male n (%)<br><br>Available n (%) | 12 (48)<br><br>25 (100) |
| Consanguinity n (%) | 25 (100) |
| Pruritus n (%) | 15 (60) |
| Age first presentation in referral centre , y<br>Available n (%) | 3.05 (0.2-5.9)<br>25 (100) |
| Treated before or at first presentation in referral centre with:<br>UDCA, n (%) | <br><br>25 (100) |
| Laboratory data at presentation in referral centre<br><br>Serum bile acids, μmol/L<br>Available n (%)<br><br>Total serum bilirubin, μmol/L<br>Available n (%)<br><br>Alanine aminotransferase, IU/L | <br><br>425 (188-663)<br>24 (96)<br><br>151 (44-529)<br>25 (100)<br><br>389 (45-1060)<br>25 (100) |

| | |
|---|---|
| Available n (%)<br><br>Aspartate aminotransferase, IU/L<br>Available n (%)<br><br>Gamma glutamyl transferase, IU/L<br>Available n (%)<br><br>INR<br>Available n (%)<br><br>Albumin, g/L<br>Available n (%)<br><br><br>Alfa Fetoprotein , ug/L<br>Available n (%)<br>Fat soluble Vitamins ;<br>Vitamin D level, nmol/L<br>Available n (%)<br>Vitamin A level, ug/L<br>Available n (%)<br>Vitamin E level, mg/L<br>Available n (%) | <br>386 (99-1141)<br>25 (100)<br><br>23 (6-99)<br>25 (100)<br><br>1.1 (1-2.8)<br>25 (100<br><br>43 (30-49)<br>25 (100)<br><br><br>4586.5 (0.9-60500)<br>23 (92)<br><br>13 (8-82)<br>23 (92)<br>207 (95-404)<br>15 (60)<br>2.2 (2.2-10)<br>21 (84) |
| Hepatocellular carcinoma, n (%)<br>Available n (%) | 4 (16)<br>25 (100) |
| Required Liver Transplantation n (%) | 21 (84) |
| Living n (%)<br>Cadaveric n (%)<br>Domino n (%) | 13(61.9)<br>5 (23.8)<br>3 (14.3) |
| Age at the time of Liver Transplantation, y | 4 (0.9-13.5) |
| Clinically significant allo-antibody formation post Liver Transplantation n (%) | 2 (10) |
| Died after Liver Transplantation n (%)<br>Died before Liver Transplantation n (%) | 2 (8)<br>4 (16) |

## 5. Discussion

### The utility of next-generation sequencing in diagnosing advanced cholestatic liver diseases in a high-consanguinity population

In the present thesis, we aimed to determine the utility of next-generation sequencing in diagnosing advanced pediatric cholestatic liver diseases in a high-consanguinity population in Saudi Arabia. This aim and the subsequent studies have resulted into one of the most comprehensive genetic analyses of pediatric cholestatic liver disease to date, conducted in a high-consanguinity population. Using next-generation sequencing as a first-tier diagnostic approach, we achieved an overall diagnostic yield of 60%, which is comparable to large-scale studies conducted internationally.

Over the last decade, there have been major developments in genetic methodologies to investigate (potentially) genetic origins of rare diseases, such as pediatric cholestatic liver diseases. Initially, NGS was performed using targeted panels which covering known cholestasis-associated genes. As shown by our results in 2017, this approach provided valuable diagnostic insights: for example, a likely causal mutation was identified in 60 of the 98 patients studied. However, the methodology used was inherently limited by its gene selection bias.

By 2021–2022, the increasing accessibility and reduced cost of sequencing allowed to adopt whole-exome sequencing (WES) as the primary diagnostic tool. We subsequently tested the value of WES in another, larger cohort (Part 2 / 2024, 299 pediatric participants from 279 families), also characterized by very high parental consanguinity. Accordingly, almost all pathogenic variants identified were homozygous, with compound heterozygous and *de novo* variants being rare. Likely causal variants were detected in 135 families (48.6%), spanning 37 genes with established or provisional associations with cholestasis. This provided the most comprehensive genetic landscape of pediatric cholestasis in a high-consanguinity population to date. The diagnostic yield varied significantly by consanguinity

and family history, underscoring the impact of population structure on the identification of recessive liver disease genes (19, 21).

NGS provided high-resolution identification of causal variants across established PFIC genes, including *ABCB11, ABCB4,* and *ATP8B1*, which collectively accounted for nearly 45% of solved cases. This aligns with prior reports emphasizing the predominance of bile acid transporter defects in genetically defined cholestasis (32). The ability of NGS to resolve complex phenotypes such as overlap between PFIC, Alagille, and biliary-tract disorders demonstrates its essential role in clinical hepatology. For instance, *TJP2*-associated PFIC and *NR1H4*-related PFIC5 were identified as significant contributors in our cohort, consistent with previous global findings (29).

Furthermore, NGS facilitates early diagnosis, reducing the need for invasive biopsies and enabling genetic counseling and family screening. The cost-effectiveness of targeted gene panels and exome sequencing has been demonstrated in multiple cohorts, including Saudi Arabia (25, 33).

**Identification and characterization of novel genetic contributors to PFIC, with a focus on *PSKH1***

The expanded use of WES enabled systematic investigation for novel disease genes. One of the most significant outcomes was the identification of PSKH1 (Protein Serine Kinase of Unknown Function 1), a previously uncharacterized gene, as a cause of a distinct hepato-renal ciliopathy. In the Part 2 / 2024 cohort, we described four unrelated consanguineous families with early-onset combined liver and renal failure. Two families shared a founder homozygous variant in *PSKH1*, and another two families had distinct homozygous variants in *PSKH1*. Clinical phenotyping showed a consistent ciliopathy presentation, and PSKH1 expression was localized to cholangiocytes in the liver and to proximal tubular epithelial cells and collective duct cells in the kidney. Interestingly, the *PSKH1*-associated phenotype overlaps clinically with *TTC21B* and *CEP290* ciliopathies (34), suggesting shared pathways regulating ciliary trafficking. The founder variant *PSKH1* p.(Arg121Trp) found in multiple

families illustrates how consanguinity uncovers rare alleles that define new disease categories.

**Characterization of animal model to study disease mechanisms and therapeutic targets**

To validate causality and investigate disease mechanisms, we characterized in detail a mouse model for PSKH1 deficiency (31), the homozygous *Pskh1*$^{mpc232}$ mice, in collaboration with the Translational Medicine program at Toronto Hospital. The clinical pathogenic mechanism of PSKH1 deficiency was ciliopathy-related and the clinical phenotype found in the described patients could be reproduced in homozygous *Pskh1*$^{mpc232}$ mice failure (21). All histological sections of kidneys evaluated from the *Pskh1*$^{mpc232}$ mice showed renal dysplasia with moderate to severe hydronephrosis. In the periportal zones of the liver there was mild to severe small oval to amorphous cell hyperplasia with or without small caliber bile duct profiles (oval cell and biliary hyperplasia) and biliary cirrhosis. Functional validation demonstrated that PSKH1 encodes a serine kinase critical for ciliary regulation. Mutant *PSKH1* variants led to defective kinase activity and abnormally elongated primary cilia in patient fibroblasts and mouse models. Mechanistically, PSKH1 interacts with splicing-factor compartments and regulates RNA processing (35). Disruption of this pathway likely impairs epithelial polarity, ciliary transport, and bile-acid secretion, explaining the dual hepato-renal phenotype observed. Taken together, the mouse model showed early-onset hepatic and renal injury replicating the human phenotype, and histopathological confirmation of biliary and renal ciliopathy features. This work established *PSKH1*-related hepatorenal ciliopathy as a new monogenic form of pediatric cholestasis. It is also tempting to speculate that the homozygous *Pskh1*$^{mpc232}$ mouse model provides a preclinical platform for testing potential therapeutic interventions and for studying the natural history of *PSKH1*-related disease. The demonstration of catalytic loss in mutant PSKH1 suggests that small-molecule allosteric activators could be a potential therapeutic direction.

This bench-to-bedside translational loop—from genomic discovery to in vivo modeling and functional confirmation is a very clear presentation of the unique position of the Center in rare liver disease research in the Middle East.

Also, the addition of *PSKH1* to the diagnostic spectrum of pediatric cholestasis has immediate implications for genetic counseling and patient management. Routine inclusion of *PSKH1* in cholestasis gene panels could improve diagnostic yield, particularly in regions with high consanguinity.

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. In current practices, AFP is considered a prognostic marker. 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 levels (Table 3). Despite advances in medical therapy, genotype driven transplantation remains the most definitive and tailor-made therapy.

**Limitations and future research direction**

Our investigations have several limitations that warrant discussion. First, the selective nature of the cohort, derived predominantly from a single high-consanguinity population, may limit the generalizability of the findings to more genetically diverse populations. While this provided a unique opportunity to uncover recessive variants and founder effects, it could have excluded sporadic cases with different genetic underpinnings.

Another limitation is the incomplete functional characterization of some novel variants. Although PSKH1 was thoroughly investigated through cellular and animal models, other identified genes with variants of uncertain significance (VUS) require further study, for example through cell and animal models. This highlights the need for more comprehensive *in vitro* and *in vivo* analyses, including, if needed, integration of multi-omics (proteomics, metabolomics), to confirm pathogenicity and elucidate molecular mechanisms.

Technological constraints also should be acknowledged. The reliance on whole-exome sequencing meant that non-coding regions and structural variations, which may contribute to PFIC, were not assessed. Future studies incorporating whole-genome

sequencing (WGS) and advanced bioinformatic tools could provide a more complete picture of the genetic landscape.

From a therapeutic perspective, while this study identified potential targets for personalized medicine, the clinical implementation of these findings remains challenging. For instance, the utility of FXR agonists and early surgical interventions requires validation through larger, multicenter clinical trials. Additionally, the feasibility of translating animal model findings to human clinical settings is not always straightforward due to interspecies differences.

Although the present work has generated major new insights, there is still more that needs to be addressed in the future. Based on the experiences and findings of the presented work, I propose that research and insights on the following themes will further increase our understanding of these rare pediatric cholestatic diseases and provide more and better insights to further improve the prognosis for patients by personalizing and optimizing the treatments, either medical or surgical. I propose the following "next steps": in research on these diseases:

1. Expanding the (2024) cohort to include genetically diverse populations to validate the broader applicability of findings.
2. Integrating WGS and transcriptomic analyses to uncover regulatory and non-coding genetic contributors.
3. Conducting longitudinal studies to assess the natural history of PFIC subtypes and their response to novel interventions.
4. Exploring gene-editing technologies, such as CRISPR/Cas9, to correct pathogenic variants in preclinical models.
5. Collaborating internationally to pool data and resources for rare genetic conditions like PFIC.

## 6. Conclusions

Our comprehensive genetic analysis of pediatric patients with advanced cholestatic liver disease has highlighted the power of next-generation sequencing as a transformative diagnostic tool. By implementing NGS, we achieved a genetic diagnosis for a significant proportion of patients, identifying pathogenic variants in the majority of cases in 2024. This thesis presents the largest WES-based cohort study of pediatric cholestasis in a high-consanguinity population. The high rate of consanguinity in our cohort facilitated the identification of homozygous pathogenic variants, contributing to the prevalence of autosomal recessive cholestatic liver diseases, notably Progressive Familial Intrahepatic Cholestasis.

A key contribution of this study was the discovery of a novel genetic etiology linked to the *PSKH1* gene, found to cause a rare hepatorenal ciliopathy (21). The identification of this gene was supported by phenotypic similarities across three consanguineous families, evidence of abnormal ciliary structure and function in patient-derived cells, and corroborating findings in a genetic mouse model.

This work not only broadens the known genetic spectrum of cholestatic liver diseases but also establishes a framework for using genetic profiling to enhance clinical diagnosis and patient management. By integrating molecular insights into clinical practice, healthcare providers can better understand disease mechanisms, predict disease progression, and tailor treatment strategies. The potential for early genetic diagnosis, as demonstrated, can guide the timing and type of interventions, ultimately improving patient outcomes and quality of life.

Future research should focus on elucidating the precise mechanisms by which *PSKH1* variants lead to ciliopathy and exploring potential therapeutic approaches. Moreover, expanding genetic panel analyses to include newly identified candidates like *PSKH1* can enhance diagnostic accuracy for pediatric cholestatic liver disease, paving the way for personalized medicine strategies that benefit both diagnosis and treatment for pediatric liver disease.

## 7. Summary

Progressive Familial Intrahepatic Cholestasis (PFIC) represents a heterogeneous group of autosomal-recessive, monogenic liver disorders characterized by impaired bile flow, chronic cholestasis, and progressive hepatic injury (1). The condition manifests clinically with varying biochemical profiles either normal gamma-glutamyl transferase (γ-GT) cholestasis, typical of transporter defects such as BSEP or ATP8B1, or high-γ-GT cholestasis, usually associated with canalicular or ductal abnormalities involving MDR3 (*ABCB4*) or tight-junction proteins (17).

Due to major genetic and diagnostic advances, the molecular spectrum of PFIC continues to expand, what has underlined its biological complexity and regional genetic diversity (15, 22). This thesis explored the genetic basis of PFIC and related pediatric cholestatic diseases in a population characterized by high consanguinity, where homozygous variants are frequent (22).

Using large-scale genomic analyses combining whole-exome sequencing, autozygosity mapping, and functional studies one of the highest molecular diagnostic yields reported worldwide was achieved for pediatric cholestasis (19, 26, 33). The profiling identified a broad range of pathogenic variants across known PFIC genes, based on two different patient cohorts, revealing both severe and moderate allelic variants. Recurrent founder variants and extended runs of homozygosity were major contributors to the genetic burden in Saudi Arabia (22, 26).

Building on this foundation, this thesis identified and functionally characterized a novel hepato-renal ciliopathy caused by variants in *PSKH1*, now recognized as a new PFIC subtype (provisionally PFIC13). Functional analyses using patient-derived fibroblasts and a Pskh1 knockout mouse model showed that loss of *PSKH1* disrupts ciliary structure and signaling, linking bile-acid regulation to hepatocellular ciliary integrity (21, 31). These findings establish *PSKH1* as a key regulator of biliary and renal epithelial homeostasis and uncover a new pathophysiological mechanism in cholestatic liver disease.

Finally, this work underscores the impact of consanguinity on amplifying rare recessive variants and sets a foundation for regional genomic medicine initiatives. The findings advocate for genotype-based treatment strategies and reinforce the emerging paradigm that precision hepatology grounded in molecular discovery and functional validation can markedly improve outcomes for children with PFIC and related disorders (20, 23).

## 8. Bibliography


1. Chiang JY. Bile acid metabolism and signaling. *Compr Physiol.* 2013;3(3):1191-212.
2. Gomez-Ospina N, Potter CJ, Xiao R, Manickam K, Kim MS, Kim KH, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. *Nat Commun.* 2016;7:10713.
3. Kong B, Zhang M, Huang M, Rizzolo D, Armstrong LE, Schumacher JD, et al. FXR deficiency alters bile acid pool composition and exacerbates chronic alcohol induced liver injury. *Dig Liver Dis.* 2019;51(4):570-6.
4. Kullak-Ublick GA. ABC transporter regulation by bile acids: where PXR meets FXR. *J Hepatol.* 2003;39(4):628-30.
5. Kriegermeier A, and Green R. Pediatric Cholestatic Liver Disease: Review of Bile Acid Metabolism and Discussion of Current and Emerging Therapies. *Front Med (Lausanne).* 2020;7:149.
6. Samur S, Klebanoff M, Banken R, Pratt DS, Chapman R, Ollendorf DA, et al. Long-term clinical impact and cost-effectiveness of obeticholic acid for the treatment of primary biliary cholangitis. *Hepatology.* 2017;65(3):920-8.
7. Trauner M, Karpen SJ, and Dawson PA. Benefits and challenges to therapeutic targeting of bile acid circulation in cholestatic liver disease. *Hepatology.* 2025;82(4):855-76.
8. Deseive S, Straub R, Kupke M, Broersen A, Kitslaar PH, Stocker TJ, et al. Impact of diabetes on coronary artery plaque volume by coronary CT angiography and subsequent adverse cardiac events. *J Cardiovasc Comput Tomogr.* 2019;13(1):31-7.
9. van Wessel DBE, Thompson RJ, Gonzales E, Jankowska I, Sokal E, Grammatikopoulos T, et al. Genotype correlates with the natural history of severe bile salt export pump deficiency. *J Hepatol.* 2020;73(1):84-93.
10. Liu L, Zhang X, Liu Y, Wang L, and Li X. Simulated In Vitro Infant Gastrointestinal Digestion of Infant Formulas Containing Different Fat Sources and Human Milk: Differences in Lipid Profiling and Free Fatty Acid Release. *J Agric Food Chem.* 2021;69(24):6799-809.

11. Verkade HJ, Thompson RJ, Arnell H, Fischler B, Gillberg PG, Mattsson JP, et al. Systematic Review and Meta-analysis: Partial External Biliary Diversion in Progressive Familial Intrahepatic Cholestasis. *J Pediatr Gastroenterol Nutr.* 2020;71(2):176-83.
12. Monte MJ, Marin JJ, Antelo A, and Vazquez-Tato J. Bile acids: chemistry, physiology, and pathophysiology. *World J Gastroenterol.* 2009;15(7):804-16.
13. Gonzales E, Gardin A, Almes M, Darmellah-Remil A, Seguin H, Mussini C, et al. Outcomes of 38 patients with PFIC3: Impact of genotype and of response to ursodeoxycholic acid therapy. *JHEP Rep.* 2023;5(10):100844.
14. Mkarem LE, Batika MAH, and Bitar R. New hope in treating progressive familial intrahepatic cholestasis in children. *World J Hepatol.* 2025;17(7):108253.
15. Amendola M, and Squires JE. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, and Amemiya A eds. *GeneReviews((R))*. Seattle (WA); 1993.
16. Akiyama H, Okamura Y, Nagashima T, Yokoi A, Muraji T, and Uetani Y. Intracranial hemorrhage and vitamin K deficiency associated with biliary atresia: summary of 15 cases and review of the literature. *Pediatr Neurosurg.* 2006;42(6):362-7.
17. Mullenbach R, Linton KJ, Wiltshire S, Weerasekera N, Chambers J, Elias E, et al. ABCB4 gene sequence variation in women with intrahepatic cholestasis of pregnancy. *J Med Genet.* 2003;40(5):e70.
18. Morotti RA, Suchy FJ, and Magid MS. Progressive familial intrahepatic cholestasis (PFIC) type 1, 2, and 3: a review of the liver pathology findings. *Semin Liver Dis.* 2011;31(1):3-10.
19. Maddirevula S, Alhebbi H, Alqahtani A, Algoufi T, Alsaif HS, Ibrahim N, et al. Identification of novel loci for pediatric cholestatic liver disease defined by KIF12, PPM1F, USP53, LSR, and WDR83OS pathogenic variants. *Genet Med.* 2019;21(5):1164-72.
20. European Association for the Study of the L. EASL Clinical Practice Guidelines on genetic cholestatic liver diseases. *J Hepatol.* 2024;81(2):303-25.

21. Maddirevula S, Shagrani M, Ji AR, Horne CR, Young SN, Mather LJ, et al. Large-scale genomic investigation of pediatric cholestasis reveals a novel hepatorenal ciliopathy caused by PSKH1 mutations. *Genet Med.* 2024;26(11):101231.
22. Abouelhoda M, Sobahy T, El-Kalioby M, Patel N, Shamseldin H, Monies D, et al. Clinical genomics can facilitate countrywide estimation of autosomal recessive disease burden. *Genet Med.* 2016;18(12):1244-9.
23. van Wessel DBE, Gonzales E, Hansen BE, and Verkade HJ. Defining the natural history of rare genetic liver diseases: Lessons learned from the NAPPED initiative. *Eur J Med Genet.* 2021;64(7):104245.
24. Saudi Mendeliome G. Comprehensive gene panels provide advantages over clinical exome sequencing for Mendelian diseases. *Genome Biol.* 2015;16(1):134.
25. Monies D, Abouelhoda M, Assoum M, Moghrabi N, Rafiullah R, Almontashiri N, et al. Lessons Learned from Large-Scale, First-Tier Clinical Exome Sequencing in a Highly Consanguineous Population. *Am J Hum Genet.* 2019;104(6):1182-201.
26. Alkuraya FS. The application of next-generation sequencing in the autozygosity mapping of human recessive diseases. *Hum Genet.* 2013;132(11):1197-211.
27. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-24.
28. Sambrotta M, Strautnieks S, Papouli E, Rushton P, Clark BE, Parry DA, et al. Mutations in TJP2 cause progressive cholestatic liver disease. *Nat Genet.* 2014;46(4):326-8.
29. Togawa T, Sugiura T, Ito K, Endo T, Aoyama K, Ohashi K, et al. Molecular Genetic Dissection and Neonatal/Infantile Intrahepatic Cholestasis Using Targeted Next-Generation Sequencing. *J Pediatr.* 2016;171:171-7 e1-4.
30. Sobreira N, Schiettecatte F, Valle D, and Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat.* 2015;36(10):928-30.

31. Potter PK, Bowl MR, Jeyarajan P, Wisby L, Blease A, Goldsworthy ME, et al. Novel gene function revealed by mouse mutagenesis screens for models of age-related disease. *Nat Commun.* 2016;7:12444.
32. Vitale G, Sciveres M, Mandato C, d'Adamo AP, and Di Giorgio A. Genotypes and different clinical variants between children and adults in progressive familial intrahepatic cholestasis: a state-of-the-art review. *Orphanet J Rare Dis.* 2025;20(1):80.
33. Shagrani M, Burkholder J, Broering D, Abouelhoda M, Faquih T, El-Kalioby M, et al. Genetic profiling of children with advanced cholestatic liver disease. *Clin Genet.* 2017;92(1):52-61.
34. Stokman M, Lilien M, and Knoers N. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, and Amemiya A eds. *GeneReviews((R))*. Seattle (WA); 1993.
35. Brede G, Solheim J, and Prydz H. PSKH1, a novel splice factor compartment-associated serine kinase. *Nucleic Acids Res.* 2002;30(23):5301-9.

## 9. Bibliography of the candidate



Dr. Mohammad Shagrani is a Consultant in Pediatric Hepatology and Transplant Hepatology at the King Faisal Specialist Hospital and Research Centre (KFSHRC) in Riyadh, Saudi Arabia. He currently serves as the Section Head of Pediatric Hepatology and Gastroenterology Transplantation at KFSHRC, where he leads one of the most active pediatric liver transplant programs in the region. In addition, he is the Head of the Pediatric Hepatology Chapter under the Saudi Association for the Study of Liver Diseases and Transplantation (SASLT), and he holds an academic appointment as an Associate Professor at Alfaisal University College of Medicine in Riyadh.

Dr. Shagrani earned his Bachelor of Medicine and Bachelor of Surgery (MBBS) degree from King Saud University College of Medicine in Riyadh in 1999. He completed his Saudi Board in Pediatrics in 2004 and subsequently joined the Prince Sultan Medical Military City in Riyadh, where he became part of the Prince Sultan Pediatric Liver Transplant Unit—the first dedicated pediatric liver disease and transplantation program in Saudi Arabia.

In 2008, Dr. Shagrani pursued advanced fellowship training at McMaster University in Hamilton, Canada, where he completed a Canadian Fellowship in Pediatric Gastroenterology, Hepatology, and Nutrition. He further enriched his expertise through

specialized training at the Children's Hospital of Western Ontario in London, Canada, focusing on pediatric liver transplantation.

Over the past two decades, Dr. Shagrani has made significant contributions to the field of pediatric hepatology and liver transplantation, particularly in the study of genetic and familial liver diseases. His research has centered on elucidating the genetic basis of these disorders to advance personalized medicine. He established the Gastrointestinal and Liver Genetic Panel—the first comprehensive national initiative for studying genetic mutations related to liver diseases in Saudi Arabia (2014–2015). Through this and related collaborations, Dr. Shagrani has played a pivotal role in the discovery of several novel genes, including KIF12 and PSKH1, the latter defining Progressive Familial Intrahepatic Cholestasis type 13 (PFIC13)—a condition now recognized internationally and sometimes referred to as "MShagrani syndrome."

Dr. Shagrani also pioneered the ABO-Incompatible Pediatric Liver Transplantation Program at KFSHRC, expanding the donor pool and improving access to transplantation for children with end-stage liver disease. He served as the Principal Investigator (PI) for the first international clinical trial in Progressive Familial Intrahepatic Cholestasis (PFIC), which culminated in the approval of the first targeted therapy for these disorders—Ileal Bile Acid Transport (IBAT) inhibitors, such as Odevixibat, now approved for use in PFIC and Alagille syndrome.

In addition, Dr. Shagrani co-founded the NAPPED Consortium, an international research network dedicated to the multidisciplinary study of PFIC and related cholestatic liver disorders. His expertise extends to metabolic liver transplantation, particularly in the management of urea cycle disorders and organic acidemias, for which his center at KFSHRC is recognized as one of the largest referral centers worldwide.

Dr. Shagrani's scholarly work has been published in leading international journals, including the Journal of Hepatology, Hepatology, Clinical Genetics, and The Lancet Gastroenterology & Hepatology. He is widely regarded as a regional and international authority and an invited

speaker at numerous scientific conferences, where he lectures on genetic cholestatic liver diseases, metabolic transplantation, and pediatric hepatology innovations.

Beyond his professional achievements, Dr. Shagrani is married and the proud father of five children four daughters and one son. He resides with his family in Riyadh, Saudi Arabia.

**Publications related to thesis:**

1. Shagrani M, Burkholder J, Broering D, Abouelhoda M, Faquih T, El-Kalioby M, et al. Genetic profiling of children with advanced cholestatic liver disease. Clin Genet. 2017;92(1):52-61.

2. Maddirevula S#, Shagrani M#, Ji AR#, Horne CR, Young SN, Mather LJ, et al. Large-scale genomic investigation of pediatric cholestasis reveals a novel hepatorenal ciliopathy caused by PSKH1 mutations. Genet Med. 2024;26(11):101231. (#co-first authors)

3. Shagrani M#, Maddirevula S#, Kumar K, Malagó M, Thompson RJ, Dezsőfi-Gottl A, Broering D. Preemptive Liver Transplantation is essential for patients with severe BSEP deficiency. Liver Transplantation ():10.1097/LVT.0000000000000860, March 24, 2026. (#co-first authors)

**Publications**

1. Al-Ashwal A, Al-Mansour S, Al-Shagrani M, Broering D, and Alhuthil R. The effectiveness of liver transplantation in reducing lipid levels in Saudi children with homozygous familial hypercholesterolemia. Front Cardiovasc Med. 2024;11:1454638.
2. Vogel GF, Mozer-Glassberg Y, Landau YE, Schlieben LD, Prokisch H, Feichtinger RG, et al. Genotypic and phenotypic spectrum of infantile liver failure due to pathogenic TRMU variants. Genet Med. 2023;25(6):100314.
3. Vogel GF, Mozer-Glassberg Y, Landau YE, Schlieben LD, Prokisch H, Feichtinger RG, et al. Genotypic and phenotypic spectrum of infantile liver failure due to pathogenic TRMU variants. Genet Med. 2023;25(6):100828.

4. Stindt J, Dröge C, Lainka E, Kathemann S, Pfister ED, Baumann U, et al. Cell-based BSEP trans-inhibition: A novel, non-invasive test for diagnosis of antibody-induced BSEP deficiency. JHEP Rep. 2023;5(7):100690.
5. Monies D, Goljan E, Assoum M, Albreacan M, Binhumaid F, Subhani S, et al. The clinical utility of rapid exome sequencing in a consanguineous population. Genome Med. 2023;15(1):44.
6. Felzen A, van Wessel DBE, Gonzales E, Thompson RJ, Jankowska I, Shneider BL, et al. Genotype-phenotype relationships of truncating mutations, p.E297G and p.D482G in bile salt export pump deficiency. JHEP Rep. 2023;5(2):100626.
7. De Franco E, Owens NDL, Montaser H, Wakeling MN, Saarimäki-Vire J, Triantou A, et al. Primate-specific ZNF808 is essential for pancreatic development in humans. Nat Genet. 2023;55(12):2075-81.
8. Thompson RJ, Arnell H, Artan R, Baumann U, Calvo PL, Czubkowski P, et al. Odevixibat treatment in progressive familial intrahepatic cholestasis: a randomised, placebo-controlled, phase 3 trial. Lancet Gastroenterol Hepatol. 2022;7(9):830-42.
9. Shamseldin HE, Shaheen R, Ewida N, Bubshait DK, Alkuraya H, Almardawi E, et al. The morbid genome of ciliopathies: an update. Genet Med. 2022;24(4):966.
10. Shagrani M, Kumar K, Baker A, Al-Awwami M, Alhussaini H, Almanea H, et al. ABO-incompatible Pediatric Liver Transplantation With Antibody and B-cell Depletion-free Immunosuppressive Protocol in High Consanguinity Communities. Transplant Direct. 2022;8(12):e1353.
11. Schulze M, Zidan A, Sturdevant M, Aljudaibi S, Shagrani M, Bzeizi K, et al. First Experience in Living Liver Donation From Donors With Sickle Cell Trait. Transplant Direct. 2022;8(6):e1332.
12. Alshehri DB, Sindi HH, AlMusalami IM, Rozi IH, Shagrani M, Kamal NM, et al. Saudi Experts Consensus on Diagnosis and Management of Pediatric Functional Constipation. Pediatr Gastroenterol Hepatol Nutr. 2022;25(3):163-79.
13. Zidan A, Aljudaibi S, Wali N, Sturdevant M, Shagrani M, Algoufi T, et al. Feasibility of Full-Right/Full-Left Split-Liver Transplant in Pediatric Deceased Donors for Pediatric Recipients. Exp Clin Transplant. 2021;19(3):273-5.

14. Zakaria HM, Alobthani S, Elsarawy A, Saleh Y, Zidan A, Alabbad S, et al. Large for size in pediatrics liver transplant using left lateral segment grafts: A single center experience. Pediatr Transplant. 2021;25(6):e14044.
15. van Wessel DBE, Thompson RJ, Gonzales E, Jankowska I, Shneider BL, Sokal E, et al. Impact of Genotype, Serum Bile Acids, and Surgical Biliary Diversion on Native Liver Survival in FIC1 Deficiency. Hepatology. 2021;74(2):892-906.
16. Troisi RI, Elsheikh Y, Alnemary Y, Zidan A, Sturdevant M, Alabbad S, et al. Safety and Feasibility Report of Robotic-assisted Left Lateral Sectionectomy for Pediatric Living Donor Liver Transplantation: A Comparative Analysis of Learning Curves and Mastery Achieved With the Laparoscopic Approach. Transplantation. 2021;105(5):1044-51.
17. Abaalkhail FA, Al Sebayel MI, Shagrani MA, O'Hali WA, Almasri NM, Alalwan AA, et al. Clinical Practice Guidelines for Liver Transplantation in Saudi Arabia. Saudi Med J. 2021;42(9):927-68.
18. van Wessel DBE, Thompson RJ, Gonzales E, Jankowska I, Sokal E, Grammatikopoulos T, et al. Genotype correlates with the natural history of severe bile salt export pump deficiency. J Hepatol. 2020;73(1):84-93.
19. Shamseldin HE, Shaheen R, Ewida N, Bubshait DK, Alkuraya H, Almardawi E, et al. The morbid genome of ciliopathies: an update. Genet Med. 2020;22(6):1051-60.
20. Shaheen R, Alsahli S, Ewida N, Alzahrani F, Shamseldin HE, Patel N, et al. Biallelic Mutations in Tetratricopeptide Repeat Domain 26 (Intraflagellar Transport 56) Cause Severe Biliary Ciliopathy in Humans. Hepatology. 2020;71(6):2067-79.
21. Maddirevula S, Shamseldin HE, Sirr A, AlAbdi L, Lo RS, Ewida N, et al. Exploiting the Autozygome to Support Previously Published Mendelian Gene-Disease Associations: An Update. Front Genet. 2020;11:580484.
22. Al-Judaibi B, Almaghrabi R, Alghamdi M, Al-Hamoudi WK, AlQahtani M, Abaalkhail F, et al. Saudi association for the study of liver diseases and transplantation position statement on liver transplantation during the COVID-19 pandemic. Saudi J Gastroenterol. 2020;26(5):233-9.

23. Kumar K, Almanea H, Broering D, and Shagrani M. Early Hepatocellular Carcinoma Associated With Fibrocystic Liver Disease in a 10-Year-Old Child: A Case Report. Transplant Proc. 2019;51(9):3147-9.
24. Brockmann JG, Broering DC, Raza SM, Rasheed W, Hashmi SK, Chaudhri N, et al. Solid organ transplantation following allogeneic haematopoietic cell transplantation: experience from a referral organ transplantation center and systematic review of literature. Bone Marrow Transplant. 2019;54(2):190-203.
25. Broering DC, Elsheikh Y, Shagrani M, Abaalkhail F, and Troisi RI. Pure Laparoscopic Living Donor Left Lateral Sectionectomy in Pediatric Transplantation: A Propensity Score Analysis on 220 Consecutive Patients. Liver Transpl. 2018;24(8):1019-30.
27.
26. Monies D, Abouelhoda M, AlSayed M, Alhassnan Z, Alotaibi M, Kayyali H, et al. The landscape of genetic diseases in Saudi Arabia based on the first 1000 diagnostic panels and exomes. Hum Genet. 2017;136(8):921-39.
27. Saudi Mendeliome G. Comprehensive gene panels provide advantages over clinical exome sequencing for Mendelian diseases. *Genome Biol.* 2015;16(1):134.
28. Troisi RI, Elsheikh YM, Shagrani MA, and Broering D. First fully laparoscopic donor hepatectomy for pediatric liver transplantation using the indocyanine green near-infrared fluorescence imaging in the Middle East: a case report. Ann Saudi Med. 2014;34(4):354-7.
29. Ouahed J, Shagrani M, and Sant'Anna A. Role of wireless capsule endoscopy in reclassifying inflammatory bowel disease in children. J Pediatr (Rio J). 2013;89(2):204-9.
30. Fayyad A, Shagrani M, AlGoufi T, ElSheikh Y, Murray J, Elgohary A, et al. Progress and outcomes of the first high-volume pediatric liver transplantation program in Saudi Arabia. Clin Transpl. 2013:77-83.

**Selected talks**

1. New Update of Acute liver failure in children. SPAC 2012, Saudi Pediatric Association, 4th conference King Fahad Cultural Centre, Riyadh - Saudi Arabia, April 23-25, 2012.
2. Pattern of Cholestasis in Saudi Children. Pediatric Transplant Program - King Fahad Specialty Hospital Dammam - Saudi Arabia, November 2012.
3. PFIC what is new? Saudi International Congress on New Frontiers Organ Transplantation (SICOT), March 2013, Riyadh - Saudi Arabia.
4. Acute Liver Failure in Children: New Update. Saudi International Congress on New Frontiers Organ Transplantation (SICOT), Riyadh - Saudi Arabia, March 4-7, 2013.
5. PFIC what we need to know. Maternity Children Hospital, Dammam - Saudi Arabia, May2014.
6. Use of Rituximab in Antibody mediated rejection status post ABO-I liver transplantation. Pediatric Hepatology Summer Course, ESPGHAN (European Society of Pediatric Gastroenterology, Hepatology & Nutrition), Italy, May 2014.
7. ABO-I Liver Transplantation in Children – Single center Experience. MESOT (Middle East Society of Organ Transplantation), Istanbul - Turkey, Sept 2014.
8. Progressive Familial Intrahepatic Cholestasis, KSA Experience. Abu Dhabi 1st International Conference in Pediatric Gastroenterology, Hepatology and Nutrition. Invited speaker by King's College Hospital Clinics, Abu Dhabi - UAE, Feb. 2015.
9. Progress and Outcome of the first high volume Pediatric Liver Transplantation Program in Saudi Arabia. IPTA (International Pediatric Transplantation Association), San Francisco, USA, March 2015.
10. State of The Art Lecture (Progress and Outcome of the first High Volume Pediatric Liver Transplant in Saudi Arabia). Invited Speaker, 4th Elite Pediatric GIT Congress, Dubai, May 2015.
11. ABO-I Pediatric Liver Transplantation Saudi Experience what is the difference? (Invited Speaker). 4th Elite Pediatric GIT Congress, Dubai, May 2015.
12. Role of Genetic Testing to personalized treatment for Progressive Familial Intrahepatic Cholestasis (Invited Speaker). International Personalized Medicine Conference from The Genome to the precision Medicine (KFSHRC Vision 2020), Riyadh - Saudi Arabia, May 2015.

13. ABO- Incompatible liver transplantation, Saudi Experience. ESOT (European Society for Organ Transplantation), Brussels - Belgium, Sept 2015.
14. Outcome of Liver transplantation for urea cycle Disorders, KFSHRC Experience (Invited Speaker). International conference of Advances in Medical Genetics - King Saud Bin Abdulaziz University for Health Sciences, Riyadh – Saudi, May 2016.
15. Antibody and B-Cell depletion free ABO-Incompatible Pediatric Liver Transplantation (Invited Speaker). International Symposium on Histocompatibility Transplantation, Riyadh - Saudi Arabia, October 2016.
16. Genetic profiling of advanced familial Liver disease. ESPGHAN - First International PFIC conference, Budapest – Hungary, June 2017.
17. Genetic profiling of advanced familial Liver Disease - Experience of High-Volume Centre. ESPGHAN, Prague, 2017.
18. Invited chairperson for the Pediatric hepatology Innovative session in ESPGHAN (European Society of Pediatric Gastroenterology, Hepatology & Nutrition). Geneva – Switzerland, June 2018.
19. Invited Speaker to present OTC - KFSHRC in EASL (European Society to study liver disease) as the chosen Centre with worldwide experience in Genetic of Familial Liver diseases. Vienna - Austria, April 2019.
20. Invited speaker: (Two talks) a-High Transaminases post liver transplantation & indications of liver transplantation. International Pediatric Liver Disease Symposium, 22-23 January 2022, King Fahad Medical City, Riyadh - Saudi Arabia.
21. Liver Transplantation for Urea Cycle Disorders and Organic Acidemia. ELTS - Egyptian Liver Transplantation Society, Cairo - Egypt, 2022.
22. Invited Speaker: What After Pediatric Liver Transplantation. Kuwait Pediatric Conference, Kuwait, 2022.
23. TJP2 single center experience. ESPGHAN, Vienna, Austria, 2023.
24. Invited Speaker: PFIC Genotype-Phenotype, Era of Target Gene Therapy Medical & Surgical. The BEST OF AASLD & SASLD (The American Association for The study of Liver Disease & The Saudi Association for the Study of Liver Disease and Transplantation), Riyadh - Saudi Arabia, 2023.

25. Invited Speaker: Liver Transplantation for Organic Acidemia, KFSHRC Experience. ESPGHAN, Helsinki, Finland, 2025.
26. Invited Speaker: Variant in MDR3 & KIF12: The Natural History of Cholangiopathies. EASL Congress (European Association for the study of the Liver), Amsterdam, Netherlands, 2025
27. Invited Speaker: Integrating Genomics into the clinical Management of Cholestatic Liver Disease. IPSEN Cholestatic Academy MENA meeting, Dubai, 2025.
28. Invited Speaker: Management of PFIC Subtypes - Adopting a personalized Approach. Dubai, 2025.

## 10. Acknowledgements

I dedicate this work to my beloved parents. To my mother, for her constant support, care and sacrifices throughout my life and especially during my medical college, without her, this would not have been possible. To the soul of my dear father, your memory continues to inspire me. I remain forever grateful for all you have given to me and our family.

This work would not have been possible without the unwavering love, patience, and faith of my soulmate and wife, Mona, who has stood beside me since our marriage on August 20, 2003. Her endless support, sacrifices, and belief in me have been the foundation of every success I have achieved. To my beloved children Leena, Deema, Reema, Yasmeen , and Khaled you are my greatest inspiration and the light that gives meaning to every challenge I face. I owe everything to my family, whose love and encouragement have carried me through every difficult moment.

I extend my deepest gratitude to my incredible team at King Faisal Specialist Hospital and Research Centre, whose dedication and commitment have made our mission possible. In particular, I would like to thank Dr. Kishwer Kumar and Dr. Sateesh Maddirevula, whose trust, hard work, and companionship have made every obstacle surmountable. Working with such devoted colleagues has been both a privilege and a source of constant motivation. I would like to express my sincere gratitude to my colleague and friend, Dr. Fowzan S Alkuraya, for his steadfast support and collaboration. I also extend my deep appreciation to our collaborators at the Translational Medicine Research Program, The Hospital for Sick Children, Toronto, ON, Canada, and The Centre for Phenogenomics, Toronto, ON, Canada.

My heartfelt appreciation goes to my mentors, who shaped my professional journey. To Professor Martin Burdelski who inspired me to enter this field and taught me the true meaning of passion and purpose your mentorship transformed my life and set me on this path. To Professor Broering, a world-renowned transplant surgeon and a true visionary, your leadership, excellence, and the world-class foundation you built at King Faisal

Hospital made it possible for us to serve children with liver disease with hope, innovation, and compassion. To my dear friend and mentor, Professor Henkjan Verkade, your wisdom, humility, and unending support over the years have been like those of an elder brother guiding me, challenging me, and opening doors to the wider world of academic discovery.

Finally, my profound gratitude goes to Dr. Antal Dezsőfi-Gottl, whose patience, understanding, and steadfast encouragement sustained me through the most demanding times. Amid the overwhelming clinical responsibilities of managing nearly one hundred pediatric liver transplants a year with only a handful of colleagues, your belief in me never wavered. When I was close to giving up, you refused to let me quit, you pushed me to the finish line with kindness and determination. Without your support, this work would never have reached completion.

To all who have walked beside me on this journey my family, my team, my mentors, and my friends this achievement belongs as much to you as it does to me. From the depths of my heart, thank you.