Diploma thesis

Hereditary Cholestasis Syndromes and Their Clinical Appearance – Review of the literature and cases at the Medical University Graz

Submitted by

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Graz, October 30, 2025

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Zusammenfassung

Hintergrund

Hereditäre Cholestase-Syndrome sind eine heterogene Gruppe seltener Lebererkrankungen, die durch genetische Defekte in Genen verursacht werden, die mit dem Transport oder Stoffwechsel von Gallensäuren in Zusammenhang stehen. Obwohl sie selten auftreten, sind diese Erkrankungen klinisch bedeutsam, da betroffene Patient*innen schwere klinische Symptome wie Gelbsucht und Juckreiz sowie weitere Komplikationen wie chronische Lebererkrankungen aufweisen können, die möglicherweise eine Lebertransplantation als ultimative Therapie erfordern. Cholestatischer Juckreiz kann die Lebensqualität erheblich beeinträchtigen. Da nun neue Behandlungsmethoden wie ASBT-Hemmer zur Verfügung stehen, ist es wichtig zu wissen, wie häufig diese Erkrankung auftritt, um zu verstehen, wie viele Patient*innen von diesen neuen Optionen profitieren könnten. Innerhalb dieser breiten Gruppe von Syndromen kann das klinische Erscheinungsbild je nach Art der genetischen Mutation, Altersgruppe und auslösenden Ereignissen wie Infektionen, Schwangerschaft oder Medikamenteneinnahme variieren, die möglicherweise die Manifestation von Symptomen bei Patient*innen mit einer genetischen Veranlagung auslösen.

Methoden

Wir haben retrospektiv Patient*innen mit Verdacht auf eine erbliche Ursache für Cholestase analysiert, die in den letzten 20 Jahren in unserer Universitätsklinik genetisch getestet wurden. Klinische Daten, Laborbefunde und genetische Varianten (z. B. Mutationen, SNPs) wurden überprüft, um Genotyp-Phänotyp-Korrelationen, das klinische Erscheinungsbild nach Alter und potenzielle Auslöser der Erkrankung zu bewerten.

Ergebnisse

Aus den Krankenakten identifizierten wir 88 Patient*innen, 64 Kinder und 24 Erwachsene, mit einer möglichen erblichen Ursache für Cholestase. Nur 53 Patient*innen wiesen einen eindeutigen cholestatischen Phänotyp auf (wie PFIC, BRIC, ICP oder LPAC). Die übrigen 35 Patient*innen hatten Anzeichen für Cholestase oder Mutationen, jedoch ohne endgültige klinische Klassifizierung zum Zeitpunkt der Untersuchung. Bei 50 von 88 Patient*innen wurden genetische Varianten identifiziert, am häufigsten im ABCB11- und ABCB4-Gen. 38 Patient*innen wiesen Mutationen ohne eindeutige klinische Diagnose auf. Das klinische Erscheinungsbild variierte je nach Alter. Pädiatrische Fälle (vorwiegend männlich (44 von

64)) waren häufiger mit ABCB11-Varianten assoziiert und zeigten Gelbsucht, während erwachsene Patient*innen (vorwiegend weiblich (16 von 24)) häufiger ABCB4-Varianten trugen und biliäre Symptome oder Gallensteinerkrankungen aufwiesen. Die Zygosität (d. h. homo- oder heterozygote Varianten) sagte das klinische Erscheinungsbild nicht konsistent voraus. Pruritus wurde zum Zeitpunkt der Diagnose nur in etwa 6 % der Fälle dokumentiert, während des Krankheitsverlaufs jedoch in etwa 22 % der Fälle, wobei Juckreiz bei Kindern mit ABCB11-Varianten und bei Erwachsenen mit ABCB4- oder ATP8B1-Varianten gehäuft auftrat. Die Behandlung war hauptsächlich symptomatisch, wobei 32 Patient*innen UDCA verschrieben wurde und nur in zwei Fällen eine spezifische antipruritische Therapie dokumentiert wurde. Ein unerwarteter Befund war das Auftreten von EBV-Infektionen als potenzieller Auslöser für cholestatische Episoden bei acht Patient*innen, von denen fünf Mutationen in ABCB11 aufwiesen.

Conclusio

Bei Patient*innen mit hohem Verdacht auf hereditäre cholestatische Syndrome führen Gentests häufig zum Nachweis von Mutationen (in unserer Studie 50 von 88), am häufigsten in den klassischen PFIC-bezogenen Genen ABCB11, ABCB4 oder ATP8B1. Die Korrelation zwischen Genotyp und Phänotyp ist jedoch weniger eindeutig, da einige Patient*innen mit einer Mutation keinen eindeutigen cholestatischen Phänotyp aufwiesen. Darüber hinaus konnten wir aufgrund fehlender Informationen über die genaue Mutation oder den Allelstatus in vielen Fällen keine umfassende Korrelation zwischen Genotyp und Phänotyp aus unseren Daten ableiten. Insgesamt waren Mutationen im ABCB11-Gen bei Kindern vorherrschend, die hauptsächlich mit Gelbsucht oder neonataler Cholestase auftraten, während ABCB4-Mutationen häufiger bei Erwachsenen, meist Frauen, auftraten und oft mit biliären Symptomen einhergingen, die unter anderem auch durch eine Schwangerschaft ausgelöst wurden. Pruritus trat bei der Erstvorstellung seltener auf, kann jedoch im Verlauf der Erkrankung häufiger auftreten. Allerdings könnte Pruritus in unserem Datensatz unterrepräsentiert sein, ebenso wie das weitgehende Fehlen über spezifische Therapien gegen Pruritus. Die Beobachtung von EBV-assoziierter Cholestase bei Patient*innen mit ABCB11-Mutationen unterstreicht Infektionen als potenzielle Auslöser bei Patient*innen mit genetischer Veranlagung.

Abstract

Background

Hereditary cholestasis syndromes are a heterogeneous group of rare liver diseases that are caused by genetic defects in genes related with bile acid transport or metabolism. Although they are rare, these diseases are clinically important as affected patients can present with severe clinical symptoms like jaundice, pruritus as well as further complications like chronic liver disease, potentially requiring a liver transplant as ultimate therapy. Cholestatic pruritus can greatly reduce quality of life. With new treatments like ASBT inhibitors now available, it's important to know how common this condition is in order to understand how many patients could benefit from these new options. Throughout this wide group of syndromes, clinical presentation can vary between types of genetic mutations, age groups, and triggering events such as infections, pregnancy or drug medications, possibly triggering the manifestation of symptoms in patients with a genetic predisposition.

Methods

We retrospectively analyzed patients with a suspected hereditary cause of cholestasis who underwent genetic testing at our university hospital throughout the last 20 years. Clinical data, laboratory findings, and genetic variants (e.g. mutations, SNPs) were reviewed to evaluate genotype-phenotype correlations, clinical presentation by age, and potential disease triggers.

Results

From medical records, we identified 88 patients, 64 children and 24 adults, with a possible hereditary cause of cholestasis. Only 53 patients presented with a clear cholestatic phenotype (such as PFIC, BRIC, ICP or LPAC). The remaining 35 patients had evidence of cholestasis or mutations but without a final clinical classification at the time of research. Genetic variants were identified in 50 out of 88 patients, most frequently in the ABCB11 and ABCB4 gene. 38 patients had mutations without a clear clinical diagnosis. The clinical presentation varied by age. Pediatric cases (predominantly male (44 out of 64)) were more often associated with ABCB11 variants and presented with jaundice, whereas adult patients (predominantly female (16 out of 24)) more often carried ABCB4 variants and presented with biliary symptoms or gallstone disease. Simply determination of zygosity status of a variant (i.e. homo- or heterozygote variants) cannot predict clinical appearance since

remaining function of the protein a critical contributing factor. Pruritus was documented in only approximately 6% of cases at the time of diagnosis but in around 22% during disease course, clustering with ABCB11 variants in children and ABCB4 or ATP8B1 variants in adults. Treatment was mainly symptomatic, with UDCA prescribed in 32 patients and in only two cases a specific antipruritic therapy was documented. An unexpected finding was the occurrence of EBV infections as a potential trigger for cholestatic episodes in eight patients, five of whom had mutations in ABCB11.

Conclusion

In patients with a high suspicion of hereditary cholestatic syndromes, genetic testing often results in the detection of mutations (in our study 50 out of 88), most often in the classical PFIC-related genes ABCB11, ABCB4 or ATP8B1. However, the genotype to phenotype correlation is less clear as some patients with a mutation did not show a clear cholestatic phenotype. In addition, due to the lack of information on the exact mutation or the allelic status in many cases, we were not able to draw a comprehensive genotype to phenotype correlation from our data. Overall, mutations in ABCB11 were predominant in children, presenting mainly with jaundice or neonatal cholestasis, while ABCB4 mutations were more common in adults, mostly females, and often associated with biliary symptoms among other causes also triggered by pregnancy. Pruritus was less frequent at initial presentation, but may become more prevalent during the disease course. However, pruritus may be underreported in our data set in line with almost no documentation on specific anti-pruritogenic therapies. The observation of EBV-associated cholestasis in patients with ABCB11 mutations highlights infections as potential triggers in patients with genetic predispositions.

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Abbreviations

ABCB	ATP-binding cassette subfamily B
ABE	Acute Bilirubin Encephalopathy
ACTH	Adrenocorticotropic hormone
ALGS	Alagille Syndrome
ASBT	Apical sodium–bile acid transporter
ATP8B1	ATPase phospholipid transporting 8B1
BAAT	CoA-amino acid N-acetyltransferase
BACS	Bile acid CoA synthase
BRIC	Benign Recurrent Intrahepatic Cholestasis
BSEP	Bile salt export pump
cAMP	Cyclic adenosine monophosphate
CDCA	Chenodeoxycholic acid
CFLD	Cystic fibrosis-associated liver disease
CFTR	Cystic fibrosis transmembrane conductance regulator protein
CLDN1	Claudin-1
CN1/2	Crigler-Najjar syndrome type 1/2
CYP7A1	Cytochrome P450 7A1 / Cholesterol 7 alpha-hydroxylase
DCA	Deoxycholic acid
DHA	Docosahexaenoic acid
DILI	Drug induced liver injury
DIOS	Distal intestinal obstruction syndrome
DJS	Dubin-Johnson-Syndrome
FGF19	Fibroblast Growth Factor 19

FXR	Farnesoid-X-receptor
IBAT	Ileal bile acid transporter
ICAM	Intercellular adhesion molecule
ICP	Intrahepatic cholestasis of pregnancy
IFNγ	Interferon gamma
JAG1	Jagged canonical Notch ligand 1
LCA	Lithocholic acid
LPA	Lipophosphatidic acid
LPAC	Low-phospholipid-associated cholelithiasis syndrome
MDR3	Multidrug resistance protein 3
MOR	μ-opioid receptor
MRP2	Multidrug resistance-associated protein 2
MVID	Microvillus inclusion disease
MYO5B	Myosin Vb
NOTCH2	Neurogenic locus notch homolog protein 2
NTCP	Na+-taurocholate cotransporting polypeptide
OATP	Organic anion transporting polypeptide
PBC	Primary biliary cholangitis
PDC-E2	Pyruvate dehydrogenase complex E2
PEX	Peroxisome biogenesis factor
PFIC	Progressive Familial Intrahepatic Cholestasis
PPAR	Proliferator-activated receptor agonist
PSC	Primary sclerosing cholangitis
PXR	Pregnane X receptor

SLC10A1	Solute Carrier family 10 member 1
SLCO1B	Solute Carrier Organic Anion Transporter Family Member 1B
TJP2	Tight Junction Protein 2
TOF	Tetralogy of Fallot
TRPV1	Transient Receptor Potential Vanilloid 1
UDCA	Ursodeoxycholic acid
UGT	UDP-glucuronosyltransferase

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1. Introduction

1.1 Definition of Cholestasis

Cholestasis is a condition characterized by impaired bile secretion as well as formation, leading to a build-up of bile components, which in turn leads to hepatocellular damage including toxicity to the biliary tree and peripheral circulation of potential harmful biliary constituents. (1)

Cholestasis can be divided into two subtypes concerning localization, into intrahepatic and extrahepatic cholestasis. Intrahepatic cholestasis can lead to damage of hepatocytes as well as bile canaliculi and bile ducts situated in the liver. Examples are the hereditary diseases Progressive familial intrahepatic cholestasis (PFIC)-1 and PFIC-2 as well as cholestasis of inflammatory origins like Primary biliary cholangitis (PBC). Extrahepatic cholestasis on the other hand can affect the common bile duct, the common hepatic duct and the extrahepatic ducts, for example cholestasis due bile stone obstruction or compression by a malignant process such as pancreatic cancer.

Cholestasis can also be classified according to origin of injury into hepatocellular or cholangiocellular / biliary-type of cholestasis. Hepatocellular cholestasis arises primarily at the canalicular membrane of hepatocytes resulting in impairment of bile secretion and formation. This includes many hereditary forms of cholestasis (see below) but also drug-induced cholestasis (e.g. by steroids) or inflammatory cholestasis (e.g. sepsis-induced cholestasis).(2) Cholangiocellular cholestasis comprises diseases such as PSC, PBC or Cystic-fibrosis related liver disease. (3)

Finally, cholestasis can be differentiated into hereditary and acquired forms (1). Hereditary forms comprise wide range of diseases related to genetic defects in bile acid and lipid transport as well as bile formation at hepatocellular and cholangiocellular/biliary duct levels, with various severities and symptoms. These can, for example, range from PFIC, which manifests in early childhood and can end in end stage liver disease to benign recurrent intrahepatic cholestasis (BRIC), which is a benign form of recurring cholestasis. It also includes cholestatic conditions such as cystic fibrosis (CF) and biliary atresia which primarily affect bile ducts. (1) The different forms of cholestasis of genetic origin will be further explained below. Acquired forms of cholestasis can be categorized by different causes (1):

- Drug-induced: Antibiotics like rifampicin, erythromycin, or oxypenicillins and other medications like glibenclamide, and chlorpromazine can cause cholestasis. (4)
- Pregnancy-related: Intrahepatic cholestasis of pregnancy (ICP) is a condition where patients develop high bile acid concentrations and pruritus without a primary dermatological condition. Pregnancies affected by the condition face increased risks, including early labor, hypoxia in the fetus, discolored amniotic fluid due to meconium, and in severe cases, stillbirth. (5) In ICP, gene variants like ABCB4 and ABCB11, as well as other genes like ATP8B1 have been found as genetic contributors in some cases. (6)
- Mechanical cholestasis is referring to a mechanical obstruction of bile flow from the liver to the duodenum. This obstruction can occur throughout the biliary tract, spanning from the hepatic ducts to the ampulla Vateri. The most common etiology for extrahepatic cholestasis is gallstones within the bile ducts, a condition known as choledocholithiasis, neoplasms are another common etiology.(7)
- Sepsis-induced cholestasis, resulting from inflammatory mediators like cytokines and endotoxins which compromise bile secretion and formation.(8)
- Viral diseases like Epstein-Barr virus or cytomegalovirus are also acquired causes of cholestasis, potentially leading to jaundice in patients, with CMV especially affecting immuno-compromised individuals.(9)
- Immune-mediated cholestasis such as PBC, primary sclerosing cholangitis (PSC) and IgG4 related cholangiopathies.

The symptoms of cholestasis may vary, from being asymptomatic to symptoms such as fatigue, pruritus or abdominal pain, which significantly lower quality of life. (10) In particular pruritus may significantly affect the quality of life of patients through disturbing daily activities as well as sleep, appetite, concentration and mood. (11)

Cholestatic liver disease is often associated with dyslipidemia (mainly hypercholesterinemia), possibly presenting itself with xanthelasma around the eyes or as palmar or tendinous xanthomata in its most pronounced appearance. The bone density may also be affected, with complications like osteopenia or osteoporosis being common. Due to a decreased level of bile acids in the intestines, the formation of micelles is reduced which subsequently leads to a malabsorption of fats and vitamins that are fat-soluble such as vitamins A, D, E and K. Deficiencies in these vitamins can lead in its most severe presentations to rachitis, xerophthalmia and a higher risk of bleeding.(12)

1.2 Enterohepatic Circulation of Bile Acids – Physiology and Functions

Enterohepatic circulation describes the movement of substances such as bile acids, starting from production and biotransformation in hepatocytes, following transport along the bile ducts and concentration in the gallbladder after which they are released into the small intestine where the majority of bilirubin and bile acids is reabsorbed in the ileum by enterocytes. (13)

Primary bile acids are synthesized in the liver from cholesterol. Cholic acid and chenodeoxycholic acid are synthesized by the hepatic enzymes' cholesterol 7-alpha-hydroxylase (CYP7A1) and sterol 12-alpha-hydroxylase (CYP8B1). Besides this classical pathway of bile acid synthesis, there is an alternative pathway, which may account for up to 30% of primary bile acid synthesis. The alternative pathway is initiated by the enzyme CYP27A1. Next, the already amphiphile bile acids with an ionized carboxylate group are conjugated to glycine or taurine through a process called N-acyl amidation via the enzymes Bile acid CoA synthase (also called BACS) and bile salt CoA-amino acid N-acetyltransferase (BAAT). The conjugation leads to a higher solubility in water and a reduced toxicity of bile acids. Subsequently, the bile acids are secreted from the liver into bile through the bile salt export pump (BSEP, encoded by the gene ABCB11). Additional substrates of organic origin like bilirubin or glutathione are mediated in their secretion by the multidrug resistance associated protein (MRP2, encoded by ABCC2). (14)

The bile acids are then stored in the gallbladder where the bile is concentrated through water absorption. The excretion of bile from the gallbladder is controlled by vagus activity as well as through the enterohormones motilin, or postprandially through cholecystokinin. In the fasting state, there is a periodical excretion of bile into the duodenum, which leads to a serum bile acids concentration of 0.2-0.7 µM in healthy individuals, compared to 4-5 µM after meal intake. Following this, 95% of the bile acids are reabsorbed in the terminal ileum through apical sodium-bile acid transporter (ASBT, synonym IBAT, encoded by the gene SLC10A2), which is a sodium-dependent transporter of bile acids.(14) Remaining bile acids which escape the uptake by the ileum, are biotransformed by the colon's microbiome, turning them into secondary bile acids. The transformation into secondary bile acids is a complex multi-enzymatic step requiring a gut microbiome. First, bile acids are deconjugated by the microbial enzyme bile salt hydrolase (BSH). The deconjugated bile acids undergo a process

of 7-dehydroxylation, through which they become 7-deoxy bile acids. Thus, the previous cholic acid becomes deoxycholic acid and the previous CDCA becomes LCA. Subsequently, these bile acids may go through further epimerization at their C-3 molecule, resulting in the formation of iso-bile acids, one example is the generation of UDCA from CDCA.(13) Secondary bile acids undergo passive diffusion and are reabsorbed in the colon. Around 5% of bile acids, which predominantly consist of DCA and LCA are lost in feces. The recirculation back to the liver occurs through the portal vein in a process that typically happens 4-12 times per day. Hereby, the bile acids interact with hepatocellular bile acid transporters such as taurocholate cotransporting enzyme (NTCP, encoded by the gene SLC10A1) and the organic anion transporting polypeptide (OATP, encoded by the gene SLCO1B1), which take-up these bile acids back into the liver. (14)

1.3 Forms of Cholestasis

1.3.1 Hereditary Cholestasis

1.3.1.1 PFIC

Diseases of the group PFIC (progressive familial intrahepatic cholestasis) are rare hereditary cholestatic syndromes, occurring with an incidence of 1 in 50,000 to 100,000 births. Despite their rarity, they stand out as one of the most prevalent causes of cholestasis in newborns and infants (Figure 1). PFIC disorders in their classic definition follow an autosomal recessive inheritance pattern and account for 10-15% of neonatal cholestasis syndromes and infants requiring liver transplantations. PFIC types 1 and 2 result from genetic defects affecting the transport systems FIC1 and BSEP, leading to the intracellular accumulation of bile acids in hepatocytes, and as a consequence causing hepatocellular toxicity with high liver transaminases and low GGT. (15) Patients with PFIC type 3 on the other had present with elevated GGT levels. (16)

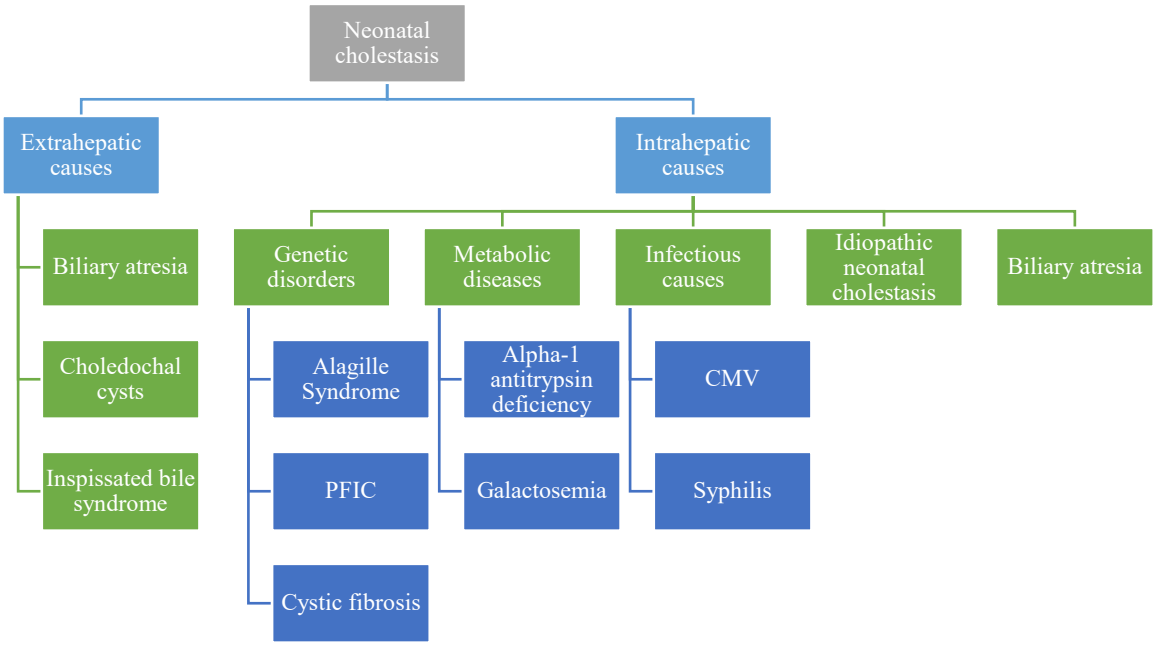

Figure 1: Categorization of the Different Causes of Neonatal Cholestasis(17-19)

It is important to note that in autosomal recessive diseases like PFIC, the phenotypes of patients may vary significantly because of different mutations, as well as patients being homozygous or heterozygous. (20) In fact, also patients with a heterozygous pathogenic variant may become symptomatic if the remaining transporter function is below a certain threshold limit. Thus, the severity of the disease is often determined by the type of missense mutations in the affected proteins. Some mutations may result in a complete loss of function, comparable to nonsense mutations, while others may have only milder effects or become only symptomatic in combination with a second trigger (e.g. a medication, hormone or cytokine, which further reduces transporter activity below the threshold for normal activity). The presence of pathogenic variants on both alleles is typically associated with an earlier onset and more severe disease course. (21)

1.3.1.1.1 PFIC-1

In PFIC-1, also referred to as Byler disease, mutations occur in the ATP8B1 gene, which encodes the FIC1 protein. This protein functions as an aminophospholipid flippase, facilitating the movement of aminophospholipids between the cytoplasmic and exoplasmic membranes of hepatocytes. This activity protects the cell membrane from the high concentration of bile salts in the canalicular lumen. However, due to mutations in the ATP8B1 gene, this process malfunctions, leading to an inability to properly manage

aminophospholipids and subsequently impacting the protective function of the cell membrane against the elevated bile salt concentration. (15, 22)

A malfunction in ATPB8B1 leads to an altered membrane lipid environment due to reduced cholesterol-to-phospholipid ratio, which leads to destabilization of the membrane (15, 22), also affecting BSEP activity, which is responsible for the export of bile acids. Consequently, hydrophobic bile acids like CDCA could exacerbate membrane injury in cells that are ATP8B1 deficient, leading to a further decline in BSEP activity. (22) In patients that have biallelic (i.e. homozygous or compound homozygous) variants in ATP8B1, the clinical phenotype is influenced by the remaining FIC1 function. While patients with benign recurrent intrahepatic cholestasis (BRIC) typically retain 5%-20% of normal healthy residual function, individuals with early-onset FIC1 deficiency are expected to present with complete loss of function.(23)

Patients with PFIC-1 not only present with a liver phenotype. Due to a broader expression of FIC1, these patients may also suffer from extrahepatic symptoms such as pancreatitis, watery diarrhea as well as short physique. This is important to know, because these clinical symptoms may prevail after a liver transplantation. (24)

1.3.1.1.2 PFIC-2

Byler's Syndrome, otherwise known as PFIC-2, is caused by a mutation in the ABCB11 gene which encodes for BSEP. BSEP is responsible for exporting bile acids from hepatocytes into the bile canaliculi (25, 26)

As the primary transporter for bile acids, any mutation within ABCB11 can result in a reduction of bile acid secretion. This decrease in secretion leads to a reduced flow and subsequent accumulation of bile salts within the liver cells, ultimately contributing to hepatocellular damage. Concerning the complications of PFIC-2, a higher occurrence of hepatocellular carcinoma, cholelithiasis, and portal hypertension has been observed. (24)

Healthy individuals express BSEP, with a higher transport capacity than required. It is assumed that damage to hepatocytes may occur when BSEP function drops to around 20-25% of normal activity. Patients with less than 20% BSEP activity due to genetic mutations, these are typically patients carrying biallelic ABCB11 variants, are prone to childhood disease, with genetic loss-of-function mutations associated with poorer prognosis. Above the

threshold of 20% BSEP activity, these include the heterozygous carriers, BSEP function is usually sufficient to prevent chronic liver injury under normal circumstances. However, when exposure to stressors such as drugs, hormones additional contributing genetic variants further reduce functional capacity or increases bile acid load, toxic bile acid accumulation in hepatocytes may occur and may then lead to acute dysfunction. Individuals with the very common V444A variant have BSEP function of around 50%. Under additional stress factors, such as drug exposure or pregnancy, functional capacity may drop below the critical 20% threshold, thereby facilitating drug induced liver injury (DILI) and ICP. (23)

1.3.1.1.3 PFIC-3

PFIC-3 is caused by defects in the ABCB4 gene, which encodes the MDR3 protein. This glycoprotein is found in the canalicular membrane of hepatocytes and functions as a floppase, facilitating the biliary secretion of phospholipids like phosphatidylcholine. Malfunctioning of this glycoprotein contributes to a shift in the biliary bile composition with non-micellar bound “free” bile-acids as a consequence of lack of biliary phospholipids. Phosphatidylcholine plays a protective role by shielding the biliary epithelium from the detergent effects of bile salts. (27) Thus, the ABCB4 mutation contributes to chronic inflammation of the biliary epithelium. The damage to the biliary epithelium consequently leads to cholestasis with elevated GGT levels. Additionally, a decrease in the concentration of phospholipids can lead to increased cholesterol crystallization, favoring the formation of gallstones. (24)

There is a wide range of cholestatic syndromes related to the malfunctioning of MDR3 apart from PFIC-3 as its most severe form of cholestasis. The range of manifestations comprises the Low-Phospholipid Associated Cholelithiasis (LPAC)-Syndrome, intrahepatic cholestasis of pregnancy (IPC), drug-induced cholestasis as well as chronic liver fibrosis and cirrhosis. Hepatobiliary disorders where MDR3 may act as a susceptibility or modifier gene are conditions like PSC, PBC and cryptogenic biliary fibrosis. Additionally, some conditions are hypothesized to involve MDR3 as a susceptibility gene, such as idiopathic adulthood ductopenia, oriental cholangiohepatitis and total parenteral nutrition-induced cholestasis. (28)

1.3.1.2 Other forms of PFIC

Apart from PFIC-1 to 3, there is a growing number of PFIC-like disorders numbered PFIC-4 to PFIC-13. PFIC-4 is a condition with a mutation in the gene TJP2. This gene encodes tight junction protein 2, a cytoplasmic protein that links transmembrane tight junction components, such as claudins, to the actin cytoskeleton. This interaction is essential for maintaining cell-to-cell adhesion. In PFIC-4, mutations in TJP2 prevent claudin-1 (CLDN1) from localizing to the bile canalicular membrane, where it normally exerts its function. As a result, impaired cell junction integrity allows bile acids to reflux into hepatocytes, leading to hepatotoxicity and cholestasis.(29) Extrahepatic manifestations have also been reported, likely reflecting the expression of TJP2 in other tissues, including the respiratory tract and the central nervous system. (30)

In PFIC-5, the gene NR1H4, is impacted by a loss of function mutation, leading to impairment of the farnesoid X receptor (FXR). Concerning the loss of function mutation, both homozygous as well as heterozygous mutations have been reported. (29)

FXR is part of a group of nuclear receptors that are ligand-activated by bile acids and is essential in the regulation of the metabolism of bile acids, as well as cholesterol, triglycerides and others. The hepatic role of this protein is, among other genes, the regulation of ABCB11 and ABCB4 gene expression through detection of intracellular bile acids. An intracellular increase of bile acids in hepatocytes leads to stimulation of FXR, resulting in transcription of ABCB11 and thus the production of BSEP proteins, having an increased export of bile acids as a consequence. (29)

FXR also has activity in the enterocytes of the ileum, stimulating the production of fibroblast growth factor 19 (FGF19) when elevation of bile acids occurs. FGF19 suppresses the enzyme CYP7A1 in the liver after undergoing enterohepatic circulation. The result of this action is the decrease in bile acid production. (29)

A defect in FXR-activity leads to a failure of BSEP-expression along with an increase in bile acid production because of non-suppression of CYP7A1. (29)

A mutation in the MYO5B gene is the cause for PFIC-6[1]. This gene encodes MYO5B, a molecular motor protein associated with the actin cytoskeleton. MYO5B plays various roles, including targeting BSEP to the canalicular membrane of hepatocytes and maintaining

[1] Please note that from PFIC-5 onwards, the numbering may vary, as there is no unified system yet.

enterocyte polarity. Mutations in MYO5B disrupt the MYO5B/RAB11A recycling endosome pathway, leading to improper BSEP localization. These disruptions may lead to cholestatic liver disease, either as a separate condition or alongside microvillus inclusion disease (MVID), a condition characterized by severe diarrhea. Patients with MVID often have more severe mutations in both alleles of MYO5B. Conversely, individuals with less severe mutations typically exhibit isolated cholestatic symptoms, with enterocyte function remaining unaffected. (29)

Overall, 13 different types of PFIC have been reported, with all of them being of autosomal recessive inheritance. The mutations and affected genes are listed in table 1:

Phenotype	Location	Gene	Reference
PFIC-1	18q21.31	ATP8B1	(31)
PFIC-2	2q31.3	ABCB11	(32)
PFIC-3	7q21.12	ABCB4	(33)
PFIC-4	9q21.11	TJP2	(34)
PFIC-5	12q23.1	NR1H4	(35)
PFIC-6[2]	3q29	SLC51A	(36)
PFIC-7	4q26	USP53	(37)
PFIC-8	9q32	KIF12	(38)
PFIC-9	15q15.1	ZFYVE19	(39)
PFIC-10	18q21.1	MYO5B	(40)
PFIC-11	15q24.1	SEMA7A	(41)
PFIC-12	15q26.1	VPS33B	(42)
PFIC-13	16q22.1	PSKH1	(43)

Table 1: PFIC-Subtypes, Genes and Chromosomal Locations.

1.3.1.3 BRIC

Benign recurrent intrahepatic cholestasis (BRIC) is a rare autosomal-recessive disease caused by a mutation in the ATP8B1 gene encoding for the FIC1 protein, the same protein affected in the PFIC-1 disease. Forms of BRIC are less severe than the PFIC disorders. (44)

[2] In omim.org, the previously mentioned MYO5B mutation has switched places to PFIC-10.

Concerning the pathophysiology of BRIC1, a reduced function of FIC1 leading to impaired stability of the plasma membrane has been suggested. Thus, translocation of phospholipids across the cellular membrane is disrupted. In BRIC2, the bile acid transporter BSEP is affected, reducing the elimination of bile salts from hepatocytes into the biliary system, leading to intrahepatic cholestasis.(45) The difference between the mutations in BRIC and PFIC lies in the extent of canalicular protein expression. Studies showed that PFIC-1 mutations result in a complete absence of canalicular protein expression, whereas BRIC1 mutations allow for the expression of residual protein.(16) The most common mutations are p.I661T (c.1982T>C), found in 79% of all BRIC1 cases with detectable alterations in ATP8B1, especially in the European population. (21)

Clinical symptoms of BRIC include pruritus attacks with an observed increase in alkaline phosphatase, followed by icterus occurring weeks later. Secondary symptoms are nausea, vomiting, steatorrhea, malabsorption, and weight loss. These attacks may last for several weeks to months. (46)

These recurrent pruritus episodes can either be spontaneous or triggered by infections or pregnancy. Cholestatic episodes are followed by asymptomatic periods, which can extend from months to several years before the recurrence of symptoms. (47)

1.3.1.4 LPAC-Syndrome

Low-Phospholipid Associated Cholelithiasis (LPAC) is a hereditary disease connected to a mutation in the gene ABCB4. Patients with LPAC-Syndrome are mostly women with a sex ratio of around 3:1, affecting predominantly young adults. A noticeable epidemiological difference in LPAC-syndrome is that it occurs in patients with low or normal body weight, patients with a higher BMI than 25 kg/m^2 being the exception at only 3%. (27)

The mutation in ABCB4, leads to reduced concentrations of phosphatidylcholine in bile, giving rise to cholesterol gallstones in bile ducts, as the phospholipid is responsible for solubility of cholesterol through formation of mixed micelles. A reduction in mixed micelles and a higher rate of simple micelles, which can solubilize less cholesterol leads to a rise in cholesterol gallstones in bile ducts. (27) However, MDR3 mutations are only found in about 30-50% of patients presenting with the clinical signs of LPAC. (48)

Clinical presentation of LPAC-Syndrome is mostly through biliary lithiasis, resulting in cholecystectomy in most cases, the majority having done surgery before diagnosis of the syndrome. Other diagnoses in rarer cases are acute cholecystitis, cholangitis or acute pancreatitis. A treatment option for LPAC-Syndrome is ursodeoxycholic acid (UDCA), which can solubilize cholesterol and may thus lower the risk of cholesterol calculi, and may further protect the biliary epithelium by reduction of the detergent effect of bile acids. (27)

1.3.1.5 NTCP Mutations - Hypercholanemia

The protein NTCP is important for the sodium dependent uptake of the majority of bile salts into hepatocytes. NTCP deficiencies lead to conjugated hypercholanemia, with one reported case showing cholestatic jaundice during infancy. (49)

In a 2021 study, an 18-year-old female patient with a homozygous mutation in the SLC10A1 gene, encoding NTCP protein, presented with chronically elevated plasma bile acid levels. However, she did not experience pruritus or elevated serum autotaxin levels. (50) In a 2022 study featuring 10 patients with NTCP deficiency, the most frequent phenotypic characteristics included hypercholanemia, loss of bone mass and vitamin D deficiency as well as defects of the gallbladder.(51)

1.3.1.6 Alagille Syndrome

Alagille syndrome (ALGS), is a genetic disease of autosomal-dominant inheritance caused by mutations in the JAG1 gene or NOTCH2 gene, both of which have a role in the Notch signal pathway. (52)

The JAG1 gene acts as a ligand for NOTCH2 and is expressed in the liver in portal mesenchymal cells, endothelial cells as well as biliary epithelial cells, whereas NOTCH2 is found in hepatoblasts during development of the liver. Mutations in JAG1 or NOTCH2 can disrupt the normal development of the biliary tree. These mutations impair JAG1–NOTCH2–mediated cell-to-cell communication between biliary epithelial cells and hepatoblasts. (52)

The classic criteria for the diagnosis of Alagille Syndrome are based on clinical presentations in five body systems. A diagnosis can be made if deficiency of the bile duct is complemented by three of these five criteria:

The first “body system” concerns the liver, with cholestasis leading to jaundice in newborns. Patients present with conjugated hyperbilirubinemia, accompanied by pale stools. (53)

The facial structure can also be affected, with patients having a broad forehead, changes in ear and nose shape. A pointed chin may lead to a generally “triangular facial structure.” (53)

Alagille Syndrome can affect the cardiovascular system, with patients showing stenosis of the pulmonary artery, pulmonary atresia as well as septal defects such as ASD (atrial septal defect), VSD (ventricular septal defect) and TOF (Tetralogy of Fallot). (53)

The vertebrae can express a characteristic shape, being called “butterfly vertebrae”. Other defects of the axial skeleton include a merging of bordering vertebrae and a spina bifida occulta. (53)

Anomalies of the ocular structures have also been recorded and include the posterior embryotoxon, characterized by a Schwalbe’s ring between iris and cornea. (53)

Alagille Syndrome can also involve the cerebrovascular system. Reported manifestations include Moyamoya syndrome, a vascular occlusive disorder of unknown cause, and cerebral aneurysms. These abnormalities in the cerebrovascular structure lead to a stroke complication in 14% of patients with Alagille Syndrome. (54)

1.3.1.7 Biliary Atresia

Biliary atresia is an often-fatal obstructive disease of the biliary tract affecting the intrahepatic, as well as the extrahepatic biliary ducts of newborns. The etiology is still unknown, although genetics may play a contributing factor. (55)

Up to one fifth of patients present with a syndrome related to biliary atresia or have another birth defect, with certain areas in the world showing a higher incidence.(56) The different syndromes are for example Biliary Atresia Splenic Malformation Syndrome. This type presents with additional abnormalities such as polysplenia, an interrupted vena cava or a continuation of the azygous vein. Yet, other syndromes are Cystic Biliary Atresia, CMV-IgM Positive Biliary Atresia and Isolated Biliary Atresia. (55)

Pathogenetic causes include defective remodeling of the biliary tract at the liver hilus during fetal development, rotavirus and reovirus type 3 associated infections, immune associated damage through a higher expression of an intercellular adhesion molecule (ICAM)-1 in bile ductuli. There are also theories about an acquired cause, as many patients with biliary atresia have pigmented stools at first, showing pale stools only later. (55)

Concerning the clinical presentation of biliary atresia, newborns show symptoms like pale stools, an increase in liver size and persisting jaundice. More than 50% of patients with this condition have initial pigmented stools, later becoming pale. Early-onset biliary atresia is defined by the presence of cholestatic jaundice and pale stools within the first two weeks of life, whereas late-onset disease presents after two weeks of age. (57) Complications of biliary atresia include enlargement of spleen and liver, portal hypertension and ascites with high mortality if not treated. (55)

Diagnostic procedures for identifying biliary atresia are by ultrasonography, whereby no or a less developed gallbladder is visible. Other methods include hepatobiliary scintigraphy, endoscopic retrograde cholangiopancreatography, MRI-scans and liver biopsy. (55)

The standard treatment is the Kasai procedure, also known as portoenterostomy. This procedure is conducted when the liver damage is not far advanced, enabling sufficient bile drainage when successful. The ultima ratio if portoenterostomy fails is a liver transplantation. (55)

1.3.2 Hereditary Jaundice due to Mutations in Hepatobiliary Bilirubin Metabolism

1.3.2.1 Dubin-Johnson Syndrome

Dubin–Johnson Syndrome (DJS) is an autosomal recessive disorder caused by a deficiency of the protein MRP2. This defect impairs the biliary excretion of organic anions other than bile acids, such as bilirubin. As a result, patients develop conjugated, non-hemolytic hyperbilirubinemia. In DJS bilirubin levels are usually elevated to levels varying between 2 to 5 mg/dl. However, in rare cases, concentrations above 10 mg/dl have been reported, with direct bilirubin being the cause for 60% of these elevations. (58)

This rare condition occurs with no particular sex predilection but is more common among Sephardic Jews. Clinically, liver parameters, blood counts, lipid levels, and serum albumin remain within normal ranges, with no significant risk of fibrosis progression or cirrhosis

development. Symptoms are generally nonspecific, including weakness and upper abdominal discomfort, while pruritus is notably absent in cases of DJS. Certain triggers, such as oral contraceptives, intercurrent illness, and pregnancy, can precipitate hyperbilirubinemia, which often leads to the diagnosis.

DJS does not require treatment. However, its diagnosis is important in order to exclude other more severe conditions of jaundice, which may lead to hepatic damage. (59)

1.3.2.2 Crigler-Najjar Syndrome

Another rare disease of autosomal recessive inheritance is Crigler-Najjar syndrome, leading to neonatal hyperbilirubinemia. The enzyme affected in this condition is UDP-glucuronosyltransferase (UGT), which facilitates glucuronidation of unconjugated bilirubin. The lack of this enzyme or reduced activity therefore leads to an increase in unconjugated bilirubin in serum. (60)

The high levels of unconjugated bilirubin overload the binding capacity of albumin, which is the typical transport protein for unconjugated bilirubin. As a consequence, the unconjugated and unbound bilirubin accumulates in locations like the skin, sclera or even the brain, as unbound bilirubin is able to pass the blood-brain barrier. (60)

From a genetic perspective, mutations in the UGT1A1 gene lead to reduced level or complete deficiency of UGT-activity. Thus, Crigler-Najjar Syndrome can be differentiated into two 2 types based on enzymatic activity (60):

Crigler-Najjar Syndrome Type 1 (CN1) represents the most severe form of this condition, characterized by a complete deficiency of UGT enzyme activity. This deficiency results from genetic anomalies such as deletions, missense mutations, exon skipping, insertions, stop codon development, or changes in splicing. (60) In CN1, total serum bilirubin levels typically rise above 20 mg/dL, sometimes even reaching 50 mg/dL. (61) Patients with CN1 often experience severe jaundice that, if untreated, can be fatal and may have serious neurological implications such as Acute Bilirubin Encephalopathy (ABE). Symptoms include sleepiness, high pitched cries, hypotonia, progressing to fever and even seizures, apnea and coma. Regular phototherapy is essential for managing the condition, while liver transplantation remains the only curative treatment. (60)

Crigler-Najjar Type 2 (CN2) on the other hand is a less severe form, caused by point mutation in UGT1A1, which leads to decreased UGT-production, and therefore less enzymatic activity (approx. 10% remaining function). (60) In CN2, total bilirubin levels typically range between 3.5 and 20 mg/dL.(61) Patients affected by CN2 typically present with intermittent jaundice initiated by stress. Because of this milder symptomatology, conservative therapy suffices, with only rare occasions of liver transplantation.(60)

1.3.2.3 Morbus Gilbert-Meulengracht

Another syndrome with hyperbilirubinemia is Morbus Gilbert Meulengracht, a mild form of non-hemolytic unconjugated hyperbilirubinemia. (62) The condition is the most common form of hereditary jaundice and more prevalent among Caucasians, with a prevalence of 2%-10% in the western hemisphere. It is predominantly inherited in an autosomal recessive manner, though autosomal dominant forms have also been reported. (63)

Under normal circumstances, unconjugated bilirubin binds to uridine diphosphate-glucuronic acid, forming soluble conjugated bilirubin that is secreted into bile. In Gilbert-Meulengracht syndrome, a defect in the UGT1A1 gene impairs this conjugation process, leading to reduced bilirubin clearance and the development of hyperbilirubinemia. (63). In contrast to CN2, the mutation typically affects the promotor region of UGT1A1 and not the coding sequence. The remaining enzyme activity is typically above 30%.

Typically, the hyperbilirubinemia in Gilbert-Meulengracht syndrome is mild or absent. However, during periods of physiological stress, such as sleep deprivation, fasting, dehydration, alcohol consumption, or exposure to general anesthesia, the levels of unconjugated bilirubin in the blood can rise to three to four times the normal physiological levels(63)

In fact, there could be a potential benefit of these higher concentrations of unconjugated bilirubin related to the antioxidant effects of bilirubin. Relative to the general population, patients with Morbus Gilbert-Meulengracht appear to have a lower incidence of malignant conditions like Hodgkin’s lymphoma or endometrial cancer. Additionally, the overall rate of mortality seems to be lower in patients with this condition. Regarding the prognosis of Morbus Gilbert-Meulengracht itself, there have been no signs of chronic liver disease development or any negative impact on patients' lives. (63)

1.3.2.4 Rotor-Syndrome

Rotor type hyperbilirubinemia, more commonly known as Rotor syndrome, is an autosomal recessive hereditary condition caused by homozygous mutations in the SLCO1B1 and SLCO1B3 genes located on chromosome 12. These mutations lead to the production of truncated proteins OATP1B1 and OATP1B3, which are important for the reabsorption of bilirubin in hepatocytes and its subsequent elimination. The reduced efficiency of these proteins results in unconjugated hyperbilirubinemia and bilirubinuria. (64). Bilirubin levels may be elevated to 5 to 8 times the upper limit of norm. (65)

In terms of epidemiology, Rotor syndrome shows no gender predilection, and its prevalence remains unknown as most individuals with the condition are asymptomatic. Symptoms may be present at birth or early childhood and may include jaundice—typically non-pruritic in Rotor syndrome—along with scleral icterus. Other cholestatic symptoms, such as fatigue, dark urine, and abdominal pain, may also manifest. A distinguishing feature of Rotor syndrome compared to other cholestatic disorders is the absence of hepatosplenomegaly, even in patients exhibiting jaundice. Another distinguishing factor between Rotor syndrome and conditions like Dubin-Johnson syndrome is the absence of black liver pigmentation, which is characteristic of Dubin-Johnson syndrome. (64)

Prognostically, Rotor syndrome is benign, with jaundice being the only persistent symptom throughout a patient's life. No specific therapy is required. Accurate diagnosis is crucial, though, to exclude more serious diseases with similar symptoms. (64)

1.3.3 Other Genetic Diseases with a Cholestatic Component

1.3.3.1 Cystic fibrosis

Cystic fibrosis (CF), is a genetic disease, impacting patients' respiratory system, as well as their digestive and reproductive tracts. The genetic mutation affects the cystic fibrosis transmembrane conductance regulator protein (CFTR), which leads to a disruption of the transmembrane cAMP activated chloride channel. This results in a reduction of chloride secretion and subsequent increase in reabsorption of sodium, through which higher amounts of water are resorbed and thicker and more viscous mucus is secreted in organs like the lungs,

the sinuses in the respiratory system, the liver, pancreas and intestines as well as the sweat glands on the skin. (66)

Concerning the gastrointestinal system, the pancreas is the most often affected organ, with thicker pancreatic mucus leading to a higher rate of obstruction and subsequently pancreatitis in acute and chronic phases. The inflammation and damage in the organ lead to the development of diabetes mellitus when the beta cells are affected. (67)

In the stomach, cystic fibrosis can lead to a reduction of bicarbonate production and thus a higher incidence of gastroesophageal reflux and peptic ulcer disease. The increased mucus production affects the intestinal tract, slowing enteral motility. This may lead to meconium ileus in newborns, whereas in adults it can lead to distal intestinal obstruction syndrome (DIOS) as well as constipation. (67)

Approximately 30% of patients with cystic fibrosis develop Cystic fibrosis-associated liver disease (CFLD), with clinical forms ranging from elevation in serum liver enzymes in 35% of individuals, hepatic steatosis (20-60%), focal (11-70%) and or multilobular biliary cirrhosis (5-15%), micro-gallbladder (30%) as well as cholelithiasis and cholecystitis in up to 10% of cases. Although rare, neonatal cholestasis can also occur, with approximately half of affected patients also presenting with meconium ileus. (68)

1.3.3.2 Zellweger Spectrum Disorder

Zellweger Spectrum Disorder is a spectrum of autosomal recessive conditions resulting from mutations in PEX genes, which are responsible for the encoding of peroxins, proteins necessary in peroxisome biogenesis. Depending on the severity and phenotype, the disorder can be classified into different groups. (69, 70)

One of them is Zellweger syndrome, also called neonatal adrenoleukodystrophy, a disease where the peroxisomes have a generalized dysfunction caused by a PEX mutation. Another disorder is caused by a mutation in the ABCD1 gene, which clinically resembles a variant of adrenoleukodystrophy. This disease is characterized by neurological and adrenal complications. Another group is related to different enzymatic dysfunctions in peroxisomes, with rhizomelic chondrodysplasia as one example. (69, 70)

The dysfunctional β-oxidation in peroxisomes dysfunctions leads to an accumulation of very long-chain fatty acids, which may lead to cholestasis (69, 70). As biotransformation in the

liver including the synthesis of lipoproteins are disturbed, the consequence is also a deficit in fat-soluble vitamins. (70)

Apart from lipid metabolism, peroxisomes are also involved in the degradation of hydrogen peroxide—a cytotoxic byproduct—and play a role in steroid hormone synthesis. Consequently, individuals with Zellweger spectrum disorder often present with elevated hydrogen peroxide levels and reduced concentrations of steroid hormones such as ACTH. The accumulation of VLCFAs and hydrogen peroxide contributes to neuronal membrane damage and demyelination. Additional clinical manifestations include cortical renal cysts, hepatic fibrosis, and central nervous system demyelination, which together account for the alternate designation of this condition as cerebrohepatorenal syndrome. (70)

Various approaches have been explored to enhance the quality of life for patients with Zellweger spectrum disorder, given the absence of a curative treatment. One such approach involves supplementation with docosahexaenoic acid (DHA), which is typically found at reduced levels in affected individuals. However, randomized controlled trials have not demonstrated significant clinical benefits from the administration of named substance.

Another therapeutic strategy involves the usage of Lorenzo oil, which has been shown to reduce VLCFA levels. However, this intervention has not demonstrated an effect on halting or slowing the development of Zellweger spectrum disorder. Due to the deficiency in fat-soluble vitamins observed in patients, cholic acid has been considered as a treatment to potentially enhance the absorption of these vitamins. However, the supporting evidence for its effectiveness remains limited as well. (70)

1.3.4 Acquired Forms of Cholestasis

1.3.4.1 Primary Biliary Cholangitis

Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by chronic inflammation of the small bile ducts, typically with granulomatous and lymphocytic features. It is the most prevalent autoimmune hepatic disease, affecting primarily women. (71)

The pathogenesis of PBC involves a deficit of immune tolerance to the E2 element of the pyruvate dehydrogenase complex PDC-E2, which is an important enzyme complex in the process of oxidative phosphorylation. This deficiency leads to immune-regulated

lymphocytic inflammation of the bile ducts, influenced by Interleukin-12 and IFNγ, resulting in chronic damage to the cells of the biliary epithelium. (71)

PBC is diagnosed by the combination of chronically elevated cholestatic serum markers, in particular alkaline phosphatase accompanied by the presence of antimitochondrial antibodies. Liver biopsy is usually not required for the diagnosis. Even though there is variety in severity of symptoms, patients experience symptoms like Sicca syndrome, pruritus, fatigue, bone pain and abdominal discomfort. (71)

PBC is treated with the bile acid ursodeoxycholic acid. (71) If the response to UDCA is insufficient, second-line therapies now consider the use of peroxisome proliferator-activated receptor (PPAR) agonists, such as bezafibrate, or more specific PPAR ligands such as Elafibranor and Seladelpar. (72)

1.3.4.2 Primary Sclerosing Cholangitis

Another acquired form of chronic cholangiopathies is primary sclerosing cholangitis (PSC), a cholestatic condition characterized by inflammation and sclerosis of larger bile ducts, with subsequent obstruction and biliary fibrosis and cirrhosis. (73)

Patients with PSC frequently have coexisting gastrointestinal conditions, with the majority experiencing inflammatory bowel disease, and they are also at an increased risk of developing cholangiocarcinoma and colorectal cancer. (73)

The etiology of PSC is not understood, however, there has been progress in understanding certain susceptibility factors, with more than 20 genes linked to the condition as well as environmental factors contributing to the disease. From an immunological viewpoint, studies suggest a significant role of adaptive immune activity in PSC, with gut-derived antigens potentially acting as triggers. (73)

To date there is no approved pharmacological treatment for PSC, but liver transplantation for those who suffer from recurrent episodes of cholangitis or progressive liver cirrhosis. However, it is important to screen PSC patients for cancer development on regular intervals with regular imaging and colonoscopy.

1.3.4.3 Sepsis-induced cholestasis

In sepsis, cholestasis can develop by various mechanisms. Hypoxic hepatitis may happen when the liver does not receive adequate oxygenation, due to cardiac or pulmonary failure during sepsis. Additionally, hepatic hypoperfusion can occur as a result of imbalanced vasoconstriction and vasodilation, alongside the influence of endotoxins and inflammatory cytokines released in sepsis. (8)

Several key transport proteins are involved in the development of cholestasis in sepsis: NTCP, which mediates the uptake of bile acids from plasma into hepatocytes; OATP, which is responsible for the import of conjugated and unconjugated bile acids as well as unconjugated bilirubin into liver cells; the canalicular bile sort export pump BSEP; MRP2, which exports conjugated bile acids into bile; and MRP3 and MRP4, which are involved in the discharge of bile acids into the bloodstream. (8)

During sepsis, TNF-α and and interleukin-1β downregulate NTCP and impair the uptake of bile acids from the blood into hepatocytes. Furthermore, the expression of export proteins such as BSEP and MRP2 declines, resulting in reduced bile flow and impaired bile acid secretion. (8)

Additionally, reduced levels of the nuclear receptor FXR impair bile acid homeostasis. Since FXR normally suppresses bile acid synthesis and upregulates transporters such as BSEP, its downregulation leads to increased bile acid accumulation in hepatocytes. This imbalance contributes to decreased bile flow and the development of cholestasis. (8)

1.4 Clinical Manifestations of Cholestasis

1.4.1 Maldigestion and Malabsorption

Cholestasis results in impaired bile flow and a lack of bile acids in the intestine. Since bile acids are essential for fat digestions, the lack of bile acids lead to fat maldigestion and fat malabsorption including fat soluble vitamins A, D, E and K. (74) Patients with maldigestion and malabsorption typically present with clinical features like osmotic diarrhea and steatorrhea.

In order to diagnose malabsorption, patients need to be physically examined and checked on whether they have osmotic diarrhea, meaning more than 250g of stool per day with an

osmotic gap of more than 60 mEq/l. Patients should be assessed on whether steatorrhea is present, meaning more than 7% of dietary fat in the patients' stool. (75) A laboratory test should include measurement of fat-soluble vitamins (A, D, E, and K) and electrolytes. To further identify the underlying cause, endoscopy with intestinal biopsies may be necessary, supported by radiologic studies such as abdominal ultrasound, CT, or MRI. (76)

Concerning therapy, vitamins, especially vitamin D (with a dose of 50–100 μg/kg/d) and K in cholestasis as well as minerals such as calcium should be supplemented.(77)

1.4.1.1 Osteoporosis

In cholestasis defects in calcium and magnesium homeostasis have been reported, which may lead to reduced bone mineral density. In addition, osteoblast dysfunction, which is potentially induced by retained bilirubin and bile acids, can result in decreased mean wall thickness and abnormalities in bone matrix formation. Malabsorption of fat-soluble vitamins further contributes to bone disease. Beyond the well-known role of vitamin D in calcium absorption (78), vitamin K is essential for processes such as the carboxylation of glutamyl residues in proteins like osteocalcin. Impairment of this function may promote osteoporosis, suggesting a link between vitamin K deficiency and bone demineralization in affected individuals. (79) Osteoporosis can be determined by diagnostic measures such as medical history of the patient, especially concerning fractures, physical examination and a bone density test with dual-energy X-ray absorptiometry (DXA), which is the current gold standard. Additionally, blood tests in order to assess vitamin D deficiency, hypocalcemia or reduced renal function as well as X-rays can be necessary. (80) Osteoporosis can be managed and prevented pharmacologically. Options include anti-resorptive therapy with bisphosphonates, receptive activator of nuclear κ-B ligand (RANKL) antibodies and selective estrogen receptor modulators (SERMs). Another approach is through stimulation of osteoblasts with teriparatide. (81) As mentioned earlier, supplementation, especially with vitamin D and calcium is also recommended. (77)

1.4.2 Cholestatic Pruritus

Pruritus is a prevalent symptom of cholestasis that drastically affects patients' quality of life. It can range in severity from mild and well-tolerable sensations to extreme burden that may cause sleep deprivation, depressive symptoms and even suicidal ideation. (82)

The condition can worsen with heat, contact with fabrics like wool and psychological stress, whereas coolness can improve symptoms. Cholestatic pruritus often shows a circadian rhythm with symptoms intensifying during nighttime. The palms and soles are the primary affected location but intensity of itching varies between patients. Unlike non-cholestatic pruritus, cholestatic pruritus is distinguished by the absence of primary skin lesions. However, secondary skin changes due to scratching—such as lichenification, excoriation, and follicular inflammation—may be observed. (82)

A comprehensive assessment of pruritus is important to exclude other underlying conditions. These may range from dermatological diseases to systemic illnesses like chronic renal failure or metabolic disorders such as diabetes or hypothyroidism. Additionally, malignant conditions like lymphoma should be considered during the diagnostic process. (82)

1.4.2.1 Mechanisms of Cholestatic Pruritus

The exact mechanisms behind cholestatic pruritus remain unclear; however, bile acids and endogenous opioids are thought to play significant roles in its pathophysiology. Elevated bile acid concentrations are often found in tissues of patients with liver disease, and pruritus has been reported in these cases. However, this hypothesis is challenged by observations that some patients with advanced liver failure no longer experience pruritus despite having highly elevated bile acid levels. Furthermore, not all patients with elevated bile acid parameters present with pruritus, suggesting that additional factors could be involved in its development. (83)

Endogenous opioids are another likely contributor to pruritus, as activation of μ-opioid receptors (MOR) promotes the symptom. In contrast, κ-opioid receptors have an inhibitory effect, and an imbalance between these pathways may underlie the condition. In cholestasis, this may be explained by the decreased opioid clearance ability of a patient with liver disease, leading to elevated endogenous opioid concentrations. Still, high opioid concentrations are not proportionate to the intensity of pruritus symptoms. (83)

The protein autotaxin, an enzyme that is responsible for the conversion of lysophosphatidylcholine into lipophosphatidic acid (LPA) is another potential candidate for causing pruritus in cholestasis. LPA stimulates TRPV1 channels, which are located on C-fiber nerve endings. This stimulation leads to an itch effect. (83) After intradermal injection of LPA in mice, increased scratching behavior was observed. (84)

Another candidate is the cytokine IL-31, which – in atopic dermatitis – has been found to be one of the main inducers of pruritus. IL-31 mediates inflammatory responses and stimulates itch. (85) Elevated baseline serum level of IL-31 have been reported among patients with PSC and PBC. (86)

Taken together, the pathophysiology of cholestatic pruritus is likely multifactorial, involving the contribution of endogenous opioids, increased bile acid levels as well as autotaxin and IL 31.(82)

1.4.2.2 Current Treatment of Cholestatic Pruritus

According to the EASL guideline, the recommended therapy for cholestatic pruritus follows a stepwise approach starting with bile sequestrants such as cholestyramine, colestipol or colesevelam. These medications are already in use for treating pruritus in PBC as the first line in American and European guidelines. Their function is the prevention of reuptake of bile acids in the terminal ileum through binding them, thus stopping the enterohepatic circulation and removing pruritogens. The recommended therapy is slowly increasing the dose of cholestyramine in order to reach 4 g up to four times a day after weeks or months of treatment.(87)

The second recommended step is the usage of rifampicin, which is a pregnane X receptor (PXR) agonist. The antipruritogenic effect of rifampicin is suggested to decrease ATX transcription through the activation of PXR. The recommended dose is to start with 150 mg, slowly increasing the dose up to 600 mg/day. Throughout the administration, the patient should have their liver function monitored, as there have been cases of hepatitis associated with rifampicin use.(87)

Oral opiate antagonists like naltrexone or butophanol are considered third-line options.(87) These drugs may alleviate pruritus by reducing peripheral and central activation of μ-opioid receptors and induce a change in balance towards κ-opioid receptor activation.(83) Side

effects of oral opiate antagonists may include withdrawal syndrome, headaches, dizziness as well as nausea.(87)

As a fourth-line therapy, SSRIs (Selective Serotonine Reuptake Inhibitors) such as sertraline may be used. Their antipruritic effects are thought to result from modulation of central neurotransmitter levels. Compared to rifampicin, SSRIs are generally better tolerated and exhibit lower hepatotoxicity. Adverse effects of sertraline are increased appetite, dry mouth, insomnia, headaches and even visual hallucinations.(87)

Bezafibrate, which is a peroxisome proliferator-activated receptor agonist (PPAR) is also being considered in cholangiopathies as anti-pruritogenic treatment. (82) PPARs like bezafibrat regulate energy metabolism, bile acid homeostasis and have immunomodulatory and anti-inflammatory properties.(88) Two other PPAR targeting compounds, Elafibranor and Seladelpar, are indicated as treatment for PBC and have recently successfully undergone phase 3 trials.(89)

While symptomatic relief is important, the primary target of any therapy remains the underlying hepatobiliary disease. Depending on the etiology, this may involve endoscopic or surgical interventions (for example, in cases of mechanical obstruction in obstructive cholestasis), discontinuation of a triggering substance in drug-induced cholestasis, or the use of pharmacological agents such as UDCA in intrahepatic conditions like intrahepatic cholestasis of pregnancy. (82)

Apart from systemic therapy, there have also been physical approaches to treat cholestatic pruritus. Here the objective is to remove the substance causing pruritus. One such approach is implementing nasobiliary drainage while performing an endoscopic retrograde cholangiopancreatography (ERCP). The goal is to disrupt the enterohepatic circulation of bile acids by preventing the reabsorption of potential pruritogens. (87)

Another method already used in atopic dermatitis is the usage of UV-light, purported to lead to a change in the release of cytokines from skin and blood. There have also been observations of histological alterations like a decrease in Langerhans cells and decline in Schwann cells and T-suppressor lymphocytes. (87)

In refractory cases where conventional measures fail, plasmapheresis has been regarded as an effective and safe therapeutic option for pruritus in cholestatic diseases such as PBC, PSC, intrahepatic cholestasis of pregnancy, and drug-induced cholestasis. By removing

pruritogens localized in tissue or plasma, this approach can lead to symptomatic improvement and is also applicable in pregnant individuals, where overall tolerance has been reported as good. (90)

The ultimate therapeutic approach is an orthotopic liver transplantation, which can be curative for patients with cholestatic conditions as it leads to renewal of normal bile flow. (87)

1.4.2.3 ASBT-Inhibitors: A Possible New Treatment?

A new group of drugs with antipruritogenic properties has been undergoing clinical trials focusing on ASBT inhibition. ASBT is responsible for the reabsorption of bile acids in the ileum during enterohepatic circulation. Pharmacological inhibition of this protein leads to interruption of the enterohepatic circulation of bile acids and can effectively reduce bile acid levels in cholestasis. As a side effect, the increased spillover of bile acids into the colon leads to diarrhea. Therefore, beside the treatment of cholestatic itch, ASBT inhibitors may also be used for patients that suffer from idiopathic chronic constipation (91).

In patients with Alagille Syndrome the ASBT inhibitor Maralixibat effectively reduces itch and has already been approved by the FDA for this indication. (92)

In PFIC patients, Odevixibat, which is another ASBT inhibitor, improved pruritus in children from 6 months to 18 years and significantly lowered their serum bile acids. (93) Odevixibat was largely well tolerated in the clinical trials. The most frequent side effects of ASBT inhibitors in the reported clinical studies were diarrhea, elevated transaminases and vomiting. One important thing to note, however, is the proper supplementation of fat-soluble vitamins, as a decline in already existing deficiency of fat-soluble vitamins has been reported in some patients receiving the high dose treatment. (93)

2. Objective

The objective of this thesis is to search for patients that are suspected with hereditary forms of cholestasis and to report on their genetic mutation and their clinical presentation. A particular focus is the presence of pruritus and the treatment to relieve itching. The pruritus focus is of particular interest since with the recent development of bile acid reuptake inhibitors, i.e. ASBT inhibitors, effective future drugs are available for the treatment of cholestatic itch.

The work consists of three parts: a literature search focused on hereditary cholestasis syndromes (with a brief overview on acquired forms of cholestasis and hereditary forms of bilirubin disorders) and hepatic pruritus as introduction, a MEDOCS-based database search for hereditary cholestasis syndromes and associated genetic mutations, and finally the clinical correlation of the cases and mutations identified in the database search with various clinical parameters and symptoms. For this, we searched for both the clinical diagnostic terms of hereditary cholestasis syndromes (i.e. PFIC, BRIC and Alagille), and directly for mutations in the relevant genes for hereditary cholestasis syndromes (i.e. ABCB11, ABCB4, JAG1). We then attempted to link the mutation to a clinical phenotype (i.e. cryptogenic or recurrent cholestasis, ICP, DILI, LPAC). The overall aim is to identify pediatric and adult patients with hereditary triggered cholestasis and record their clinical presentation retrospectively, particularly with regard to pruritus.

3. Methods

3.1 Study Design and Source of Data

We conducted a retrospective study of patients with presumable hereditary cholestatic liver diseases at the Medical University Hospital Graz over a 20-year period (January 2004 to April 2024). Patient identification was enabled through the assistance of the Institute for Medical Informatics, Statistics and Information (IMI) from the Medical University of Graz. Identification of potential cases was based on search terms as outlined below in “3.2. Patient population”. The IMI provided cases as a list of patient identification numbers and the relevant search term. Patient data were then investigated using the hospital documentation system MEDOCS. All relevant hepatological records were screened, and the relevant information was extracted into a Microsoft Excel spreadsheet. The dataset underwent

pseudo-anonymization before further analysis so that patient identifiers were not linked to clinical data. Access to personally identifying information was limited to individuals directly involved in this thesis. Ethical approval (36-081 ex 23/24) was granted by the Ethics Committee of the Medical University of Graz.

3.2 Patient Population

Adults and children (<18 years) with one or more of the following confirmed or suspected diagnoses were eligible: hereditary cholestasis, PFIC (all types), BRIC, Alagille Syndrome, biliary atresia, LPAC syndrome, Dubin-Johnson syndrome, Crigler-Najjar syndrome, Rotor syndrome, or mutations/suspected mutations in ABCB4, ABCB11, ATP8B1, JAG1.

Inclusion was independent of sex, age, comorbidities or any laboratory parameters. Exclusion criteria were insufficient information in medical records, irrelevance to the listed hereditary conditions, acquired forms of cholestasis or duplicate cases (e.g. same patient in the children and adult cohort after transition).

For further analysis, patients were separated into two groups dependent on the time at diagnosis: adults (⩾18 years) and pediatric patients (<18 years).

3.3 Data Collection

The following variables were extracted from medical records:

- Genetic data – if available – such as mutated gene, identified mutations, and zygosity (homozygous or heterozygous).
- Clinical data including the date and clinical symptoms at first contact with the Department of Gastroenterology & Hepatology for adults or the Department of General Pediatrics for children, age at diagnosis, pruritus, therapy received, disease progression, and last follow-up.
- Hepatologic laboratory parameters including ALT, AST, GGT, alkaline phosphatase (AP), bilirubin, and bile salts if available (first, highest, and last available values).
- Other clinical features included the presence of pruritus during lifetime, concomitant diseases, and relevant cofactors (e.g., medications, infections).

4. Results

Phenotypes and Genotypes of the Patient Cohort

According to our search criteria we initially identified 97 patients with a presumable hereditary cholestatic liver disease and/or mutation in genes associated with hereditary cholestasis. 9 patients were excluded for various reasons: two patients with too little information on the case, one patient was a duplicate in the children and adult cohort, and six patients had irrelevant diagnoses not associated with hereditary cholestasis. Of the remaining 88 individuals, 64 were children and 24 adults at time of diagnostic work-up.

On a clinical-diagnostic level, one child was diagnosed with PFIC-1, although no specific mutation was recorded. One child had PFIC-2, and another was suspected of PFIC-3, though no definitive diagnosis was available. One additional pediatric patient was suspected of having PFIC without further classification or mutation data.

Four children were diagnosed with Alagille syndrome, with two more suspected cases lacking definitive confirmation in the medical records. Seven children were diagnosed with biliary atresia; in one of these cases, a JAG1 mutation was identified, and the diagnosis was later revised to Alagille syndrome.

Seven children were diagnosed with Gilbert's Syndrome, with one additional suspected case. One child was suspected of having DJS, though the diagnosis remained unconfirmed.

Among 26 children with genetic information but no clear clinical diagnosis, 21 carried ABCB11 mutations (9 homozygous, 11 heterozygous, and 1 with undocumented zygosity). Three children had ABCB4 mutations (two heterozygous, one not specified). The remaining two children included one with a heterozygous NOD2 mutation and one with microdeletion syndrome (zygosity unclear). Genetic testing has been performed due to suspected hereditary cholestasis. Hereditary cholestasis has been suspected due to multiple reasons: early presentation with cholecystolithiasis, neonatal cholestasis and prolonged jaundice, as well as exacerbated liver enzymes after infections such as EBV.

Twelve additional children were suspected of hereditary cholestasis, but no mutation or definite diagnosis could be made.

Clinical Findings	Children
PFIC-1	1
PFIC-2	1
PFIC-3	0
Suspected PFIC-3	2
PFIC (not further specified)	1
Alagille-Syndrome	4
Suspected Alagille-Syndrome	1
Biliary Atresia	7
LPAC-Syndrome	0
Suspected LPAC-Syndrome	0
Crigler-Najjar-Syndrome	0
Gilbert's Syndrome	7
Suspected Gilbert's Syndrome	1
Suspected Dubin-Johnson-Syndrome	1
Mutations are present, but no clear clinical diagnosis	26
Mutation in ABCB11	21
Mutation in ABCB4	3
Mutation in NOD2	1
Microdeletion syndrome	1
Cryptogenic Cholestasis (neither clinical diagnosis nor mutation identified)	12
Total number	**64**

Table 2: Number of Pediatric Patients Sorted by Clinical Findings

For the 24 adult patients, one patient was diagnosed with PFIC-3 during pregnancy. Two patients had LPAC syndrome, with one additional suspected case. One adult was diagnosed with CN2. One individual was suspected of having Alagille Syndrome with DILI as a differential diagnosis. Three adults were diagnosed with Gilbert's Syndrome.

Among 12 adults with genetic information but without a final clinical diagnosis, nine carried ABCB4 mutations (two heterozygous, the rest not further specified). One patient had an ABCB11 mutation (zygosity not documented), and two patients had ATP8B1 mutations (zygosity not reported). As with the pediatric cases, genetic testing was done in adults with suspected hereditary cholestasis. Suspicion also arose because several individuals presented with conditions such as ICP, recurrent or early onset choledocholithiasis, recurrent biliary pancreatitis, unexplained chronic cholestasis or DILI, .

Three adults were suspected of hereditary cholestasis. Of these, one was diagnosed with ICP, one with DILI, and one remained classified as suspected hereditary cholestasis without a confirmed diagnosis or genetic finding.

Clinical Findings	Adults
PFIC-1	0
PFIC-2	0
PFIC-3*	1
*additional presentation with ICP	1
Suspected PFIC-3	0
PFIC (not further specified)	0
Alagille-Syndrome	0
Suspected Alagille-Syndrome	1
Biliary Atresia	0
LPAC-Syndrome**	2
** additional presentation with ICP	1
Suspected LPAC-Syndrome	1
Crigler-Najjar-Syndrome	1
Gilbert's Syndrome	3
Suspected Gilbert's Syndrome	0
Suspected Dubin-Johnson-Syndrome	0
Mutations are present, but no clear clinical diagnosis	12
Mutation in ABCB4* **	9
**** presented with ICP**	4
Mutation in ABCB11	1
Mutation in ATP8B1	2
Cholestasis, neither clinical diagnosis nor mutation identified	3
ICP	1
DILI	1
Suspected hereditary cholestasis	1
Total number	**24**

Table 3: Number of Adult Patients Sorted by Clinical Findings

On an overall genetic level, 50 out of 88 patients had a mutation found in a specific cholestasis-related gene, with mutations in ABCB11 dominating in 32 individuals (2 adults, 30 children). Among these, eight children and one adult were finally diagnosed with Gilbert's Syndrome (i.e. mutations in the UGT1A1 promotor as likely reason for jaundice). The second most common group of mutations affected the ABCB4 gene (13 adults, 3 children), with six adults diagnosed with ICP, one case of PFIC-3 and two cases of LPAC. Six mutations were detected in the UGT1A1 gene (0 adults, 6 children) (of which all patients also had a mutation in ABCB11 and were diagnosed with Gilbert's Syndrome), two cases of JAG1 mutations (0 adults, 2 children) defining Alagille syndrome, two in ATP8B1 (2 adults, 0 children) (of which one patient also had a mutation in ABCB11). Both patients with ATP8B1 were diagnosed with DILI. Two pediatric patients were diagnosed with Alagille Syndrome (with no further information on the case), another presented with a NOD2 mutation with no further diagnosis being documented. One pediatric case was diagnosed with microdeletion syndrome, which is a group of syndromes consisting of microdeletions

of nearby gene loci in a specific chromosome region (94). Furthermore, 7 adults and 26 children were suspected of having hereditary cholestasis, however no genetic testing has been done at the current point (Figure 2).

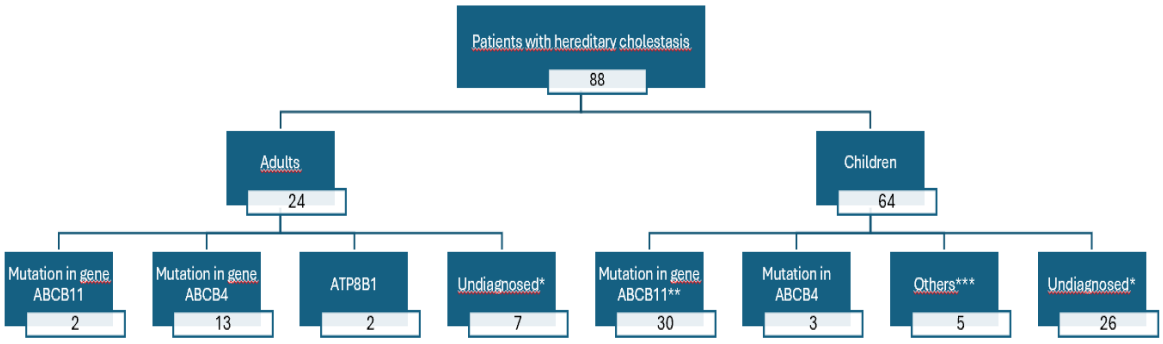

Figure 2: Patients with Possible Hereditary Cholestasis with Specific Mutations Sorted by Age at Diagnosis
**suspected as hereditary cholestasis but no specific diagnosis made yet through genetic testing*
***There is one patient who has both a mutation in ABCB11 and in ABCB4*
****Mutations in the following genes: 1 patient with Alagille, 2 patients with JAG1, 1 patient with NOD2, 1 patient with a micro deletion syndrome in 17q12, 1 patient with ATP8B1*

Sorting the patients further by sex, the majority of adult patients were female (16 out of 24 patients), whereas the majority of underage patients were male (44 out of 64 patients) (Figure 3).

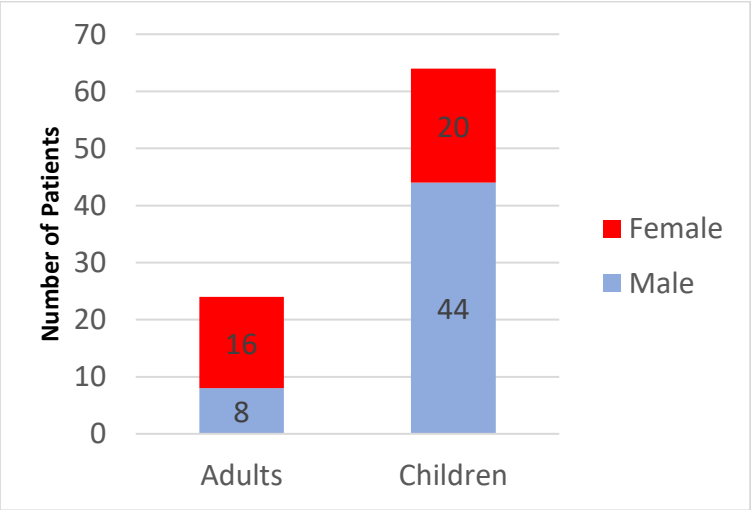

Figure 3: Patients with Possible Hereditary Cholestasis Sorted by Sex and Age at Diagnosis

Among adult females, seven cases of ICP were reported. All but one patient (for whom no further genetic data are available) carried an ABCB4 mutation. Regarding zygosity, two were heterozygous, while in the remaining four cases it was unclear whether the mutation was monoallelic or biallelic. Among these, one female patient was later diagnosed with LPAC. All females diagnosed with ICP were below 40 years old at the time of diagnosis, the youngest patient was 25 years of age.

Genotype of Patients with Jaundice at Diagnosis

We further analyzed patients who presented with visible jaundice as most remarkable sign of cholestasis the time of diagnosis (Figure 4). Among the 88 patients, 22 (25%) showed signs of visible jaundice, the majority of whom were children (20 patients). Of the two adult patients with jaundice, one had a homozygous mutation in ABCB11 with the common V444A polymorphism (meaning the replacement of the amino acid valine by alanine at position 444 of the gene ABCB11 (95)), while the other patient had a missense mutation in ABCB4 with c.3227G > A (meaning a substitution of guanine to adenine at position 3227). It has not been documented whether this is mono- or biallelic, but the patient was diagnosed with PFIC-3 assuming that the mutation is homozygous or compound-heterozygous. Among the 20 pediatric cases, 11 had mutations in ABCB11, five were homozygous, five heterozygous, and one undiagnosed patient who has not undergone genetic testing. All the homo- and heterozygous patients were diagnosed with the common ABCB11 polymorphism V444A. From these, six patients were diagnosed below the age of one, with four of them being documented with prolonged neonatal jaundice. In six children, the presence of mutations could not be determined. The remaining cases included two patients with JAG1 mutations featuring Alagille syndrome and one with microdeletion syndrome.

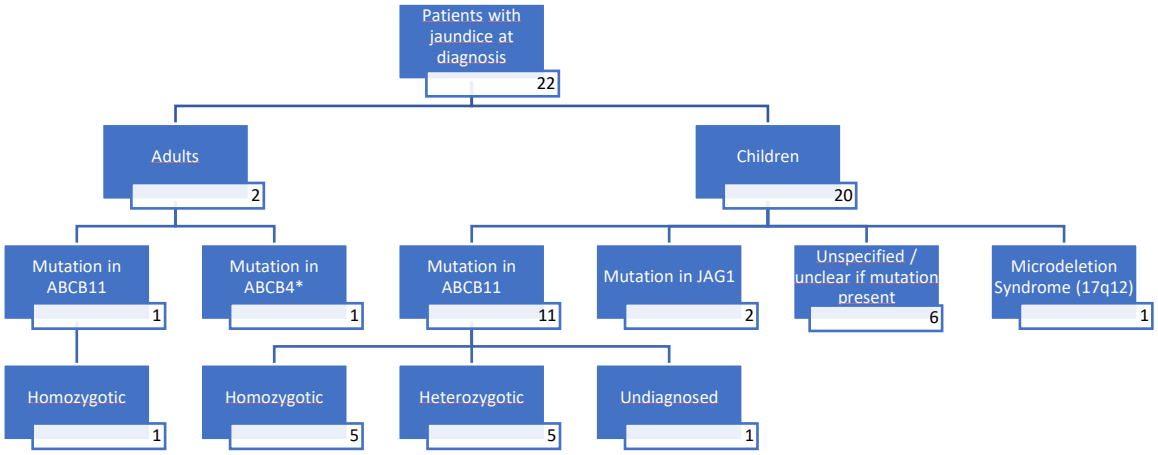

Figure 4:Overview of Patients with Jaundice at Diagnosis

A noteworthy finding was that jaundice was triggered by infection in eight patients, specifically due to an Epstein-Barr virus (EBV) infection (Figure 5). Further analysis revealed that five patients who experienced jaundice had mutations in ABCB11, three were heterozygous and two homozygous. Additionally, one patient had a heterozygous mutation in NOD2, while the remaining two had no identified genetic diagnosis.

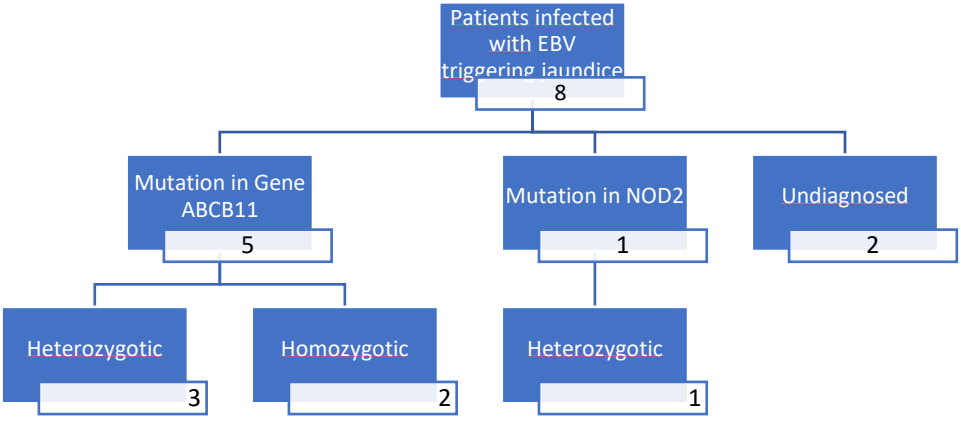

Figure 5: Overview of Patients Infected with EBV Triggering Jaundice and Mutations

Genotype of Patients with Pruritus at Diagnosis

Next, we determined the patients who reported pruritus as a symptom (Figure 6). Overall, nineteen out of eighty-eight patients showed pruritus during their disease course. Two of

them, however, likely had other cause of pruritus than cholestatic liver disease. One pediatric patient diagnosed with a heterozygous mutation in ABCB11 reported having hand, foot, and mouth disease with pruritus present during this diagnosis, the other individual was affected by scabies (this patient was also diagnosed with a heterozygous mutation in ABCB11.

The remaining seventeen patients consist of eight children and nine adults. Of the eight children, two had a homozygotic mutation in ABCB11, two had a heterozygotic mutation in ABCB11, in one patient only the mutation in ABCB11 was known without further information. Two children had an unknown cause for pruritus (the patients were still included because of a later suspicion of having a hereditary cholestatic disease, however there is no further documentation present) and one patient was diagnosed with a mutation in JAG1, thus resembling Alagille syndrome.

The adults had a different spectrum of mutations, with four patients being diagnosed with a mutation in ABCB4 and one patient in ATP8B1. The other four did not have a specific mutation.

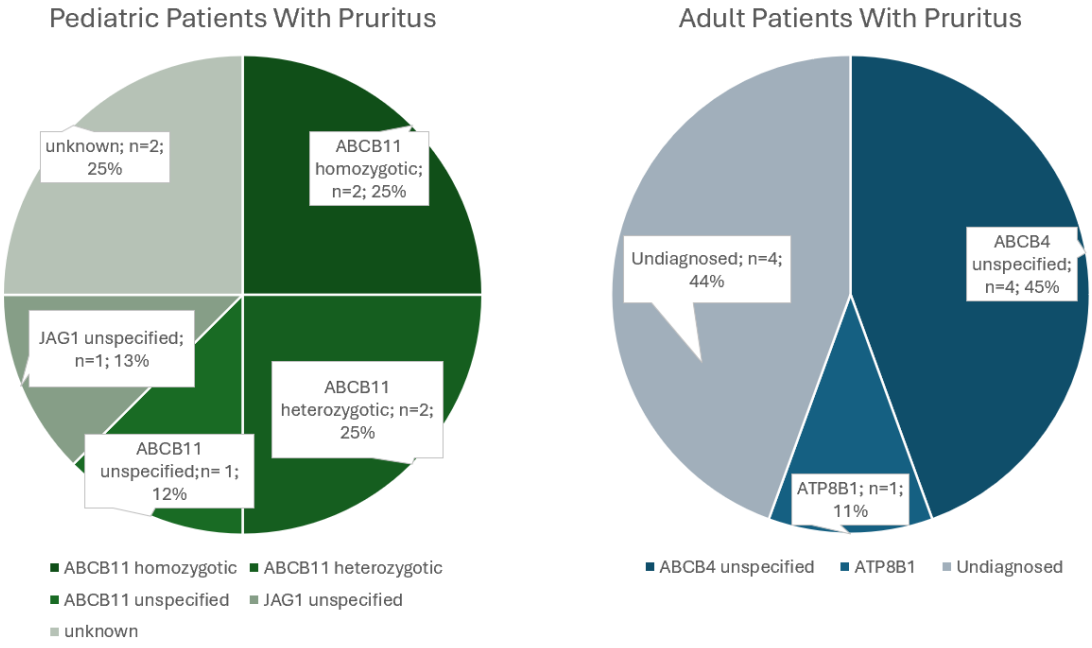

Figure 6: Patients which Reported Pruritus Sorted by Age and Mutation

Symptoms of Patients at Diagnosis

Apart from pruritus and jaundice, a wide range of different symptoms could be observed, with some patients presenting with multiple symptoms (Figure 7).

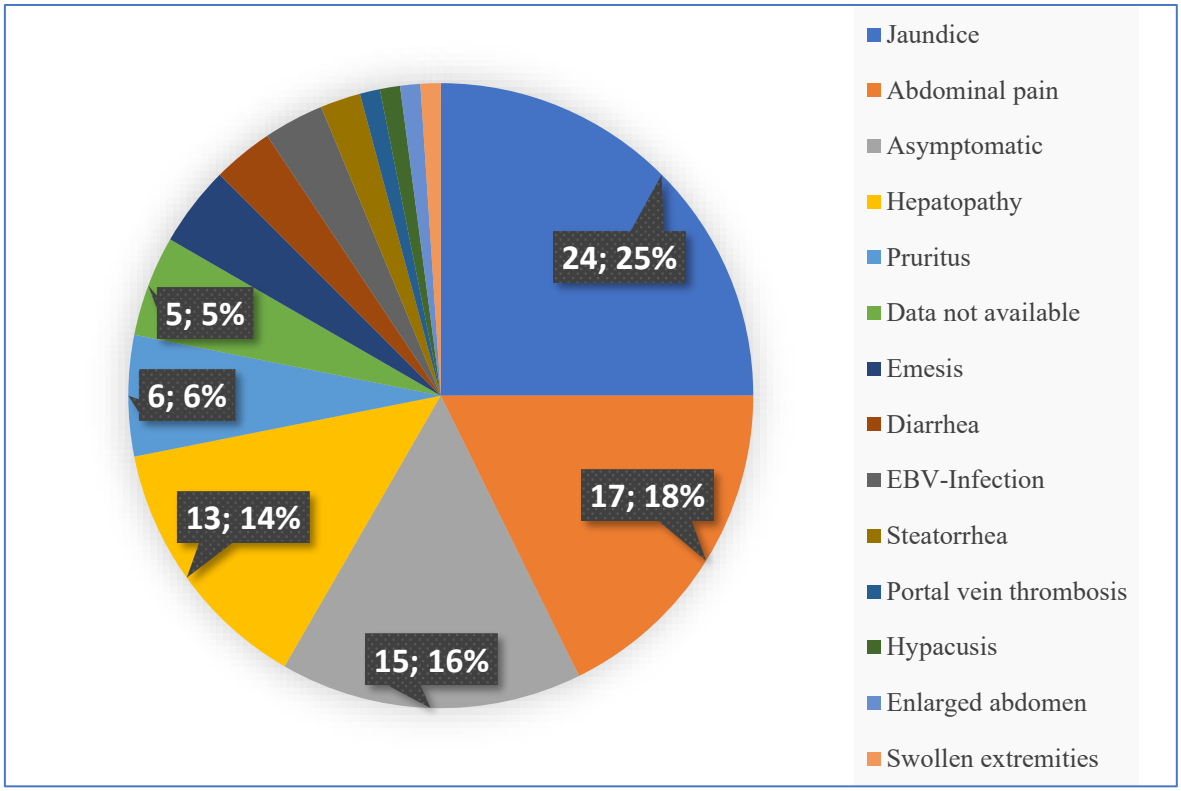

Figure 7: Most Common Symptoms at Diagnosis; Please note that this graphic is representative of the aforementioned eighty-eight patients. The absolute number of symptoms is higher because some patients reported multiple.

The most frequently reported symptom at diagnosis was jaundice, occurring in 25% of cases, followed by abdominal pain in 18%. Asymptomatic patients made up the third largest group at 15%. An additional 8% presented with hepatopathy (which includes elevated liver enzymes), and 5% showed unspecified signs of cholestasis that were not further detailed. Pruritus was noted in 6% of cases, emesis was reported in 4%. Less common symptoms included diarrhea and clinical signs of Epstein–Barr virus (EBV) infection, and steatorrhea in 2%. Rare symptoms, each seen in 1% of patients, included hypacusis and neck pain (though no genetic testing has been done on the patient – differential diagnoses include drug induced toxicity or Alagille Syndrome), swollen extremities, portal vein thrombosis, and abdominal distension.

Use of Ursodeoxycholic Acid as Therapy

UDCA was administered in 32 out of 88 patients, including 19 children and 13 adults. UDCA treatment was initiated across a range of genetic mutations, with considerable diversity among pediatric patients. Among the children receiving UDCA, three had homozygous ABCB11 mutations, two had heterozygous mutations, and one had an ABCB11 mutation that was not further specified. Additional cases included patients with JAG1 mutations, microdeletion syndrome, one with an unspecified ABCB4 mutation, and another with a heterozygous ABCB4 mutation. Nine children were treated with UDCA despite having either undiagnosed or unclear genetic findings. Among the adults, three patients had heterozygous ABCB4 mutations, eight had unspecified ABCB4 mutations, and two had no confirmed genetic diagnosis. Of these patients, seven (who were first diagnosed when they were children) are documented to have discontinued UDCA

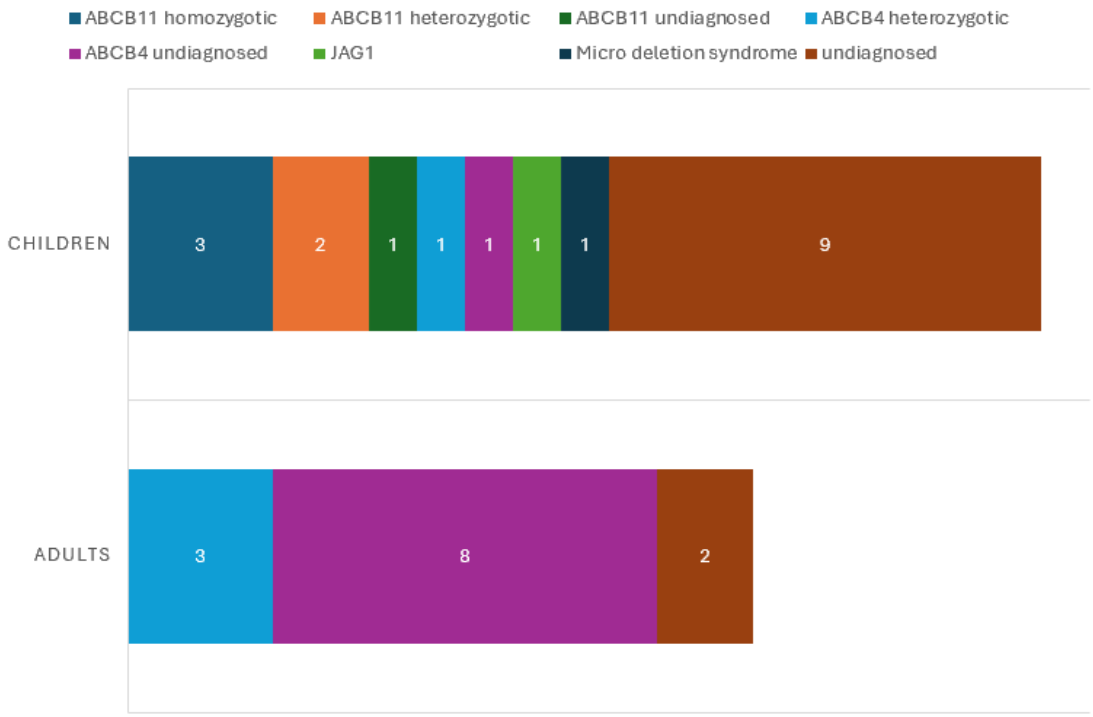

Figure 8: Patients with UDCA Therapy Sorted by Age and Mutation

Regarding therapy of cholestatic pruritus, only two out of 9 adult patients with pruritus are documented to have received specific treatment for pruritus, one having a mutation in ATP8B1 (treated with cholestyramine, naloxone, naltrexone and sertraline) whereas no mutation has been diagnosed in the other individual (treated with cholestyramine and

naltrexone). No reports on children with pruritus and their anti-pruritogenic therapy could be found in our review of medical records.

5. Discussion

Our search for patients with presumable hereditary cholestasis or mutations in genes typically related to genetic cholestasis revealed 88 cases. In these 88 patients the most frequent mutations were in ABCB11 and ABCB4. Mutations in ABCB4 were more frequent in adults, whereas ABCB11 mutations dominated in pediatric cases. This distribution aligns with the established role of ABCB11 mutations in early-onset diseases such as PFIC-2 and BRIC2, where first episodes can occur before the age of six months (96, 97). In contrast, ABCB4 mutations are more often associated with adult-onset conditions. For example, LPAC syndrome is often diagnosed retrospectively, since patients present with recurrent biliary symptoms even after cholecystectomy. Early diagnosis of LPAC is further complicated by the fact that many patients initially have asymptomatic cholelithiasis. (98)

Interestingly, zygosity did not seem to influence whether patients with ABCB11 mutations presented with jaundice at diagnosis. Among the 12 documented cases, six were homozygous, five heterozygous, and in one patient zygosity was not determined. This observation highlights the marked phenotypic variability, which depends on the type of mutation and the remaining function of the mutated protein (96). A similar pattern was observed in patients with pruritus. Among children with ABCB11 mutations, two homozygous and two heterozygous patients presented with this symptom.

ABCB11 mutations can result in a range of phenotypes, from milder cases like BRIC-2 to more severe forms like PFIC-2. The phenotype largely depends on the remaining activity of the mutated BSEP protein. BSEP has high capacity, and it is suggested that 20-25% of BSEP activity is sufficient for normal function. The common variant V444A, which is present in up to three quarters of the worldwide population (97, 99), reduces BSEP function to 50% (23). We found that 29 patients in our cohort had this particular mutation, meaning reduced baseline BSEP function. Additional triggers may have further reduced BSEP function leading to presentation with cholestasis.

For ABCB4, the second frequently mutated gene in our cohort, the phenotype to genotype is more directly correlated. Whereas the remaining protein function of 25% is sufficient for

BSEP, this is likely not the case for MDR3. Even heterozygous ABCB4 variants can be predisposed to cholangiopathy and chronic liver disease. Evidence suggests a more linear relationship between MDR3 activity and clinical phenotype, with reduced function even in heterozygotes being sufficient to cause disease. Importantly, biliary pathology has been reported across all genotype severities, including milder variants. (23) We found heterozygotes with LPAC syndrome or pregnancy-related cholestasis, yet no documented cases of homozygous patients. One patient had a missense mutation in ABCB4 with c.3227G > A with probably significantly reduced function and was diagnosed with PFIC-3.

The presentation of patients differed by age: children, mostly male (44/64) showed more often signs of jaundice and ABCB11-associated disease, whereas in our adult group patients were predominantly female (16/24) and more often had ABCB4 variants in combination with biliary pain. This finding reflects other clinical studies focusing on adults with ABCB4-related liver diseases, reporting higher rates especially among young females. (100) One possible explanation for the female predominance among adult patients is the well-documented triggering of cholestasis during pregnancy in individuals with a genetic predisposition, such as ABCB4 mutations. (101) In our cohort, 7 out of 24 adult patients were diagnosed with ICP, with six of them documented to carry a mutation in ABCB4, further underlining the central role of ABCB4 dysfunction in pregnancy-related cholestasis. The early-onset presentation in pediatric patients is consistent with canalicular transporter defects such as PFIC. These patients typically present with neonatal or infant cholestasis, jaundice, or pruritus. Thus, in children, suspicion is raised mainly by jaundice or neonatal cholestasis, while in adults it is biliary pain and gallstone-related symptoms.

Jaundice was the most common initial symptom, although it occurred in only 25% of patients. In our cohort, pruritus was infrequent at the time of diagnosis in only around 6% of cases but present in approximately 22% across the disease course. Concerning genetics, pruritus in children mostly clustered with ABCB11 variants, whereas in adults, patients with mutations in ABCB4 and ATP8B1 were affected. With regard to therapy, UDCA was the most frequently used treatment, administered to 32 out of 88 patients. However, it is not specifically antipruritic and was discontinued in many cases. Only two patients were documented to have received antipruritogenic treatment with opioid antagonists, suggesting undertreatment or under-recognition and highlighting the need for additional therapeutic options in the future. Apparently, pruritus and its therapy were underreported in our study. As pruritus can be a severe factor in quality of life, recently approved antipruritogenic

medications like ASBT-inhibitors could be useful. Apart from these, standard options include bile sequestrants like cholestyramine, rifampicin and SSRIs (93).

Interestingly, we found several patients who had Ebstein-Barr Virus infection exacerbating cholestasis in patients with an underlying mutation. Eight patients in our cohort, five presenting with a mutation in ABCB11 showed signs of cholestasis as part of an EBV-infection. Compared to the general clinical presentation, EBV is a rare cause for cholestasis with severe cholestasis in cholestatic EBV hepatitis only documented in 5% (102). In our relatively small cohort, EBV appears to be clustered with patients having ABCB11 mutations. This suggests that EBV may act as a trigger for cholestasis in genetically susceptible patients. (102)

Limitations

Due to data protection and safety regulations, detailed information on the exact type and allelic status of mutations was not retrievable for all patients. This restricted our ability to establish robust genotype–phenotype correlations. Furthermore, most genetic analyses were limited to the classical PFIC1–3 genes, without broader cholestatic panels, which may have missed additional relevant variants.

Due to this thesis being a retrospective analysis, data availability was limited. Follow-up was not possible, as many patients had their last contact with our institution several years ago, and their current clinical status remains unknown. In addition, our hospital documentation system (MEDOCS) was only implemented in 2004, leaving incomplete or missing records for patients in our cohort who were born before that time. For many adult patients, only minimal information about their first presentation was available, often restricted to document scans. In some cases, particularly among international patients who were seen only once, there was no further information concerning their clinical status.

As a referral cohort from a tertiary center, our sample may over-represent more severe or complex cases and is not necessarily representative of population-level hereditary cholestasis, possibly introducing referral bias. The relatively small cohort size, which includes numerous different subtypes of hereditary cholestasis, limits firm conclusions regarding genotype–phenotype associations.

Conclusion

Our findings underline the clinical and genetic heterogeneity of hereditary cholestasis, with ABCB11 and ABCB4 mutations being most frequent, but with highly variable phenotypes that cannot be explained by genotype alone. Pregnancy, infections, and other environmental factors potentially act as disease modifiers, especially in patients with a predisposition (e.g. V444A polymorphism in our cohort). Pruritus, which is a major determinant of quality of life, was found to be underreported and undertreated in this cohort. This is particularly important to address in future clinical visits and documentations, since potent antipruritogenic medication are nowadays available. For improved diagnosis and management, more comprehensive genetic testing should be considered, along with systematic clinical documentation of symptoms such as pruritus and treatment responses. Future studies should integrate genotype and clinical data to refine diagnostic algorithms and allow earlier identification of at-risk patients, thereby managing timely intervention with emerging therapies such as ASBT-inhibitors.

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