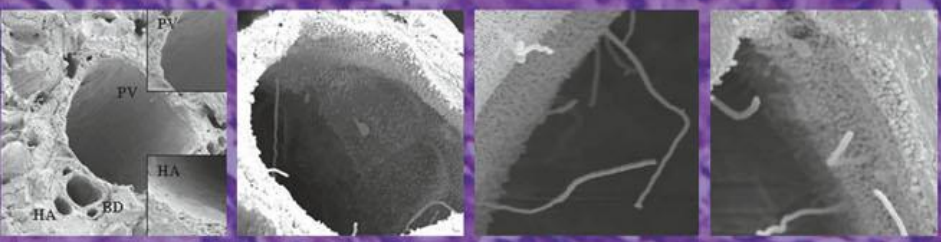
Fibrocystic Diseases of the Liver

Edited by

Karen F. Murray, MD
Anne M. Larson, MD



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To my husband Bernie and children Michael and Katie, for their love, understanding, and ongoing encouragement and support; to my parents for their love and encouragement; and to Dr. Jay Perman for rousing my interest in pediatric gastroenterology, guidance in academic pediatrics, and for his friendship.

Karen F. Murray, MD

Preface

It has been recognized for centuries that cystic pathology of the liver and kidneys, and occasionally other organs, can occur in the same individual or within families and may have grave consequences. We have learned over time that the inheritance pattern seen in many of these conditions honors Mendelian genetics and that some conditions have non-cystic syndromic associations. Our ability to categorize these conditions has historically relied upon descriptive features observed postmortem, surgically, or through radiological techniques. Over the last decades, however, our understanding of the embryology, cellular pathogenesis, and genetic basis of these conditions has permitted clearer clinical diagnoses and the ability to prognosticate and offer therapies.

This is the first text to focus entirely on the fibrocystic diseases that affect the liver and the state-of-the-art research that underlies our current understanding of these conditions. We have brought together experts in the related fields of hepatic fibrocystic disease. The book provides a clear, in depth, and well-illustrated understanding of the embryology and development of the ductal plate, cholangiocyte biology, and the role of the biliary cilia in the pathogenesis of these conditions. An update review of the complex genetics of these disorders is reviewed in detail to allow further understanding of the molecular pathogenesis of the conditions as well as the clinical phenotypes encountered. Additionally, the text reviews the radiological and pathological methods important in diagnosis, the many clinical manifestations of these conditions and associated features and syndromes, the potential complications encountered in caring for affected individuals, and the treatments available to ameliorate the symptoms, progression, or complications of these diseases.

The text has incorporated a wealth of figures to illustrate the concepts described and to serve as reference for pathological and radiological findings of these conditions.

This book's purpose is to serve as a reference for those caring for patients with fibrocystic diseases affecting the liver and to offer an authoritative analysis of the cause of these conditions, their clinical manifestations, and the available strategies for managing for them. Additionally, it is the hope of the editors that this text will provide a

unique conglomeration of the knowledge to date of these conditions and hence serve as the nidus for further research advancements in the understanding and treatment of these conditions.

Seattle, 2009 ***Karen F. Murray, MD***
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I Pathophysiology and Development

1 Embryology and Development of the Ductal Plate

Clifford W. Bogue, MD

CONTENTS

Summary

Proper development of both the intrahepatic and the extrahepatic biliary tracts is critical to the proper functions of the liver related to bile production and excretion. Many disorders that result in fibrocystic disease of the liver have, at their origin, a defect in the early development of the liver. In particular, disordered development of the ductal plate can lead to severely debilitating hepatobiliary disorders such as biliary atresia and fibrocystic disease of the liver. In this chapter, I review current knowledge of the embryological development of the extrahepatic and intrahepatic biliary trees with a focus on the genes and molecular pathways that have recently been shown to play

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critical roles in biliary development. Although the identification of the genetic pathways involved in biliary development is still quite incomplete, recent progress has shown that there are a number of transcription factors and signaling pathways that regulate both hepatoblast differentiation and biliary morphogenesis. Building on this exciting new knowledge will provide new avenues for both the diagnosis and the treatment of debilitating diseases that, up to this point, have no definitive treatment or cure.

Key Words: Ductal plate, Embryology, Morphogenesis, Bile duct

INTRODUCTION

Bile production is an essential function of the liver. Bile, which is produced by hepatocytes, is initially secreted into bile canaliculi. The canaliculi are connected to a network of intrahepatic bile ducts (IHBD), which transport bile into the extrahepatic biliary tract (EHBT), comprised of the hepatic, cystic, and common bile ducts plus the gallbladder. Both the IHBD and the EHBT are lined by specialized epithelial cells termed cholangiocytes or biliary epithelial cells (BEC). Over the past several years, much attention has been paid to the early events in liver development, such as endoderm development and hepatic specification (reviewed in [1]). Much less is known about the differentiation of bile duct epithelium and morphogenesis of the biliary tract. However, recent progress by our laboratory as well as others has shed light on some molecular mechanisms of biliary tract differentiation and morphogenesis.

LIVER SPECIFICATION AND BUDDING

During embryogenesis, the primitive gut forms during gastrulation and is gradually patterned along its anterior–posterior axis into the fore-, mid- and hindgut domains. Present in the foregut are the common endodermal precursors of the liver, pancreas, thyroid, and lung. A series of reciprocal tissue interactions between the foregut endoderm and the adjacent mesoderm progressively subdivides these broad primitive gut tube domains into smaller regions that ultimately give rise to discrete organs (Fig. 1.1). In mammals, the liver is derived from both the endoderm of the ventral foregut and the adjacent mesenchyme in the septum transversum. In the mouse, fate-mapping experiments reveal that the liver arises from lateral domains of endoderm and the developing foregut [2, 3] as well as from a small group of endodermal cells tracking down the ventral midline [3]. In the mouse

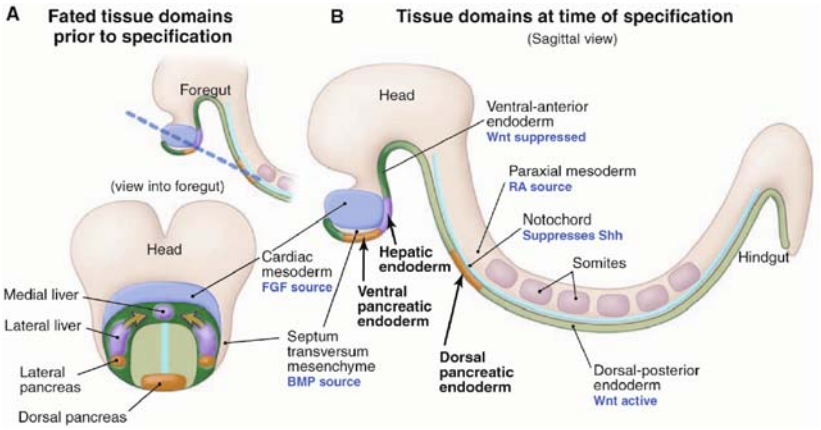

Fig. 1.1. Cell domains and signals for embryonic liver and pancreas specification. (**a**) Fate map of progenitor cell domains before tissue induction; view is into the foregut of an idealized mouse embryo at E8.25 (three- to four-somite stage). *Arrows* indicate movement of lateral progenitor regions toward the ventral-medial region. (**b**) Sagittal view of a mouse embryo several hours later than in (a) showing the positions of the newly specified liver and pancreas tissue domains. Signals and cell sources that pattern the endoderm are shown. *Dashed blue line* indicates plane of view in (**a**). Reproduced from Zaret and Grompe [5] with permission from the American Association for the Advancement of Science.

at E8.5 [corresponding to about 3 weeks of human gestation (see Table 1.1)] the foregut undergoes morphogenetic closure during which the medial and lateral domains come together as the hepatic endoderm is specified. Based on embryo tissue recombination experiments as well as genetic experiments in a number of vertebrates species, including chick, frog, mouse, and zebrafish, both the liver and the pancreatic domains of the foregut are specified within the endodermal epithelium by inductive signals from nearby mesodermal cells (reviewed in [4, 5]). Fibroblast growth factor (FGF) from the cardiac mesoderm and bone morphogenetic protein (BMP) from the septum transversum mesenchyme (STM) coordinately induce the underlying endoderm to adopt a hepatic fate [6, 7].

Another key factor expressed in the mesoderm that plays an important role in hepatic specification is *Wnt* [8]. Initially, broad suppression of mesodermal *Wnt* and *Fgf4* signaling in the foregut permits liver and pancreas induction, whereas mesodermal *Wnt* signaling in the posterior gut suppresses liver and pancreas tissue fate and allows development of the intestine. Studies of mice with a mutation in *Hhex* reveal that the positioning of the ventral foregut relative to the cardiac domain is

Table 1.1
Timing of human and mouse liver development. The major developmental events that occur in liver development in humans and mice are shown, along with the corresponding embryonic age at which these events occur in each species

Human	*Mouse*	*Developmental event*
18 day embryo	E8.5	Thickening of foregut endodermal epithelium
23 day embryo	E9.5	Endodermal cords of hepatoblasts invade mesenchyme
8–12 weeks	E13.5–15.5	Periportal hepatoblasts express cytokeratin and ductal plate forms
12 weeks	E16.5	Ductal plate remodeling begins
25 weeks	E17.5	Ductal plate becomes discontinuous, bile ducts begin forming
35 weeks	Postnatal day 1–2	Most portal tracts have 1–2 bile ducts
Birth–4 weeks postnatal	Postnatal day 2–6	Maturation of biliary tree to >7 generations, complete regression of ductal plate

important for the ventral foregut to receive the correct FGF signal from the adjacent cardiac mesoderm and thus determines whether specific endodermal cells will be induced to either a liver or a pancreatic fate [9]. In addition to *Fgf* and *Bmp*, it appears that retinoic acid signaling, most likely from mesodermal cells in the paraxial region of the embryo, helps to further refine domains which will give rise to the liver and pancreas [10].

One major question that arises upon the identification of various mesodermal signaling factors that are necessary for liver specification is what endodermally derived transcription factor networks preexist and are necessary for liver specification and induction. Using genetic analysis, it has been shown that the forkhead box-containing transcription factors *Foxa1* and *Foxa2* are both required for hepatic specification in the mouse [11]. In embryos deficient for both of these genes in the foregut endoderm, no liver bud is formed and there is no expression of the hepatoblast marker alpha-fetoprotein (AFP). As well, $Foxa1^{-/-};Foxa2^{-/-}$ endoderm failed to induce hepatic genes in culture

in the presence of exogenous Fgf2. Thus, this study provides compelling genetic evidence that the *Foxa* factors endow the endoderm with the ability to respond to inductive signals from surrounding mesoderm. More recently, it has been shown that the gene *Hnf1b* (*vhnf1, TCF2*) plays a crucial role in hepatic specification of the foregut endoderm. Previously, this was difficult to prove because *Hnf1b*$^{-/-}$ mouse embryos died before gastrulation due to a defect in the formation of the extra-embryonic visceral endoderm. However, Lokmane et al. generated *Hnf1b* null embryos with a wild-type extra-embryonic endoderm through the use of tetraploid embryo complementation [12]. Embryos derived via tetraploid embryo complementation that are deficient in *Hnfb1* have severe liver hypoplasia and the liver that does form is composed almost entirely of cells derived from the mesenchyme – there was no expression of genes that are markers of endodermally derived hepatoblasts. Furthermore, when *Hnf1b*$^{-/-}$ endoderm is cultured with Fgf, there is no expression of albumin, indicating an inability to respond to Fgf signaling and a subsequent failure of hepatic specification. The function of *Hnf1b* in early hepatic induction is conserved in other vertebrates as evidenced by similar failure of liver induction in zebrafish embryos [12].

Once the hepatoblasts are specified, these endodermal cells undergo a morphologic transition from a cuboidal shape to a columnar one. Subsequently they become a pseudostratified columnar epithelium within the foregut. The basal lamina surrounding hepatic pseudostratified epithelium then breaks down and the cells proliferate and migrate into the surrounding stroma. The homeobox transcription factor *Hhex* controls the morphogenetic process by which the hepatoblasts become pseudostratified and is also important for the migration of hepatoblasts into the septum transversum [13]. Other genes that are also critical for this important step of bud formation include the homeobox transcription factor *Prox1*, *Onecut1*, and *Onecut2* [14, 15].

OVERVIEW OF BILIARY TRACT DEVELOPMENT

Development of the IHBD is similar in humans, rats, and mice [16]. IHBD development in the mouse begins at E13.5–14.5 when hepatoblasts close to the portal vein mesenchyme begin to express biliary-specific cytokeratins (reviewed in [17]). These cells are considered bile duct precursor cells [18]. At E15.5, these biliary precursor cells form a continuous layer of single cells around the portal vein, termed the ductal plate (Figs. 1.2 and 1.3). By E16.5, the ductal plate is bilayered and 1 day later (E17.5), focal dilations appear between the two cell layers and these dilations will give rise to bile ducts and the rest of

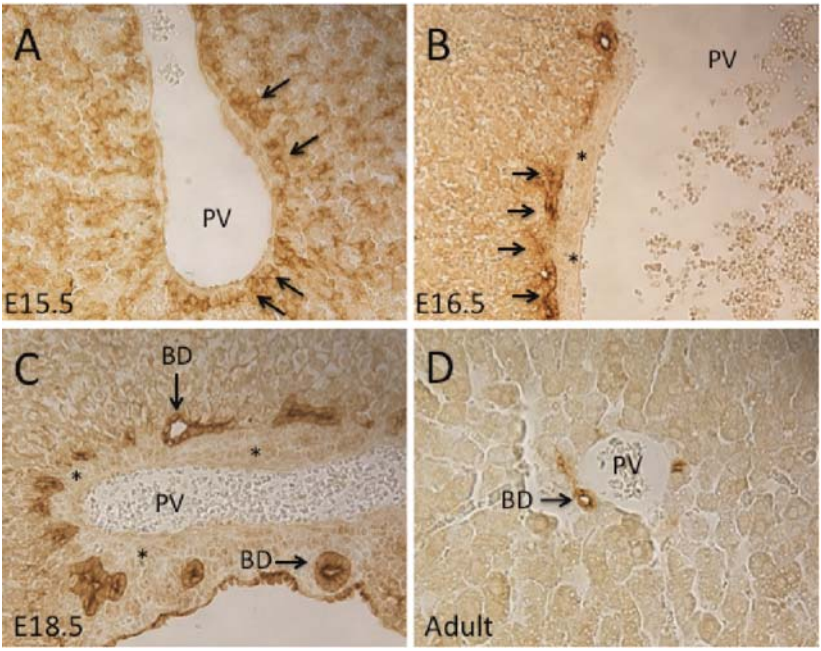

Fig. 1.2. Formation of the Ductal Plate. Cytokeratin immunohistochemistry on wild-type mouse liver showing the formation of the ductal plate. (**a**) At E15.5, cytokeratin staining is seen in hepatoblasts throughout the developing liver. However, there is increased cytokeratin expression in cells adjacent to the portal vein and these cells are beginning to form a continuous layer around the portal vein (*arrows*). (**b**) By E16.5, the developing ductal plate is composed of a bilayer of cytokeratin-expressing cells (*arrows*). At certain areas, focal dilatations appear which will become mature bile ducts. (**c**) At E18.5, the ductal plate becomes discontinuous and bile ducts are clearly present (*arrows*) and have become incorporated into the portal mesenchyme. (**d**) By the first postnatal week, the ductal plate has completely regressed and there are one to three bile ducts surrounding each portal vein (*arrow*). BD = bile duct, PV = portal vein, * = portal mesenchyme.

the ductal plate progressively regresses. At the time of birth or shortly thereafter, the ducts are incorporated into the portal mesenchyme and connect to each other forming an intricate interconnected network of ducts. It is generally agreed that BDE differentiates from hepatoblasts that are bipotential cells capable of developing into both hepatocytes and BDE (reviewed in [17, 18]). This is based upon the expression of specific immunohistochemical markers as well as transplantation experiments showing that BDE derived from early hepatoblast cell lines

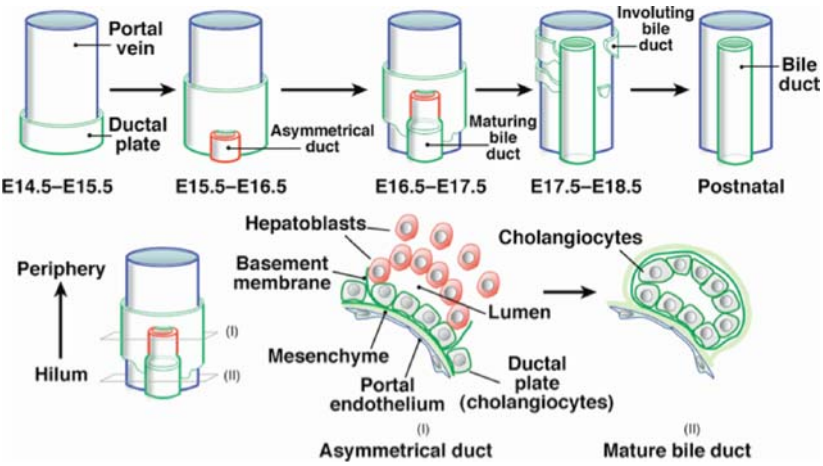

Fig. 1.3. Morphogenesis of the intrahepatic bile ducts. During bile duct development, the cholangiocytes first form a ring of cells (ductal plate) around the branches of the portal vein. Hepatoblasts then come into contact with the ductal plate and delineate the lumen of transiently asymmetrical ducts. When the hepatoblasts on the parenchymal side of the asymmetrical ducts differentiate to cholangiocytes, the ducts become symmetrical (totally delineated by cholangiocytes) and surrounded by portal mesenchyme. The ducts grow from the hilum toward the periphery of the liver lobes, which is reflected by the observation that sections at different levels along the hilum-periphery axis reveal different levels of duct maturation. Reproduced from Antoniou et al. (in press) [61] with permission from Elsevier B.V.

(i.e., bipotential mouse embryonic liver cells or BMEL cells) can differentiate into both hepatocytes and BDE in vivo [19, 20]. Interestingly, no direct genetic lineage studies have ever been performed to conclusively establish that all BDEs are derived from hepatoblasts.

HEPATOBLAST DIFFERENTIATION INTO BILIARY EPITHELIAL CELLS

Several recent studies have shed important light on the factors involved in cell-fate decisions in the hepatoblast. Clotman et al. showed convincingly that *Onecut1*$^{-/-}$ mice have ductal plate malformations (DPM), develop biliary cysts, and have agenesis of the gallbladder [21]. In particular, the livers of *Onecut1*$^{-/-}$ mice showed premature differentiation of BECs with a greater percentage of hepatoblasts differentiating into BECs. Additionally, they noted cord-like structures containing BECs extending into the hepatic parenchyma instead of being spatially limited to the areas adjacent to portal veins. Their results suggest that

Onecut1 normally restricts the differentiation of hepatoblasts into BECs quantitatively, temporally, and spatially. These authors also showed that Onecut1 transactivated the *Hnf1b* promoter in vitro, implicating *Hnf1b* as a molecular target of Onecut1. In turn, liver-specific deletion of *Hnf1b* using floxed mice bred to AFP-Cre mice revealed a critical role for *Hnf1b* in bile duct development [22]. Additional data from zebrafish studies also indicate that *Onecut1* and *Hnf1b* function within the same genetic pathway that regulates biliary development [23]. However, the zebrafish studies only support a role for *Onecut1* in bile duct morphogenesis not in hepatoblast differentiation into BECs.

Several other genes have been implicated in BEC differentiation. The expression of the *Cebpa* gene, which governs the transcription of hepatocyte-specific genes, is suppressed in periportal hepatoblasts, those cells that subsequently differentiate into BECs [24]. In $Cebpa^{-/-}$ mice, hepatocyte differentiation is suppressed and the livers of these mice develop pseudoglandular structures throughout the liver parenchyma [25]. Many of the cells in these pseudoglandular structures express high levels of *Onecut1* and *Hnf1b*, suggesting that, in the absence of *Cebpa*, the biliary differentiation program is activated, perhaps via Cebpa regulation of *Onecut1* and *Hnf1b* expression. Additionally, in the $Cepba^{-/-}$ liver, the development of *Hnf4a*-negative pseudoglandular structures preferentially near portal veins argues for the presence of biliary-inducing factors/structures in the periportal region.

The gene *Tbx3* is a member of the T-box gene family, which is a family of genes defined by a common DNA-binding T-box domain and known to play important developmental roles [26]. Functional analysis of *Tbx3* both in vivo and in vitro indicates that it plays an important role in hepatoblast proliferation and differentiation. In particular, $Tbx3^{-/-}$ livers were small, the early hepatoblasts showed a markedly decreased proliferation rate compared to wild-type mice, and the expression of Cepba and Hnf4a was decreased, while the expression of Hnf1b and Onecut1 was increased, suggesting that hepatoblast differentiation was skewed toward the biliary lineage [27, 28]. Tbx3 is known to act as a repressor of *Cdkn2a* ($p19^{Arf}$) and hepatic epithelial cells from E12.5 $Tbx3^{-/-}$ mice showed growth arrest as well as increased expression levels of *Cdkn2a* and the biliary markers CK7 and CK19 [28, 29]. Inhibiting *Cdkn2a* using short hairpin RNA returned hepatoblast proliferation to near-normal levels. As well, overexpression of *Cdkn2a* in wild-type hepatic epithelial cells suppressed proliferation and increased CK19 and CK7 expression, establishing a link between *Cdkn2a*, cellular proliferation, and hepatoblast differentiation. However, it remains unclear if there is a direct link between decreased hepatoblast

proliferation and differentiation that is biased toward BECs since mice deficient for the forkhead gene family member *Foxm1b* show decreased hepatoblast proliferation and decreased numbers of BECs [30].

More recently, Clotman and colleagues extended their studies on the role of Onecut transcription factors in liver cell-fate decisions by generating mice that had mutations in both *Onecut1* and *Onecut2* [31]. They found that the absence of both transcription factors led to hepatoblasts differentiating into hybrid cells that display characteristics of both hepatocytes and BECs as evidenced by the expression of both Hnf4a and cytokeratin. Interestingly, this finding was associated with disruption of the normal *Activin/Tgfb* expression gradient in the liver. In wild-type liver, there is a gradient of *Activin/Tgfb* expression such that *Activin/Tgfb* signaling is highest near the portal vein and decreases as the distance from the portal vein increases. In *Onecut1*$^{-/-}$*/Onecut2*$^{-/-}$ mice, this gradient was perturbed resulting in increased *Activin/Tgfb* signaling at a distance from the portal vein, which led to the dysregulated differentiation of hepatoblasts into "hybrid cells" with molecular characteristics of both hepatocytes and BECs. Thus, it appears that there is a gradient of *Activin/Tgfb* signaling around the portal vein that is modulated by Onecut factors and is required for segregation of the hepatocytic and biliary lineages. In addition, the expression level of the *Tgfb/Bmp* pathway component *Smad5* is elevated in early BECs that form the ductal plate, while expression of chordin, an antagonist of the *Tgfb/Bmp* pathway, is high in hepatoblasts that do not contribute to the ductal plate [32]. This suggests that activation of *Tgfb/Bmp* signaling correlates with hepatoblast differentiation into BECs.

The Notch signaling pathway has been shown, in multiple studies, to be necessary for normal bile duct development. The Notch pathway is an evolutionarily conserved signaling pathway in both vertebrates and invertebrates that mediates changes in gene transcription via association with the DNA-binding protein Rbpj (also known as RBP-Jκ). In general, Notch regulates patterning in the early embryo by giving fate instructions to adjacent cells, usually through the Hes/Hey family of transcriptional repressors [33, 34]. In humans, genetic mutations in *Jag1*, a Notch ligand, and in the *Notch2* receptor have been identified in patients with Alagille syndrome [35–37]. Patients with Alagille syndrome have intrahepatic bile duct paucity as well as cardiovascular and skeletal abnormalities. In vivo, as well as in vitro, studies in mice confirm a requirement for Notch signaling in biliary development but, until recently, the specific role that Notch signaling plays in biliary differentiation was unclear [38–41]. Some studies implicated Notch signaling in the regulation of hepatoblast differentiation [32, 39, 42]. On the other hand, mice with genetically engineered mutations in

Notch2 or in the Notch target gene *Hes1* have altered bile duct morphology but apparently normal BEC induction [38, 40]. In an elegant series of experiments that has helped clarify the role of Notch signaling in biliary development, Zong showed that Notch signaling acts during embryogenesis to coordinate both biliary differentiation and bile duct morphogenesis [43]. The strength of this study is the investigators' approach using targeted deletion of the *Rbpj* gene to circumvent possible functional redundancy in the Notch signaling pathway. *Rbpj* is a necessary effector of canonical Notch signaling and its deletion abrogates all Notch signaling [44]. Early deletion of *Rbpj* in hepatoblasts resulted in a decreased number of BEC precursors and bile ducts, while activation of the Notch pathway using a notch intracellular domain transgene led to ectopic biliary development throughout the liver. Thus, using targeted gene deletion as well as over- and misexpression studies in vivo, these investigators convincingly show that Notch signaling plays a critical role in both biliary differentiation and bile duct morphogenesis.

As mentioned previously, the homeobox gene *Hhex* is critical for liver bud formation (see above). Expression studies revealed that *Hhex* mRNA has been detected in the liver bud, the embryonic liver, the gall bladder, and the extrahepatic bile duct [45, 46]. Furthermore, *Hhex* is expressed in the adult liver in hepatocytes and IHBD cells and *Hhex* has been shown to regulate the in vitro expression of the *ntcp* gene, which encodes a bile acid transporter expressed in adult hepatocytes [45, 47, 48]. These findings suggest that *Hhex* has important roles during later stages of hepatobiliary development and/or function. To address the function of *Hhex* in the liver after liver bud formation, Hunter et al. derived a *Hhex* conditional null allele using the Cre-loxP system and employed the *Foxa3-Cre* [11] and *Alfp-Cre* [49] transgenes to eliminate *Hhex* in the hepatic endoderm at distinct developmental stages [50]. Elimination of *Hhex* in the hepatic diverticulum (*Foxa3-Cre;Hhex*$^{d2,3/-}$) resulted in a severely hypoplastic and cystic liver, with major defects in the development of the extrahepatic biliary tract. The liver-enriched transcription factors *Hnf4a* and *Onecut1* were absent in hepatoblasts of *Foxa3-Cre;Hhex*$^{d2,3/-}$ embryos at E13.5, which resulted in major defects in hepatic epithelial development. By late gestation, there were many "hybrid" cells expressing both Hnf4a and cytokeratin, similar to the "hybrid" cells seen in *Onecut1*$^{-/-}$*/Onecut2*$^{-/-}$ mouse livers, indicative of a hepatoblast differentiation defect. *Hhex* also plays a critical role in bile duct morphogenesis (see below).

Other signaling pathways that are thought to have a role in hepatoblast differentiation include *Fgf* and *Wnt/Ctnnb1(β-catenin)*. In the chicken liver, both *Fgf2* and *Fgf7* induce differentiation of hepatoblasts

to BECs in conjunction with *Bmp4* and extracellular matrix components in vitro [51]. There are no in vivo studies to confirm these findings so the role of Fgfs and Bmps in BEC development remains obscure. Several studies suggest a role for *Wnt/Ctnnb1* in hepatoblast proliferation and cell survival [52, 53]. A role for *Wnt/Ctnnb1* in biliary differentiation has also been suggested by both in vivo and in vitro studies. Treatment of E10 liver cultures with anti-sense oligonucleotides to *Ctnnb1* resulted in the absence of casein kinase-positive biliary cells [53]. Conversely, treatment of early liver explants with Wnt3a-conditioned media primarily led to proliferation and differentiation of CK-19$^+$ cells, suggesting a bias toward biliary differentiation [54]. Liver-specific deletion of *Ctnnb1*, achieved by crossing mice with a floxed *Ctnnb1* allele to mice expressing Cre-recombinase under the control of the *Foxa3* promoter, resulted in a hypoplastic liver with defects in both hepatocyte and biliary differentiation [55]. Since there is a defect in the formation of both hepatocytes and BECs in these mice, it remains unclear from these studies if *Ctnnb1* plays a specific role in biliary fate determination or if it plays a more global role in hepatoblast survival. However, using a similar Cre-loxP mouse gene-targeting approach to activate ectopic *Ctnnb1* expression in the embryonic liver by inactivating the *Ctnnb1* suppressor Apc1, Decaens showed that elevated levels of *Ctnnb1* in hepatoblasts inhibited hepatocyte development, while biliary cell development did occur [56]. Interestingly, normal ductal plate structures did not form and ectopic ducts with BECs formed throughout the liver, suggesting that *Ctnnb1* may affect bile duct morphogenesis as well as hepatoblast differentiation. The mechanisms by which *Wnt* signaling may regulate hepatoblast differentiation remain unknown.

BILIARY TRACT MORPHOGENESIS

The biliary tract is composed of both an extrahepatic portion, which consists of the hepatic ducts, the cystic duct, the gallbladder and the common bile duct, and the intrahepatic portion, which consists of the intrahepatic bile ducts. The current data suggest that the extrahepatic biliary tract (EHBT) develops from an outpouching of the ventral foregut just caudal to the liver bud and closely associated with the ventral pancreatic bud [57]. There appear to be common mechanisms involved in the development of the EHBT and the pancreas. Mice in which the expression of transcription factor *Hes1* is deleted have gallbladder agenesis and severe agenesis of the EHBT and the BECs of the EHBT transdifferentiate toward the pancreatic lineage [58, 59]. Since

Hes1 is a key target of the Notch pathway, this suggests that Notch signaling is necessary for normal EHBT development and may be involved in repressing the pancreas phenotype in endodermal cells that would otherwise form the EHBT.

It was originally thought that the IHBDs originated as a branch of the EHBT near the porta hepatis and subsequently grew into the liver in an infiltrative manner. However, Vijayan and Tan studied paraffin-embedded tissue from human embryos or fetuses between 5.5 and 16 weeks of gestation [60]. This tissue was serially sectioned and the images were aligned and used for generating three dimensional images of the extrahepatic and intrahepatic biliary systems. Their study indicated that IHBDs developed from the ductal plate, which remains in contact with the extrahepatic bile duct from a primitive form through a remodeling period to its definitive tubular structure. Thus, the IHBDs form exclusively within the context of the ductal plate rather than by elongation and branching of the extrahepatic bile duct. This process takes place in a proximal-to-distal gradient starting at the porta hepatis and moving to the periphery of the liver. Morphologic analysis of EHBT development suggests that the convergence of the EHBT and the IHBD is at the hepatic ducts (reviewed in [57]). This is supported by genetic evidence in mice that have a liver bud specific deletion of *Hhex*. In these mice, in which *Hhex* is deleted at E9.5 in the foregut and liver bud, there is complete absence of the EHBT and the hepatic ducts are present [50]. Interestingly, the EHBT was replaced with tissue that morphologically resembled duodenum, suggesting that the cells of the EHBT may have the ability to transdifferentiate into duodenum as well as pancreas.

As mentioned earlier, morphogenesis of the IHBD begins as cholangiocytes form a single continuous layer of cells around a portal vein called the ductal plate. Shortly thereafter, focal areas of the ductal plate become bilayered and develop luminal structures that form the mature bile ducts. One important step in this process, about which little is known, is the mechanism responsible for determining the location within the ductal plate that mature bile ducts will form. It is unlikely that this is a stochastic process because, if it were, the result would be the formation of biliary structures at different locations within the ductal plate (in reference to the proximal – distal axis), resulting in a discontinuous biliary tree. It is tempting to speculate that the location of bile duct formation within the ductal plate is influenced by the presence of a biliary structure located more proximally in the liver but there have been no studies to specifically address this issue. Alternatively there could be predetermined locations within the ductal plate where bile ducts are formed, perhaps due to local signals emanating from the

portal vein or the portal vein mesenchyme. This would suggest that there is heterogeneity in the expression of signaling molecules in the portal mesenchyme such that only specific and predetermined locations within the portal vein mesenchyme give rise to signals that induce bile duct formation. However, the mechanism by which ducts elongate along their longitudinal axes remains obscure.

Until recently, very little was known about the transcriptional control of bile duct morphogenesis and biliary cyst formation. Studies over the past several years have begun to elucidate a network of transcription factors that are critical for biliary morphogenesis. Interestingly, many of these genes play important roles in both biliary morphogenesis and differentiation of hepatoblasts into BDE. Therefore, it is often difficult to establish if aberrant duct morphogenesis is due to a particular gene's role in regulating bile duct formation or is secondary to abnormal hepatoblast differentiation, or both. For instance, both *Onecut1*$^{-/-}$ embryos and embryos with a liver-specific deletion of *Hnf1b* fail to form morphologically normal IHBD in addition to displaying defects in biliary cell differentiation and defective EHBT development [21, 22]. Additionally, as mentioned above, *Onecut1* appears to act upstream of *Hnf1b* and to directly regulate its transcription [21]. *Onecut2*$^{-/-}$ mice also had cyst-like abnormalities of the ductal plate, which was more pronounced in *Onecut1*$^{-/-}$*;Onecut2*$^{-/-}$ mice, suggesting a genetic interaction between these two transcription factors that controls bile duct morphogenesis [31]. Notch signaling has also recently been shown to be necessary for both biliary fate determination and morphogenesis [43]. In this study, the authors' detailed analysis of biliary development also revealed novel mechanism for duct morphogenesis that relies upon sequential differentiation of adjacent layers of precursor cells. This potentially new mode of tubulogenesis involves the formation of radially asymmetric duct structures that are the precursors of symmetric, mature bile ducts (Fig. 1.3). Also, this study demonstrated that Notch directly regulates tubulogenesis because ectopic expression of the Notch intracellular domain (NICD) throughout the liver parenchyma resulted in ectopic tubule formation in the hepatic lobules. *Cepba*$^{-/-}$ mice show abnormalities of bile duct morphogenesis as well as biliary cell differentiation [25]. Like mice ectopically expressing NICD in the hepatic parenchyma, tubule formation was not limited to the area of the ductal plate. Instead, many epithelial luminal structures formed throughout the liver. This uncoupling of biliary differentiation and epithelial lumen formation suggests separate and distinct roles for *Cepba* in repressing biliary differentiation and in regulating bile duct development/epithelial lumen formation. Similarly, gene-targeting studies of the homeobox gene *Hhex* suggest separate roles in biliary

differentiation and morphogenesis, depending on the timing of deletion [50]. When *Hhex* is deleted in the foregut and liver bud at E9.5, there is abnormal hepatoblast differentiation as well as the formation of many epithelial luminal structures and cysts throughout the liver parenchyma. However, the luminal structures and cysts are lined by three cell types – cells that express only hepatoblast/hepatocyte-specific protein (Hnf4a) and no biliary protein (CK-19), cells that express both hepatoblast/hepatocyte and biliary proteins, and cells that only express biliary proteins. In mice in which *Hhex* is deleted in the liver at E11.5–12.5, hepatoblast differentiation proceeds normally yet the mice have biliary morphogenesis defects as evidenced by the presence of ductal plate malformations and cyst formation. Most recently, deletion of the transcription factor *Sox9* in the liver led to a delay in the maturation of the early asymmetrical primitive bile ducts into mature symmetrical ducts [61]. *Sox9* appears to act downstream of *Onecut1* and upstream of *Hes1* and *Cebpa* as well as to interact with TGFB signals in the developing liver. This study also supports the concept that bile duct tubulogenesis proceeds in a radially asymmetric fashion.

CILIA – A POTENTIAL LINK BETWEEN DEVELOPMENT AND DISEASE

In many instances of abnormal biliary morphogenesis, the ductal plate forms biliary cysts that persist into adulthood, suggesting a defect in epithelial tubulogenesis that is similar to that seen in both human polycystic kidney and liver disease. Thus, there may be common mechanisms leading to the formation of both renal and hepatic cysts. Evidence strengthening this association is the fact that humans with some forms of polycystic kidney disease (PKD) develop hepatic cysts and the fact that several mouse and rat mutant strains with PKD also have hepatic cysts, including the *Pck* rat [62, 63], the *inv* mouse [64], TG737 mouse (*Ift88* mutation) [65], and the *cpk* mouse [66]. Interestingly, the evidence that has accumulated to date indicates that alteration in the development and/or function of primary cilia is a universal abnormality underlying renal cystic disease [67, 68]. Each cholangiocyte, like each renal epithelial cell, contains a single cilium on its surface [63]. In the PCK rat strain, mutation of the *Pkhd1* gene (fibrocystin) leads to hepatic cysts and is associated with abnormalities in ciliary morphology, thereby raising the possibility that cilia, and therefore the genes involved in ciliary biogenesis and function, are central to bile duct morphogenesis and hepatic cyst formation. There are many other examples of genes that are implicated in biliary tract morphogenesis/cyst formation whose function has been also associated with ciliary

biogenesis/function (either in the liver or elsewhere), including *Onecut1*, *Hnf1b*, *Ift88*, *Pkhd1*, *Invs* [69, 70]. However, the developing ductal plate and BECs have not been examined for the presence or absence of cilia in any of these mutants. The only data available to support a potential role for cilia in BEC differentiation and/or bile duct morphogenesis are from an analysis of the livers from human embryos with the clinical diagnosis of Meckel syndrome (or Meckel syndrome), an autosomal recessive disease consisting of a combination of renal cysts, anomalies of the central nervous system, digit abnormalities, and ductal plate malformations that are thought to be due to defective ciliary biogenesis/function [71]. Many of the specimens evaluated showed classic ductal plate malformations, hepatoblast differentiation defects, and absent cilia. While it is not clear whether the differentiation defect led to the ciliary defect or vice versa, the end result was ductal plate malformation. Candidate genes thought to be causative for Meckel syndrome are all involved in ciliary biogenesis and/or function, leading to the strong possibility that cilia play a major role in development of the biliary system.

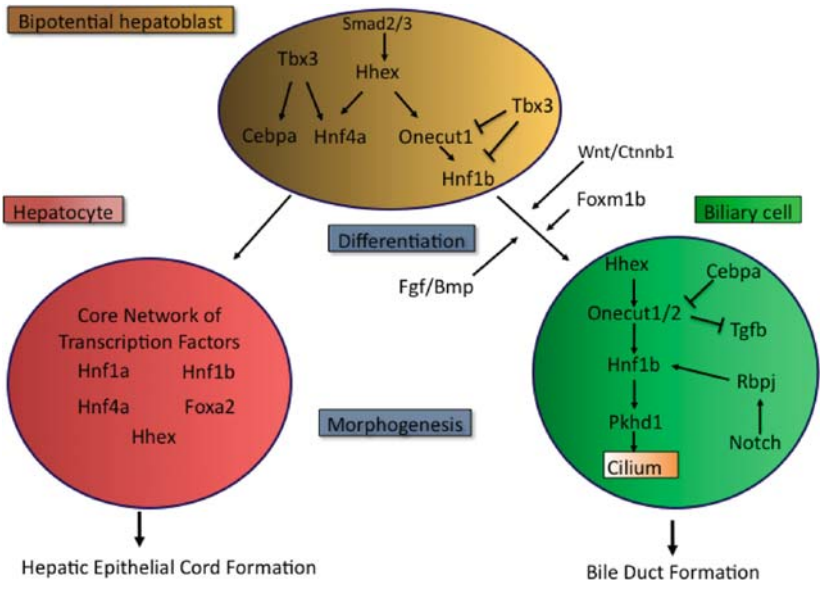

Fig. 1.4. Summary of the genetic factors involved in hepatoblast differentiation and bile duct morphogenesis. Shown are both stimulatory and inhibitory interactions. See text for details.

In summary, much recent progress has been made in elucidating the genetic factors and pathways that play critical roles in early liver

bud formation, hepatoblast differentiation, and bile duct morphogenesis. This newly acquired information, summarized in Fig. 1.4, allows us to begin to understand the molecular control of key developmental events in liver development as well as the underlying basis for many forms of fibrocystic disease of the liver. But this picture is far from complete. Future research focused on early hepatobiliary development will provide the biological platform upon which we can build new diagnostic and therapeutic approaches to combat many debilitating liver diseases in humans. As well, these advances will provide much needed insight into critical biological processes such as cell signaling, the role of cilia in development, and epithelial tube formation, which will also have broad and important implications for diseases and developmental disorders in organs other than the liver.

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2 Cholangiocyte Biology as Relevant to Cystic Liver Diseases

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CONTENTS

Summary

Polycystic liver diseases are hereditary disorders that affect the biliary epithelium, often in conjunction with the renal tubule epithelium. Characterized by the progressive formation of cysts

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throughout the liver and kidney, they can often lead to severe life-threatening complications. Polycystins and fibrocystin, the defective proteins in the dominant and in the recessive form of the disease, respectively, are mainly expressed in the primary (nonmotile) cilia of cholangiocytes, the epithelial cells that line the intrahepatic biliary tree. Important clues for understanding the pathogenesis of cystic diseases come from understanding the biology and pathobiology of cholangiocytes. In this chapter, cholangiocyte function and morphology is first briefly described, with particular emphasis on the regulation of their secretory properties and the complex intercellular signaling. Then, we discuss a number of possible mechanisms leading to cyst formation and progressive growth of the cysts. In both autosomal dominant and recessive forms, liver cysts arise from an aberrant development of intrahepatic bile duct epithelium. During cyst expansion, different factors, including excessive fluid secretion, extracellular matrix remodeling, increased proliferation of the epithelial cells lining the cyst, and aberrant hypervascularization around the cyst wall, variably take part in promoting progressive cyst growth. Many of these factors act via autocrine mechanisms. Each of them represents a possible target for therapies aimed at reducing the growth of liver cysts.

Key Words: Cholangiocytes, ADPKD, ARPKD, Polycystin-1, Polycystin-2, Fibrocystin, VEGF, Cilium, Ductal plate malformation

INTRODUCTION

Polycystic liver diseases are genetic disorders that affect mainly the bile duct and the renal tubule epithelia. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited diseases, occurring in 1:400 to 1:1,000 individuals; it is characterized by the formation of multiple cysts in the kidney, liver, and pancreas. Although synthetic liver function is usually preserved in ADPKD, severe cyst complications (mass effect, hemorrhage, infection, or rupture) may develop and thus require urgent liver transplantation. Autosomal recessive polycystic kidney disease (ARPKD) and its liver-related phenotypes Caroli disease (CD) and congenital hepatic fibrosis (CHF) are, by contrast, rare disorders with an estimated prevalence of 1:20,000 live births. CD and CHF are characterized by recurrent acute cholangitis and severe portal hypertension due to an excessive peribiliary fibrosis that can be further complicated by the development of biliary malignancies.

The even rarer "isolated" polycystic liver disease (PCLD) is phenotypically similar to ADPKD, except that the kidney is not affected. In all cases of cystic liver disease, the pathologic condition targets the biliary epithelium, justifying the inclusion of these forms among the genetic cholangiopathies [1].

ADPKD is caused by mutations in one of two genes, *PKD1* (polycystic kidney disease 1) (85–90% of the cases) or *PKD2* (10–15%) encoding for polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Polycystins act as mechanoreceptors, chemoreceptors, and calcium (Ca^{2+}) channels, able to sense changes in apical flow. ARPKD/CD and CHF are caused by mutations in the *PKHD1* (polycystic kidney and hepatic disease 1) gene, encoding for fibrocystin, a protein whose functions remain largely unknown. Polycystins and fibrocystin are expressed in the primary cilia of cholangiocytes. In secretory epithelia, primary nonmotile cilia are involved in the regulation of multiple epithelial functions including secretion, proliferation, differentiation, and interactions with cell matrix. In the liver, cilia are preferentially expressed by cholangiocytes. Although the impact of ciliary dysfunction on cholangiocyte physiology is unknown, animal models with defects in ciliary proteins, such as polycystins, fibrocystins, and polaris, show different degrees of biliary dysgenesis. In the liver, both ADPKD and ARPKD/CHF/CD are morphologically characterized by aberrant development of the biliary epithelium that retains an immature, ductal plate-like architecture with the formation of multiple biliary microhamartomas that progressively dilate to macroscopic cysts, scattered throughout the liver parenchyma.

The isolated polycystic liver disease (PCLD) on the other hand is caused by mutations in *PRKCSH,* a gene coding for protein kinase C substrate 80 K-H, also called hepatocystin, or in the *SEC63* gene. *SEC63* encodes for a component of the molecular machinery regulating translocation and folding of newly synthesized membrane glycoproteins. Hepatocystin and *SEC63* are not expressed in cilia, but in the endoplasmic reticulum, thus cystic diseases of the liver can also be caused by defects in proteins that are not expressed in cilia.

Diseases of the biliary epithelium caused by single-gene defects that alter a critical physiologic process provide an invaluable clue for understanding epithelial function and pathophysiology. As a consequence, in the last few years, interest for polycystic liver diseases has consistently grown. In this chapter we will review the aspects of cholangiocyte biology that more closely relate to the pathogenesis and treatment of cystic diseases of the liver.

MORPHOLOGY AND SECRETORY FUNCTIONS OF THE NORMAL BILIARY EPITHELIUM

The biliary epithelium forms a branching system of conduits within the liver where bile flows from the hepatocytes to the gallbladder and intestine. The biliary tree is organized in a complex tridimensional network that starts at the canals of Hering, located at the limiting plate with hepatocytes, and forms tubules (<15 μm in diameter) which gradually converge to create ducts of progressively larger size (up to 300–800 μm): interlobular, septal, major ducts, and hepatic ducts embedded into the portal space [2]. The biliary tree is lined by cholangiocytes. These are epithelial cells with absorptive and secretory properties that actively contribute to bile formation, regulating its volume, pH, and composition according to physiological needs. Ductular secretion may account for about 40% of bile flow in humans, a percentage that can be rapidly increased during the digestive phase.

The morphology of cholangiocytes, as well as their function, varies along the biliary tree: cholangiocytes in the small interlobular bile ducts are cuboidal epithelial cells, but become columnar and mucus-secreting in larger ducts approaching the extrahepatic portion [3]. This morphological heterogeneity also corresponds to a functional regional specialization: cholangiocytes lining the large interlobular and major ducts are mostly involved in secretory functions. Conversely, cholangiocytes in the smaller bile duct branches, cholangioles and ducts of Hering, perform other important biological properties such as the ability to proliferate in response to liver damage, participate in the inflammatory response, and undergo limited phenotypic changes. Furthermore, liver progenitor cells are believed to arise from subpopulations of cholangiocytes residing in the canal of Hering [4]. This functional specificity is substantiated by the fact that most cholangiopathies show a site-restricted bile duct injury. For instance, primary biliary cirrhosis (PBC) targets specifically the interlobular bile ducts, whereas primary sclerosing cholangitis (PSC) affects the larger intrahepatic and extrahepatic ducts. Interestingly, the “small duct” variant of PSC, where damage is restricted to the finest branches of the biliary tree, has distinct clinical manifestations.

The biliary tree runs along the portal spaces between the hepatic lobules, in close vicinity to a branch of the portal vein and to one or two branches of the hepatic artery. While portal blood perfuses hepatocytes in the hepatic lobules, the cholangiocyte blood supply is provided by the hepatic artery. Branches of the hepatic artery, at the periphery of the liver lobule, create a peribiliary vascular plexus (PBP), a network of

capillaries which nourishes the cholangiocytes and eventually merges into the hepatic sinusoids.

Bile formation starts at the hepatocyte canalicular membrane, with the secretion of bile acids, other organic and inorganic solutes, electrolytes, and water. As the primary bile flows through the bile ducts on its route toward the duodenum, its composition is regulated by the intrahepatic bile duct epithelium that reabsorbs fluids, amino acids, glucose, and bile acids, while secreting water, electrolytes, and immunoglobulin A (IgA) [5, 6]. Fifteen years of investigation have partly unveiled the complexity of the transport function of cholangiocytes and of its regulation (see Fig. 2.1). Here we will limit our discussion to the aspects that may be relevant for understanding cystogenesis in the liver.

Ultimately, secretion and alkalinization in the bile ducts is mainly associated with a net flux of chloride (Cl^-) and bicarbonate (HCO_3^-) into the lumen which induces the secretion of water and regulates bile pH. In contrast with hepatocytes, where the major driving force for bile production is the active secretion of bile acids by adenosine triphosphate (ATP)-driven transporters, cholangiocytes secrete fluid and electrolytes in response to paracrine or endocrine stimuli. A number of different ion channels and transporters have been identified and shown to be specifically located at the basolateral or apical membrane. As in all mammalian cells, the driving force for facilitated membrane transport in cholangiocytes is provided by the Na^+/K^+ ATPase, which actively extrudes sodium (Na^+) from the cell and, together with potassium (K^+) channels, maintains the transmembrane potential. At the basolateral side, the Na^+ gradient regulates the Na^+/H^+ exchanger isoform 1 (NHE1) and the Na^+:HCO_3^- symporter (or Na^+-dependent Cl^-/HCO_3^- exchanger in humans, NCHE) which mediate the reabsorption of HCO_3^- necessary for acid extrusion, while the $Na^+/K^+/2Cl^-$ cotransporter (NKCC1), a major determinant of fluid secretion, mediates the chloride uptake into the cell. On the apical side of the cell, Cl^- efflux is mainly mediated by a cyclic adenosine monophosphate (cAMP) activated, slow conductance, Cl^- channel encoded by the cystic fibrosis transmembrane conductance regulator (CFTR). The opening of chloride channels (CFTR) in the apical membrane leads to an efflux of Cl^- and the generation of a osmotic gradient which induces the release of water into the lumen through aquaporines (AQP-1 and AQP-4). The Cl^- gradient regulates the Na^+-independent Cl^-/HCO_3^- exchanger (AE2) which extrudes bicarbonate into the bile providing biliary alkalinization, in accordance with intracellular pH. Other carriers such as the Na^+-dependent glucose transporter (SGLT1), the glutamate transporter, and the ileal bile acid transporter (iBAT) expressed on the apical membrane of cholangiocytes mediate the reabsorption of

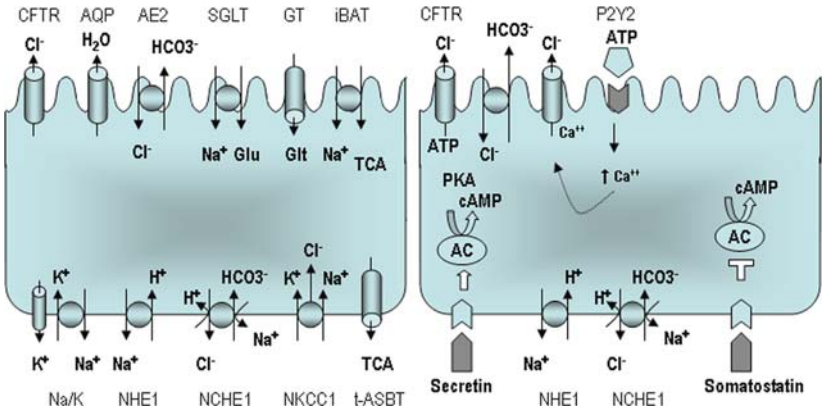

Fig. 2.1. Secretory function of cholangiocytes and its regulation. *Left*: secretion and alkalinization in bile ducts is ultimately associated with a net flux of Cl^- (which induces fluidification) and HCO_3^- (alkalinization) into the lumen mediated by specific transporters localized to the apical or basal membrane of cholangiocytes. The Na^+/K^+ pump creates the membrane potential necessary for cell homeostasis and maintains the Na^+ gradient across the membrane necessary for facilitated transports. At the basolateral side, the Na^+/H^+ exchanger NHE1 and the $Na:HCO_3^-$ symporter NCHE1 mediate the reabsorption of HCO_3^- into the cell and the acid extrusion, while chloride uptake occurs through the $Na^+/K^+/Cl^-$ cotransporter NKCC1. On the apical side, the Cl^- is released into the bile by cystic fibrosis transmembrane conductance regulator (CFTR) inducing a parallel osmotic movement of H_2O through aquaporines. The Cl^-/HCO_3^- exchanger AE2 (located at both the basolateral and the apical membrane) mediates carbonate release into the bile. Specific Na^+-dependent apical carriers, GT and iBAT, mediate the reabsorption of glutamate and taurocholate, respectively. Biliary acids are then secreted in the peribiliary plexus via t-ASBT. *Right*: Choleretic hormone secretin stimulates cAMP production by adenylate cyclase with consequent activation of CFTR via PKA mediated phosphorylation. Cl^- released by CFTR promotes HCO_3^- secretion by AE2 and bile alkalinization. Similarly luminal purinergic nucleotides can activate a Ca^{++}-dependent Cl^- channel and stimulate secretion. In contrast, somatostatin decreases bile secretion and alkalinization by adenylate cyclases inhibition.

biliary constituents, such as glucose and glutathione breakdown products and conjugated bile acids. This is particularly important because bile acids can stimulate proliferation of biliary epithelial cells. Biliary bile acids are then secreted in the peribiliary plexus via t-ASBT, a truncated isoform of the apical sodium-dependent bile acid transporter (ASBT), or via MRP3 (multidrug-resistant protein 3), a p-glycoprotein. This cholehepatic circulation of bile acids is also important in the overall regulation of bile secretion.

The secretory function of the bile ducts is finely regulated by rapid hormone-mediated signaling. The net amount of fluid and secreted HCO_3^- is determined by the integration of different pro-secretory (secretin [5], glucagon [7], VIP [8], acetylcholine [9], bombesin [10]) and anti-secretory (somatostatin [11], endothelin-1 [12]) stimuli. All these hormone signals ultimately act on the adenylyl cyclases (ACs), the transmembrane enzymes that regulate the intracellular level of the second messenger cAMP, converting ATP to cAMP. Secretin, the main choleretic hormone, increases cAMP/PKA (protein kinase A). This activates CFTR, and consequently stimulates Cl^- and HCO_3^- efflux and inhibits the Na^+/H^+ exchanger (NHE)-dependent Na^+ absorption [13, 14]. Cholinergic agonists, β-adrenergic agonists, and HCO_3^--mediated signals also regulate bile secretion through the cAMP and PKA pathway. ACs may thus represent an important means of integration of multiple secretory signals. So far nine different isoforms of AC have been identified (AC1-9), each displaying tissue-specific expression and regulation. Interestingly the AC6 isoform was found to be located in cholangiocyte cilia, thus further suggesting a correlation between ductal bile secretion and ciliary function [15].

The secretory functions of the biliary epithelium are also regulated by molecules (such as bile salts, glutathione, and purinergic nucleotides) secreted by hepatocytes into the canalicular bile and delivered to receptors and transporters located in the apical membrane of cholangiocytes [5]. For instance, ATP, which is released into the bile by hepatocytes or by cholangiocytes themselves, can bind to apical P2Y2 purinergic receptors and stimulate apical Ca^{2+}-activated Cl^- channels and basolateral Na^+/H^+ exchanger (NHE-1), thus promoting Cl^- efflux into the bile and basolateral HCO_3^- influx [16]. Certain bile acids may also stimulate cholangiocyte secretion of HCO_3^- by inducing ATP dependent Cl^- secretion by CFTR and purinergic activation of apical Ca^{++}-activated or volume-activated Cl^- channels [17].

CHOLANGIOCYTE REACTION TO DAMAGE

Cholangiocytes possess receptors for a number of cytokines, chemokines, and growth factors and angiogenic factors that enable an extensive cross talk with other liver cell types, including hepatocytes, stellate cells, and endothelial cells [6]. This property becomes particularly relevant when the liver or the biliary tree is damaged. In fact, the cholangiocyte compartment can significantly expand in response to liver injury. Cholangiocyte proliferation occurs in most pathologic conditions, including cholestasis, viral hepatitis, and hepatic necrosis, and represents a key mechanism of regeneration and repair,

which ensures the integrity of the biliary tree following liver damage. These "reactive" or "activated" cholangiocytes are believed to arise from a progenitor cell compartment located in close contact with the smallest radicals of the biliary tree, the terminal cholangioles at the canals of Hering. Reactive cholangiocytes show a less differentiated secretory phenotype, but acquire the capability to secrete a number of proinflammatory and chemotactic cytokines and growth factors. They can recruit inflammatory and mesenchymal cells and induce them to proliferate and to produce extracellular matrix (ECM) components [18]. There are, in fact, intimate contacts and exchange of signals between mesenchymal cells and reactive cholangiocytes. While mesenchymal cells are considered the effectors of fibrosis, reactive cholangiocytes are considered the "pacemaker of liver fibrosis" [19]. The list of cytokines, chemokines, inflammatory factors and growth factors, and receptors that mediate the epithelial/mesenchymal cross talk in the liver is continuously increasing. It includes interleukin-6 (IL-6), IL-8, tumor necrosis factor-α (TNFα), interferon-γ (IFNγ), monocyte chemotactic protein-1 (MCP-1), cytokine-induced neutrophil chemoattractant (CINC), and nitric oxide, which regulate the immune activity of lymphocytes and polymorphonuclear cells. Reactive cholangiocytes also produce growth factors such as vascular endothelial growth factor (VEGF), endothelin-1 (ET-1), platelet-derived growth factor-BB (PDGF-BB), transforming growth factor-β2 (TGF-β2), and connective tissue growth factor (CTGF).

In addition to establishing paracrine communications with mesenchymal cells, cholangiocytes may also participate in the generation of liver fibrosis through a process of epithelial to mesenchymal transition (EMT). EMT is a process of cellular reprogramming whereby epithelial cells acquire some of the phenotypic and functional characteristics of mesenchymal cells, such as the expression of fibroblast-specific markers (FSP-1, vimentin), the ability to migrate by locally dismantling the basement membrane upon which the epithelial sheet resides, and the ability to generate different connective tissue components (fibronectin, collagen, elastin, tenascin). EMT may thus contribute to the accumulation of activated fibroblasts in association with the loss of bile ducts. This biological process has also been described in the pathogenesis of organ fibrosis in the kidney [20] and lung [21]. Recent studies suggest that EMT may also be involved in liver fibrosis [22, 23].

In response to liver injury, reactive cholangiocytes also acquire a neuroendocrine-like phenotype and express receptors that enable them to respond to regulation by neural terminations. In fact, reactive cholangiocytes express β1 and β2 adrenergic receptors, the M3 acetylcholine receptor [24], serotonin 1A and 1B receptors [25]. During cholestasis,

cholangiocytes can also directly secrete serotonin, thus further limiting the growth of bile ducts by an additional autocrine inhibitory loop. Furthermore, in experimental cholestasis, cholangiocytes secrete neuropeptides, such as nerve growth factor (NGF), that can stimulate cholangiocyte proliferation [26]. A large number of other regulatory neurotransmitters and neuropeptides are expressed by reactive cholangiocytes, but will not be mentioned here. The role of neuroendocrine signaling in cyst growth in the polycystic liver diseases has not been experimentally addressed yet.

MECHANISMS OF CYSTIC LIVER DISEASES: CILIA AND BEYOND

A novel concept in biliary physiology is that primary cilia are involved in the regulation of fundamental biological activities including cell differentiation, proliferation, and secretion [27, 28]. The presence of primary cilia at the apical domain of cholangiocytes has long been known; however, their role in biliary physiology remained undefined. The discovery that mutations in several proteins relevant to ciliary function are associated with cystic diseases in several organs, including kidney, liver, and pancreas, strongly revived interest in the function of this organelle [29].

Primary cilia of cholangiocytes, as opposed to cilia of lung epithelial cells, are nonmotile, but can be bent in response to changes in luminal fluid flow, thereby transducing a mechanical force into an intracellular calcium signal [30]. Masuyk et al. investigated ciliary function in the biliary epithelium using microperfused rat intrahepatic bile duct units (IBDU) and showed that changes in luminal flow increased $[Ca^{2+}]_i$ and inhibited forskolin-stimulated cAMP production [15]. These changes were significantly inhibited by removal of cilia with chloral hydrate or by silencing of ciliary protein (such as PC1 or PC2) or of adenylyl cyclases 6 (AC6) [15, 31]. PC2 is believed to function as a nonselective Ca^{2+} channel, activated by PC1 through its C-terminus, while AC6 is a Ca^{2+}-inhibitable AC expressed in cilia, which interacts with PC2. Ciliary dysfunction in ADPKD would thus reduce intracellular calcium levels, thereby increasing AC6 activity and the levels of cAMP. It is well known that cAMP stimulates cholangiocyte secretion through the Src/Ras/MEK/ERK1-2 pathway and thus can promote cyst formation. Additionally, PC1 may have a direct transcriptional effect mediated by the proteolytic cleavage and nuclear translocation of its carboxy-terminal tail to the nucleus [32]. In kidney cells, PC1 was also shown to regulate the signal transducer mTOR [33, 34]. The

constitutive activation of both these pathways results in progressive cyst growth [33].

Much of the attention has been focused on ciliary function; however, it should be noted that morphologic alterations of cilia have not been consistently reported in cystic liver diseases. Recent electron microscopy studies on human ADPKD liver described heterogeneous abnormalities on the apical surface of cyst epithelium, depending on cyst size [35]. The epithelial cells lining small cysts (1 cm) showed a relatively normal apical surface with cilia and microvilli represented in the expected number and size. On the other hand, the apical surface of medium-sized cysts (2–3 cm) showed areas free of microvilli with rare and shortened cilia, while large hepatic cysts (3 cm) totally lack microvilli and primary cilia. These progressive morphological abnormalities of primary cilia and microvilli may represent a mechanic effect of enhanced endoluminal pressure during cyst growth, rather than a primary consequence of defective ciliary proteins.

Most "ciliary proteins" are not exclusively expressed in cilia. For example, PC2 is also strongly expressed in the endoplasmic reticulum (ER), where it interacts with ryanodine or InsP3 receptors to regulate ER and cytoplasmic calcium levels. Furthermore, the proteins encoded by the *PRKCSH* and *SEC63* genes are not expressed in cilia, but in the ER. Defects of other enzymatic activities associated with the ER, such as xylosyltransferase 2, an initiator of heparin sulfate and chondroitin sulfate biosynthesis, have been linked to the development of renal and liver cysts [36].

It is interesting that cyst formation is not a common reaction of the biliary epithelium to liver damage. In obstructive cholestasis the biliary epithelium reacts by forming multiple branching tubules, while in inflammatory biliary disease the epithelium forms a ramified mesh of reactive cells that for the most part lack a lumen. On the other hand, conditional PC1 or PC2 mice in which the genetic deletion is induced after birth develop multiple cysts in the liver, indicating that polycystins remain key determinants of biliary architecture during adult life.

An important property of epithelial sheets is planar cell polarity – the capacity to orient the axis of cell division in such a way that the growth of the epithelial sheet is "polarized" within the plane of the cell sheet. This means, for example, that the epithelial cells of the kidney align their mitotic spindle along the tubule axis so that the daughter cell will be inserted in a way that elongates the tubule rather than increases the size of its lumen. Interestingly, in rodent models with low Pkhd1 expression, the orientation of the mitotic spindle is distorted [37]. Pkhd1 is also localized to the basal body [29, 38–40], a sub-cellular organelle that originates from the mother centriole in the

centrosome and is responsible for the assembly of the cilium. Centrioles organize the mitotic spindle and serve as microtubule organizing center (MTOC). An anomalous cell division would result in tubule enlargement rather than tubule elongation. Direct experimental evidence for this model in cholangiocytes has not yet been produced.

CELLULAR MECHANISMS OF LIVER CYST FORMATION AND GROWTH

Mechanisms leading to the progressive growth of liver and kidney cysts are being actively investigated. In both autosomal dominant and recessive forms, liver cysts arise from an aberrant development of intrahepatic bile duct epithelium. During cyst expansion different factors, including excessive fluid secretion, extracellular matrix remodeling, increased proliferation of the epithelial cells lining the cyst, and the pericystic vasculature, variably take part to promoting the progressive cyst growth.

Altered Biliary Developmental Program (Ductal Plate Malformation)

The developmental role of polycystins is evident from studies in genetically modified mice and was clear to early pathologists that recognized a morphology suggestive of a blockage in ductal plate maturation. They classified cystic liver disease as a malformative condition.

The ontogenesis of the intrahepatic biliary tree begins around the 8th week of gestation and proceeds centrifugally from the ileum to the periphery of the liver. Still immature at birth, the biliary tree completes its development during the first year of life. Its formation starts when the periportal hepatoblasts surrounding branches of the portal vein undergo a phenotypic switch and assemble into a sheath of small flat epithelial cells, called "primordial ductal plate." Over the following weeks, some segments of the ductal plate perimeter are duplicated by a second layer of cells (double layered ductal plate), while the remaining single layer portions are deleted by apoptosis. The double layered ductal plate then dilates and starts to form a tubular structure which is incorporated into the mesenchyme of the developing portal space (migratory stage) and later undergoes a branching process to form the biliary tree [41]. Ductal plate remodeling during fetal and postnatal development is thus a fine balance between proliferative and apoptotic processes. A failure in ductal plate remodeling causes a number of developmental cholangiopathies, hence classified as ductal plate malformations (DPM) of which PKD is one [42].

However, in humans, cysts appear to develop throughout adult life. Mice with conditional knockout of *Pkd1* or *Pkd2* show a progressive formation of liver and renal cysts reminiscent of human diseases even when the induction is performed weeks after birth [43–45]. This indicates that there is also a role for polycystin in maintaining a normal biliary architecture during adult life. It is interesting to note that there are fundamental structural differences between ADPKD and ARPKD liver cysts. In ADPKD, the nascent cysts detach from the original duct and form autonomous structures that no longer communicate with the duct; in ARPKD, cysts mostly remain open. This fundamental difference helps to explain the different clinical manifestations between ARPKD/CHF/Caroli and ADPKD.

Altered Epithelial Fluid Secretion

Studies performed by Everson et al. on ADPKD patients have shown that hepatic cysts are able to generate ion secretion under basal conditions and when stimulated with secretin [46]. The increased intraluminal pressure may contribute to cyst expansion as secretion into the closed cyst would stretch the lining epithelial cells and induce proliferation. In cell culture models of epithelial cysts, increasing intraluminal pressure increased the rate of cell proliferation [47, 48]. Stretch may activate apical secretion of purinergic agonists [49], major players in the regulation of cholangiocyte secretion and proliferation [16, 50]. Studies in kidney cyst cells from both ARPKD and ADPKD have shown that the cyst epithelium releases substantial amounts of ATP in culture [51] and expresses P2X and P2Y purinergic receptors, along with Ca^{2+}-stimulated Cl^- channel activation [52], leading to cystic fluid accumulation. It is presently unclear if these findings apply to cholangiocytes as well and if purinergic signaling is actually different from the normal epithelium. Cystic cholangiocytes appear to have an altered intracellular Ca^{2+} homeostasis, so without direct experimental evidence, it is difficult to predict the overall role of purinergic activation.

On the other hand, there is important cross talk between the cAMP and the Ca^{2+}-dependent pathways. Increased cellular cAMP content due to the reduced Ca^{2+}-dependent inhibition of AC6 would favor CFTR-dependent secretory events. It is interesting to note that the severity of ADPKD was milder in two cases in which the diseases coexisted with cystic fibrosis [53], a disease that impairs CFTR-dependent Cl^- secretion [54]. Consistent with these reports and the role of CFTR-dependent secretion, small molecule CFTR inhibitors slowed cyst growth in experimental polycystic kidney disease [55, 56].

In spite of the evidence of active fluid secretion in cystic kidney and liver disease, there is no definitive proof that unregulated fluid secretion is actually the major pathophysiologic mechanism leading to cyst growth in the liver. Furthermore to account for the very slow growth rates of the cysts, the net difference between absorption and secretion should be very subtle and constant in the face of increasing intraluminal pressure [57].

Cholangiocyte Proliferation

Most observations indicate that increased proliferative activity of the cystic epithelium may be the major determinant of cyst growth. As discussed earlier, cholangiocytes lining liver cysts present functional similarities with the reactive ductules, and express a vastly similar array of growth factors, growth factor mediators, cytokines, and chemokines. While in reactive ducts this property facilitates progenitor cell-mediated liver repair, their expression in cystic cholangiocytes likely represents the phenotypic and functional signature of a relative loss of differentiation. For example, we have shown that the pattern of angiogenic factors expression by cystic cholangiocytes in ADPKD is similar to that of the ductal plates.

Increased epithelial levels of cAMP is one of the factors determining the increased proliferative activity of cystic cholangiocytes and fluid secretion [58–60]. The increased cAMP level in cystic epithelia may be related to changes in the intracellular Ca^{2+} homeostasis and the cross talk with the Ca^{2+}-inhibitable adenylate cyclase 6. The relevance of cAMP in promoting cholangiocyte growth is demonstrated by the fact that in vivo treatment of normal rats with the adenylate cyclase (AC) stimulator forskolin induces cholangiocyte proliferation in association with increased activity of protein kinase A (PKA) and the activation of the cAMP/PKA/Src/ERK1/2 cascade [61]. Inhibition of cAMP production has been exploited as a therapeutic strategy in ARPKD. In particular somatostatin and its analogs, such as octreotide, were used to inhibit the secretin-induced increases in cAMP levels observed in cholangiocytes from bile duct-ligated (BDL) rats [62]. Somatostatin represses AC function through its receptor SSTR2, which is expressed in the liver only by cholangiocytes [63]. Masyuk et al. showed that octreotide, given in vivo to PCK rats, reduced liver and kidney weight, hepatic and renal cyst volume and fibrosis, and diminished the rate of cell proliferation in hepatic and renal epithelia [60].

The cystic fluid from patients with ADPKD also contains elevated levels of cytokines and growth factors that could potentially promote

cell proliferation and cyst expansion [64–66]. These include high levels of IL-8, IL-6, EGF, and VEGF [65, 67, 68]. Histological studies have shown a marked over-expression of estrogen receptors, insulin-like growth factor (IGF), IGF-receptor, growth hormone receptor, and pAKT [35, 68, 69]. Estrogens and IGF-1 are major factors able to induce proliferation of cyst epithelium, given their capability to activate specific proliferative and/or survival pathways. Like cAMP, estrogens [70], IGF-1 [71] and VEGF [71] may promote cholangiocyte growth via the ERK pathway, which is the main pathway of regulation of cholangiocytes proliferation. However, the strong immunoreactivity for IGF1, IGFR-1, and pAKT in liver cysts from ADPKD patients also indicate activation of the PI3-kinase pathway. Through this pathway IGF1 can activate mTOR (mammalian target of rapamycin) and thus promote cell proliferation via cyclins. In fact, phospho-mTOR is over-expressed in the liver cystic epithelium of ADPKD patients and mouse models. It is interesting to note that mTOR can also stimulate HIF1α-dependent VEGF secretion.

Cystic cholangiocytes over-express VEGF, angiopoietin-1, and their cognate receptors VEGF receptors 1, 2 and Tie-2 [68]. Thus, VEGF and angiopoietin-1 may exert an important autocrine proliferative effect and promote the growth of liver cysts.

Autocrine and Paracrine VEGF Signaling

Ductal plate malformations are frequently associated with an abnormal vasculature ramification: a morphologic feature known as "pollard willow pattern" which derives from an alteration in the normal relationship between bile ducts and portal vascular structures. The close anatomic relationship between intrahepatic bile ducts and hepatic arterial vascularization is already evident during the developmental stages and it appears to be crucial for the maintenance of the integrity and function of the biliary epithelium [72]. In immunohistochemical studies, Fabris et al. investigated the expression of angiogenic growth factors (VEGF and angiopoietins) and their cognate receptors during biliary and arterial development in humans and showed that, during development, VEGF released by cholangiocytes promotes the angiogenesis of the PBP in close vicinity to the maturing bile ducts [73]. Likewise, the PBP support is also fundamental during ductular reaction in response to liver damage or disease. Indeed, in many forms of liver injury, cholangiocyte proliferation is accompanied by an increase in number of the surrounding vascular structures [74]. In rat models of cholangiocyte proliferation

(common bile duct ligation), Gaudio et al. observed that a marked proliferation of the PBP became apparent only after the extension of the bile duct system occurred, underscoring the role of proliferating cholangiocytes in directly promoting angiogenesis [75]. Indeed they showed a significantly higher expression of VEGF in cholangiocytes isolated from BDL rats compared to normal rats [71].

VEGF is one of the most potent angiogenic factors and its role in vascular proliferation associated with tumor growth or wound healing has been widely documented in different organs. Also in human diseases related to developmental ductal plate malformation (i.e., PKD) the dysmorphic bile ducts are surrounded by hyperplasic vascular structures. Fabris et al. showed that in the cystic biliary epithelium of fibropolycystic liver diseases (ADPKD and Caroli disease), VEGF and angiopoietin-1 are markedly up-regulated, together with their receptors VEGFR2 and Tie2, and their enhanced expression is closely related to the microvascular density around biliary cysts [68]. In polycystic diseases, the biliary epithelium retains thus an immature phenotype characterized by up-regulation of VEGF and angiopoietins. The growing cysts stimulate angiogenesis to meet their need of vascular supply for metabolic support.

VEGF production is controlled in most tissues by hypoxia-inducible factor 1 (HIF-1), one of the key regulators of oxygen homeostasis. HIF-1 is a transcription factor active in hypoxic conditions, and the loss of microvillar structure and decreased microvillar density in ADPKD liver cyst epithelium are also features consistent with an ischemic damage [35]. However, isolated cystic cholangiocytes also overproduce VEGF, indicating that this is the direct result of the loss of PC1 and PC2 function, rather than the consequence of the cystic epithelium becoming hypoxic (C. Spirlì et al., submitted). Mice deficient in PC2, in particular, show a severe liver phenotype with higher proliferation rate of the cystic epithelium and higher expression of VEGF and its receptor VEGFR-2, pERK1/2 and HIF1α, suggesting that PC2 acts as repressors of the Raf/MEK/ERK cascade in physiological conditions and the lack of its function leads to activation of this pathway and the consequent increase in proliferation (C. Spirlì et al., submitted).

The significance of this effect is even more relevant in light of the fact that many of the growth factors that stimulate cholangiocytes appear to act through this pathway. The list of HIF-1-regulated genes is ample and includes genes coding for proteins involved in angiogenesis, energy metabolism, erythropoiesis, cell proliferation and viability, vascular remodeling, and vasomotor responses. Interestingly, HIF-1α

transcription can also be stimulated in normoxic conditions by a number of growth factors, cytokines, and extracellular mediators (IL-1, IL-6, EGF, HGF, TGFβ, 17-β-estradiol, IGF-1) which can stabilize or phosphorylate HIF-1α via PI3K/AKT/tuberin/mTOR or Raf/MEK/ERK or STAT3.

Extracellular Matrix Remodeling

In the normal liver ECM is scarce, and it is essentially composed of elastin, fibronectin, collagen type I, and collagen types III, IV, V, and VI in low quantity [76]. However, progressive accumulation of ECM components with an altered composition in the portal tract is a common feature in ADPKD, where a remodeling of the ECM is a prerequisite to allow the expansion of the cyst wall [76, 77]. These ECM abnormalities have also been found in the renal interstitium. The progressive establishment of fibrosis within the renal interstitium as well as within the hepatic portal space is particularly abundant in ARPKD, where dense fibrotic tissue is formed in close vicinity to aberrant bile ducts. In congenital hepatic fibrosis, Ozaki et al. recently showed that connective tissue growth factor (CTGF) is diffusely retained in the heparan sulfate proteoglycan web in the portal tract where it can be responsible for non-resolving fibrosis due to persistent activation of many mast cells and portal myofibroblasts [78]. Not surprisingly, the growth of liver cysts requires the enhanced degradation of ECM induced by an altered interplay between matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMP). MMPs are typically involved in the breakdown of extracellular matrix in embryonic development as well as in tissue repair and remodeling. IL-8, a cytokine that has been shown to be up-regulated in the liver cyst epithelium of animal models of ARPKD, stimulates MMP2 and MMP9 production by endothelial cells and portal myofibroblasts [79, 80].

Studies in PKD rodent models [81, 82] and in cultured mouse renal tubular cells [83] showed that MMP2 was consistently increased in cystic epithelial cells: in the PKD mouse model (C57BL/6 J-*cpk*), Rankin et al. observed an increased kidney cystic cell content of MMP-2 both in vivo [81] and in vitro [83]. Once isolated, cultured cells were able to migrate through collagen gels, further indicating they possess proteolytic activity. In addition, high MMP2, MMP9, MMP3, TIMP1, and TIMP2 levels were found in the culture medium [83]. Thus, increased expression of MMPs contributes to the overall reorganization and restructuring of the ECM necessary for cyst expansion.

NEW THERAPEUTIC STRATEGIES IN PREVENTING CYST GROWTH

In this chapter, we reviewed the aspects of cholangiocyte physiology and pathophysiology that are relevant for polycystic liver diseases. In particular, we have reviewed the anatomy and the function of the biliary tree and the mechanisms of cholangiocyte secretion and its regulation. We have discussed the role of some of the chemokines, cytokines, and growth factors expressed by cholangiocytes in pathologic conditions and in polycystic liver diseases. Many of these factors play a role in liver cysts growth. We have also discussed the signaling pathways that mediate cholangiocyte function. Each pathway represents a potential target for therapies aimed at reducing the growth of liver cysts. For example, the VEGF and HIF-1α signaling pathways could be targeted by inhibiting VEGFR2 or using blocking antibodies to VEGF receptors. We have shown that the treatment of ADPKD mice models with VEGFR-2 inhibitors significantly reduced liver cysts area and decreased the VEGF-induced pERK-1/2 activation providing a proof of concept for the potential use of anti-angiogenic therapy in polycystic liver diseases (C. Spirlì et al., submitted).

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3 Cholangiocyte Cilia and Basal Bodies

Tatyana V. Masyuk, PhD, Anatoliy I. Masyuk, PhD, and Nicholas F. LaRusso, MD

CONTENTS

Summary

Primary cilia are nonmotile, tubular organelles extending from the cell plasma membrane. They are important in maintaining normal cell physiology and in many clinical disorders (i.e., cilia-related diseases or ciliopathies) associated with abnormalities in ciliary structure and/or function.

In the intrahepatic bile ducts, primary cilia extend from the cholangiocyte apical plasma membrane into the ductal lumen. Cholangiocyte cilia are sensory organelles that recognize the current state of bile, i.e., its flow, chemical composition, and osmolality, and transmit the related information into intracellular signaling, i.e., function as mechano-, chemo-, and osmosensors. In contrast,

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in ciliopathies, the structural and/or functional abnormalities of these organelles in cholangiocytes lining liver cysts are associated with activation or down-regulation of several intracellular signaling pathways, in particular, cAMP and [Ca2+]i, leading to hepatic cystogenesis.

Key Words: Cholangiocyte, Primary cilia, Cholangiociliopathies

GENERAL INTRODUCTION TO CILIA

Cilia and flagella are present in many organisms from *Chlamydomonas reinhardtii* to *Caenorhabditis elegans* to mammals. Structurally cilia (single *cilium,* Latin for "eyelash") and flagella are identical; the only differences are in their length, number per cell, and type of movement [1, 2]. The term "cilia" was originally used to describe a large number of relatively short structures such as seen in *Paramecium*; the term "flagellum" was applied to a single long structure in cells such as sperm [3]. Cilia are centriole-derived organelles consisting of a set of microtubules surrounded by a membrane [2, 4–6]. Based on the microtubule arrangements, cilia are classified as 9 + 2 (nine peripheral microtubule doublets arranged around a central core that contains two central microtubules) and 9 + 0 (contain only nine peripheral microtubule doublets). Multiciliated cells usually possess motile 9 + 2 cilia, while nonmotile 9 + 0 (called primary) cilia are present in uniciliated cells [1, 2, 5, 7, 8]. However, some evidence suggests that this rule has exceptions. For example, single motile cilia with 9 + 0 arrangement are present in the embryonic node, while olfactory neurons possess many immotile 9 + 2 cilia [2]. Protozoans (e.g., *Chlamydomonas, Paramecium*, sea urchin embryos) have only motile cilia and use these organelles for locomotion or for moving liquid over their surface, while nematodes (e.g., *C. elegans*) have only primary cilia on their sensory neurons. In contrast, all types of cilia are found in mammals [9–11].

Mammalian motile cilia are usually present in large numbers on cell surfaces, such as epithelial cells lining airways, ependyma and the choroid plexus in the brain, oviduct and epididymis of the reproductive tracts. These cilia beat in an orchestrated wavelike fashion and are involved in movement of mucus in the lung and cerebrospinal fluid in the brain or in transport of ovum and sperm along the reproductive tracts [12].

The term "primary cilia" originates from the process of ciliogenesis in airway epithelial cells. During differentiation, these cells start out

with a single cilium, called a "primary cilium", which has only a transitory existence [3, 13]. At a later stage of the development, numerous motile cilia (i.e., "secondary" cilia) are produced forming a ciliated border [13]. Thus, by analogy with the initial single (primary) cilium of the airway cells, a single cilium is called "a primary cilium."

The primary cilium is a solitary, nonmotile, long, tubular organelle extending from the plasma membrane of the cell. With few exceptions (e.g., nucleated blood cells, hepatocytes), primary cilia are ubiquitous in vertebrates. They are found on epithelial cells of the bile ducts, kidney tubules, the pancreas, and the thyroid gland as well as on non-epithelial cells such as chondrocytes, fibroblasts, smooth muscle cells, and neurons.

While the existence of primary cilia has been known for over a century, the interest of biomedical scientists in these organelles has increased only recently triggered by recognition that cilia are important in maintaining normal cell function and that many clinical disorders (i.e., cilia-related diseases or ciliopathies) arise from aberrant formation or function of cilia. Ciliopathies include but are not limited to polycystic kidney diseases (PKD), situs invertus, infertility, respiratory diseases, hydrocephalus, blindness, obesity, polydactyly, and diabetes. The pleiotropic nature of these disorders reflects the different functions of cilia in cell physiology [1–4, 6, 8, 9, 12, 14–18].

In this chapter, we discuss the structure and functions of cholangiocyte cilia in normal and pathological conditions and the role of these organelles in the development of ciliopathies in the liver, which we call the cholangiociliopathies [19].

STRUCTURE OF MAMMALIAN CILIA

Structurally any type of cilium is cylindrical in shape and consists of (i) the microtubule-based membrane-covered axoneme; (ii) the matrix; (iii) the axoneme tip; and (iv) the basal body (Fig. 3.1) [2, 3, 9, 20].

The *axonemes* of motile and primary cilia extending from the cell surface into the extracellular space are highly ordered structures of nine peripheral microtubule doublets arranged around a central core that contains (motile cilia) or does not contain (primary cilia) two central microtubules (Fig. 3.1). Each doublet consists of a complete microtubule A and incomplete microtubule B which shares part of its wall with the A-tubule. In motile cilia, the A-tubule of one doublet and the B-tubule of adjacent doublet are contacted by dynein arms, which generate a bending force by sliding one doublet past another. The outer microtubules interact with the central pair of microtubules via radial

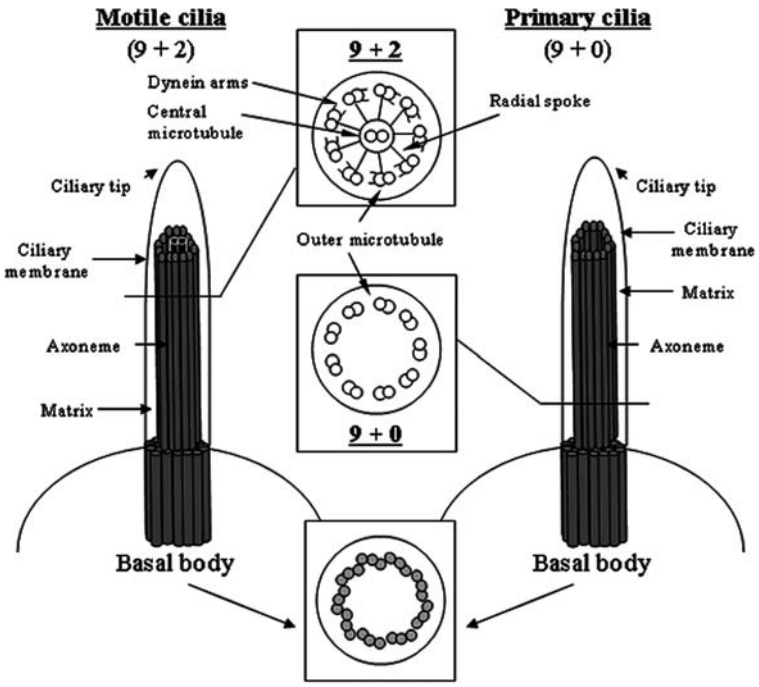

Fig. 3.1. Architecture of motile and primary cilia and basal body. The basal body in both types of cilia is the same and consists of 9 triplet microtubules. In motile cilia, the axoneme is composed of 9 doublet peripheral microtubules and a central pair of microtubules with a 9 + 2 arrangement. Dynein arms that have ATPase activity necessary to generate power for ciliary motility are attached to microtubules. Each microtubule connects by radial spokes to the sheath surrounding the central pair of microtubules. Primary cilia have 9 doublet peripheral microtubules, while the central pair is lacking (i.e., 9 + 0 axonemal arrangement).

spokes. In primary cilium, radial spokes and dynein arms are missing [2–4, 9, 14, 20–23].

The *ciliary membrane* is continuous with the plasma membrane (Fig. 3.1) but is different in lipid and protein composition. The ciliary membrane contains a number of receptors and channels at a higher density compared to the plasma membrane [3, 4, 23, 24]. Hundreds of proteins are expressed in cilia (complete list can be found on the Ciliomics and Cilia Proteome Web pages: www.sfu.ca/~leroux/ciliome_home.htm and www.ciliaproteome.org). Signaling proteins localized to this organelle include channels such as TRPV; the proteins associated with polycystic kidney and liver

diseases – polycystin-1 and -2 and fibrocystin; receptors such as SSTR3, serotonin, angiopoietin (Tie-1 and Tie-2), Smoothened, PDGF (PDGFα), and $P2Y_{12}$ [1, 16, 23, 25–33].

The *matrix* contains the intraflagellar transport (IFT, see below) machinery necessary to assemble and maintain the ciliary axoneme (Fig. 3.1). It is also likely that the matrix contains the components of the intracellular signaling pathways [1, 3, 12, 23, 34].

The *basal body* of a primary cilium is a mature mother centriole (Fig. 3.1). In multiciliated cells, axonemes of motile cilia emerge from multiple newly formed centrioles [3, 9, 13, 20]. Structurally, the basal bodies and the centrioles are identical and consist of nine triplet microtubules. The junction between the axoneme and the basal body is called the transition zone or transition region that is thought to function as a filter regulating the passage of molecules into or out of the axoneme [2, 4, 5, 9]. Current evidence suggests that ciliary-targeted proteins are transported from the Golgi as vesicles, secreted at the base of the cilia, docked at the transition zone, and then loaded onto the transport machinery for delivery to a cilium [1, 35]. Many proteins related to ciliary functions (for example, fibrocystin, inversin, polycystin, MKS1, BBS proteins) are accumulated in the vicinity of the basal bodies [12]. Several mechanisms involved in triggering of proteins to the ciliary membrane have been proposed. One of them is protein myristoylation that results in association of protein with lipid rafts and facilitate its ciliary targeting/retention [36]. Recent studies have identified specific ciliary targeting motifs in some proteins, such as Smoothened, SSTR3, polycystin-2, and the odorant responsive cyclic nucleotide-gated channel, CNGB1b [1, 34, 37, 38]. However, absence of these motifs does not exclude targeting of proteins to cilia [16, 31]. Ciliary targeting is also dependent on protein phosphorylation [1, 39].

Cilia in Normal Cholangiocytes

Primary cilia were first described in cholangiocytes more than 45 years ago (i.e., in 1963) [40]. Occasionally these organelles were also seen in the fat-storing cells (Ito cells) of diseased livers [41] but have never been seen in hepatocytes. In the portal triad of normal rats no visible cilia are present in hepatocytes, portal vein, and hepatic artery (Fig. 3.2a). These organelles are observed only in the intrahepatic bile ducts (Fig. 3.2b) extending into the ductal lumen from the cholangiocyte apical plasma membrane.

Interestingly, along the biliary tree axis, ciliary length is proportional to bile duct diameter: in large bile ducts, they are longer compared to small ducts (7.35 ± 1.32 μm and 3.58 ± 1.12 μm, respectively;

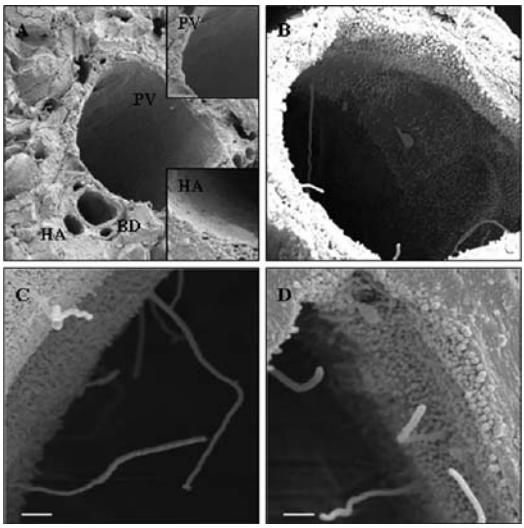

Fig. 3.2. Primary cilia in whole rat liver. (**a**) No visible cilia are present in the portal vein (PV) or in the hepatic artery (HA). Magnification: ×900. *Insets* on the *right* show close up view of hepatic artery and portal vein wall. (**b**) Cilia were observed only in the intrahepatic bile ducts (BD) as shown by scanning electron microscopy (SEM). Magnification: ×6,000. (**c**) In large bile ducts cilia are ∼2 times longer than in small ducts (**d**). Scale bars: 1 mm. Reproduced from (Am J Physiol Gastrointest Liver Physiol, 2006) with permission from American Physiological Society/hotwire press [42].

Fig. 3.2c, d). Since the biliary tree is heterogeneous with regard to the diameter of bile ducts, composition of proteins, and responses to proliferative stimuli, it is possible that in small and large bile ducts cholangiocyte cilia might perform different functions [42].

In *intrahepatic bile ducts* isolated from normal rats by microdissection, a single primary cilium ∼7 μm in length is clearly observed on the cholangiocyte apical membrane by scanning electron (Fig. 3.3a, b), transmission immunofluorescent (Fig. 3.3c), and confocal microscopy (Fig. 3.3d).

In cultured normal rat cholangiocytes (NRCs) and normal mouse cholangiocytes (NMCs), cilia progressively grow over time (up to day 14 of post-confluence) to the maximal length of ∼7–10 μm (Fig. 3.4).

Transmission electron microscopy of longitudinal sections (Fig. 3.5a, b) and cross section (Fig. 3.5c) demonstrates the 9 + 0 axonemal arrangement of cholangiocyte cilia (Fig. 3.5c, inset).

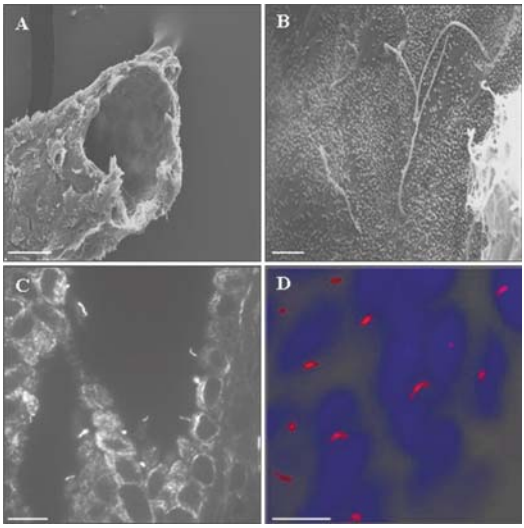

Fig. 3.3. Primary cilia in microdissected intrahepatic bile ducts. **a** and **b**: SEM of bile duct lumen (**a**) shows that cholangiocyte primary cilia (**b**) extend from the apical plasma membrane. Presence of cilia stained with ciliary marker anti-acetylated a-tubulin is confirmed in isolated bile ducts by transmission (**c**) and confocal (**d**) immunofluorescent microscopy. Scale bars: 50 mm (**a**), 1 mm (**b**, **c**), 10 mm (**d**). Reproduced from (Am J Physiol Gastrointest Liver Physiol, 2006) with permission from American Physiological Society/hotwire press [42].

Cilia in Cholangiociliopathies

Cholangiocytes are the target of a large group of acquired and inherited liver diseases, i.e., the cholangiopathies [43]. A number of cholangiopathies are caused by mutations in the genes encoding ciliary-associated proteins that account for the structural and functional characteristics of cholangiocyte primary cilia. We propose this group of hepatic disorders be called "cholangiociliopathies" [19, 44]. The cholangiociliopathies are characterized by liver cysts and/or hepatic fibrosis and include but not limited to autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive PKD (ARPKD), nephronophthisis (NPHP), Bardet–Biedl syndrome (BBS), and Meckel syndrome (MKS) (also *see* Chapter 4). The list of cholangiopathies in general and of the cholangiociliopathies, in particular, consistently grows.

The mechanisms involved in the pathogenesis of cholangiociliopathies are obscure. Over the past years, several hypotheses for cyst development have been proposed. One of them, the ciliary hypothesis

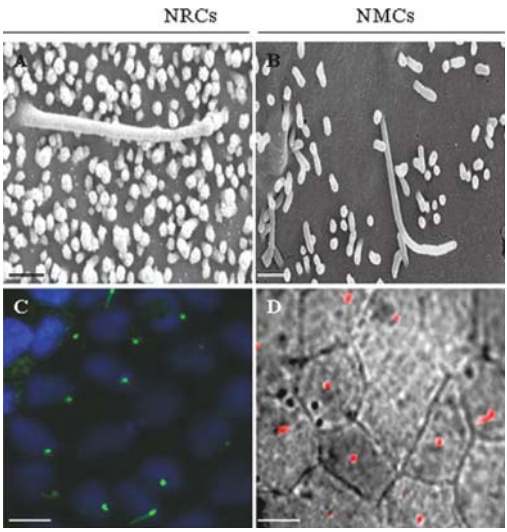

Fig. 3.4. Presence of cilia in cultured normal rat cholangiocytes (NRCs) and normal mouse cholangiocytes (NMCs) is shown by SEM (**a, b**), confocal immunofluorescent (**c**, *green*), and merge image of immunofluorescent and transmission microscopy (**d**, *red*). Scale bars 1 μm (**a**, **b**) and 10 μm (**c**, **d**). Nuclei are stained with DAPI (**c**, *blue*). Reproduced from (Am J Physiol Gastrointest Liver Physiol, 2006) with permission from American Physiological Society/hotwire press [42].

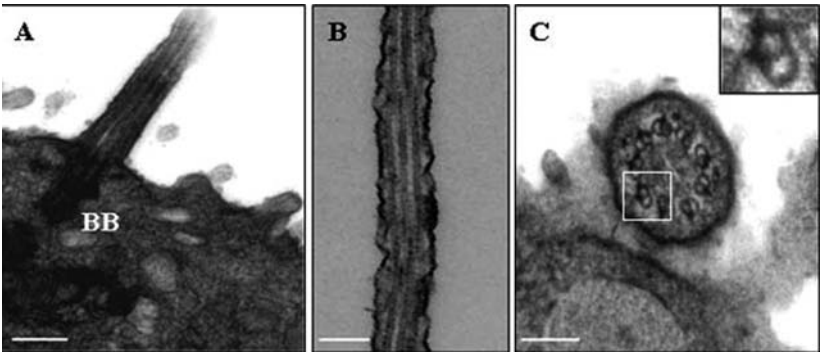

Fig. 3.5. Structure of cholangiocyte cilia by transmission electron microscopy. Longitudinal (**a**, **b**) and cross sections (**c**) show that cholangiocyte cilia have 9 + 0 axonemal structure. Scale bars: 50 mm (**c**) and 100 nm (**d**, **e**). BB – basal body. Reproduced from (Am J Physiol Gastrointest Liver Physiol, 2006) with permission from American Physiological Society/hotwire press [42].

of cystogenesis, is based on the following observations: (i) many identical proteins are localized to cilia, basal body, and centrosomes; (ii) some of these proteins form functional complexes with each other and are involved in similar signaling pathways; and (iii) ciliary-associated proteins are widely expressed in different organs and tissues and their mutations lead to a similar phenotype such as cyst formation and/or fibrosis.

Proteins implicated in the development of ciliopathies are known to affect ciliary function and/or ciliary morphology. In ADPKD, disruption of polycystin-1/polycystin-2 complex (proteins that are involved in disease development) in renal cilia is believed to be a major cause of cyst formation in kidney while the structure of the cilium itself is not affected. In contrast, in ARPKD, the morphology of these organelles in cholangiocytes lining liver cysts is abnormal with bulbous extensions present on the tip or membrane of cilia. Cystic cilia are ~2 times shorter (3.35 ± 1.96 μm) than in normal cholangiocytes (6.63 ± 1.32 μm) ranging in length from 0.21 to 6.27 μm (Fig. 3.6).

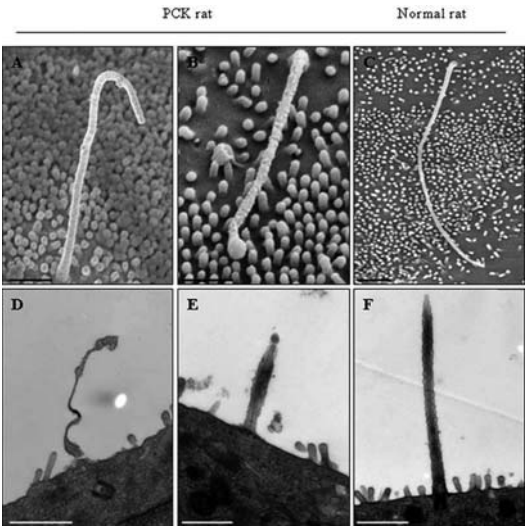

Fig. 3.6. Scanning (*top*) and transmission (*bottom*) electron microscopy of cholangiocyte cilia in normal and PCK rats demonstrates that cilia in the PCK rat are heterogeneous in length (**a** and **b**) and malformed (**d** and **e**) compared to normal cholangiocytes (**c** and **f**). Scale bars: 1 μm (**a**, **c**, **d** and **f**); 500 nm (**b**, **e**). Reproduced from (Am J Pathol, 2004) with permission from the American Society for Investigative Pathology and Stanford University Libraries/highwire press [81].

Ciliogenesis

Formation of any type of cilia (i.e., ciliogenesis) requires dynamic intracellular remodeling that consists of four major stages: (i) generation of centrioles; (ii) migration of the mother centriole to the cell plasma membrane; (iii) docking of the mother centriole to the plasma membrane (i.e., formation of the basal body); and (iv) formation of ciliary axoneme (Fig. 3.7) [20]. The primary cilium arises from the preexisting centriole, whereas motile cilia require production of multiple centrioles de novo. Once formed, centrioles migrate to the cell surface and give rise to many cilia [5].

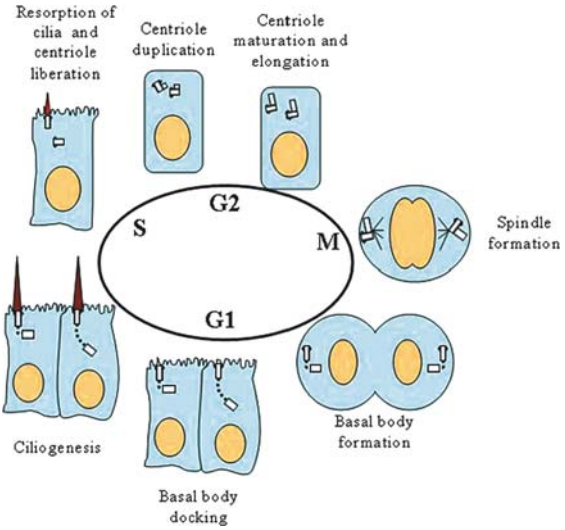

Fig. 3.7. Schematic representation of ciliogenesis. Entry into the cell cycle is preceded by ciliary resorption and liberation of centrioles. During S phase of the cell cycle and after ciliary reabsorption, centrioles are duplicated following their maturation during G2/M. Cilia are assembled when cells exit the cell cycle from mitosis into G1 phase.

Centrosome, Centrioles, and Pericentriolar Material

Centrosomes, the only non-membranous organelles in vertebrate cells, are located near the cell center in close proximity to the nucleus and composed of four main structures: centrioles, pericentriolar material (PCM), γ-tubulin complexes, and fibers (Fig. 3.8) [45, 46]. An animal cell typically possesses two centrioles – the mother centriole (the older of two) and the daughter centriole that are linked by interconnecting fibers, surrounded by PCM and positioned at 90° angel to each other. The centriole is composed of the barrel-shaped array of

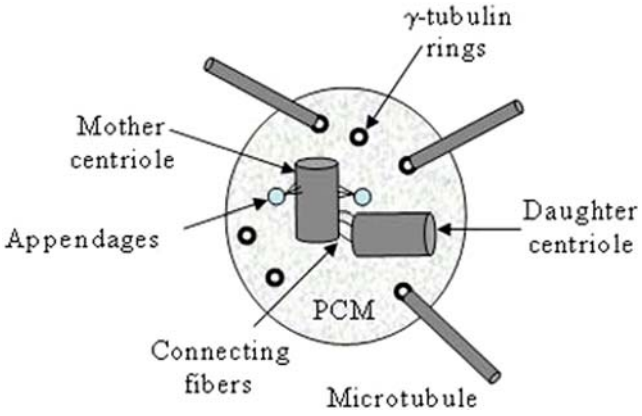

Fig. 3.8. Structure of centrosomes. Centrosomes are located near the cell center in close proximity to the nucleus and composed of four main structures: mother and daughter centrioles, pericentriolar material (PCM), γ-tubulin complexes responsible for microtubule nucleation, and fibers that are composed of *Sfilp* and *centrin* and act as a connection between elements of centrosome.

nine microtubules that are triplets. They form a cylindrical core with a slight twist and composed of complete A-tubule and incomplete B- and C-tubules [5, 20, 47]. Centrioles are typically 300–500 nm long and ~150–200 nm in diameter [5, 48]. They are structurally different in the pair: the mother centriole has a set of appendages at the distal end, whereas the daughter centriole does not. The appendages are essential for anchoring of microtubules, and the daughter centriole acquires them in the late G2 phase of the cell cycle [46, 49–51] (Fig. 3.8).

CENTROSOME FUNCTIONS

Recent data suggest that centrosomes comprising of hundreds of proteins are important organelles not only for microtubule nucleation and organization but also for cell cycle progression, migration, ubiquitin-proteosome degradation, cell polarity, and cilia formation [3, 5, 45–47, 50, 52–54]. During the cell cycle, centrosomes play different roles (Fig. 3.7). In the interphase, they serve as microtubule organizing centers and function as a scaffold to direct organelles and vesicles trafficking. During mitosis, centrosomes form the mitotic spindle that is required for cell entry into the S phase [46], although they are not essential for this process. Finally, centrosomes are involved in the formation of primary cilia. In the G1/G0 phase of the cell cycle, followed centrioles duplication, the mother centriole is recruited to the apical

membrane to become a basal body and to generate a primary cilium [47, 49, 50, 52–56].

Consistent with their many functions, centrosomes have been implicated in a variety of human diseases. Abnormalities in the centrosomes number, their size, and morphology have been observed in nearly all human tumors, including breast, colon, liver, bone marrow, cervical, and prostate cancer [49, 54, 57, 58].

Centriole Duplication

A cell inherits one centrosome with two centrioles (Fig. 3.7). Duplication of the centrosome is initiated in the G1–S phase of the cell cycle and is controlled by the cyclinE–Cdk2 pathway. Following physical separation of the centrioles, the daughter centriole (pro-centriole) is formed in the close proximity of each preexisting centrioles. The pro-centrioles continue to elongate and to mature by recruiting additional PCM proteins [5, 20, 47, 49, 50, 56, 59].

Centriole Migration and Docking

In the G1/G0 phase of the cell cycle the mother centriole is recruited to the plasma membrane to become a basal body and to generate a primary cilium (Fig. 3.7) [5, 20, 47, 56, 60, 61]. The mother centriole is ultrastructurally distinguished from the daughter centriole by the presence of distal and sub-distal appendages which are essential for the membrane docking [13, 20].

Factors that control the centriole migration and docking are unknown. The involvement of actin–myosin network, the activation of different signaling pathways, the planar cell polarity proteins, and the helix forkhead/winged family of transcription factors have been all shown to play an important role in this processes [51, 62–64].

Formation of the Ciliary Axoneme

The final step of ciliogenesis (i.e., the formation of the ciliary axoneme) includes the following: (1) initiation (during this steps, the distal end of the mother centriole is associated with the concave–convex vesicle called the primary ciliary vesicle); (2) elongation (during this step, axonemal microtubules grow from the distal part of the basal body against the membrane of the primary vesicle forming ciliary bud); (3) fusion (during this step, a primary cilium is emerged as a result of fusion of the ciliary bud with cell plasma membrane); and (4) the final elongation of the ciliary axoneme [48].

Recently the regulation of ciliogenesis has received a great attention. Many proteins, transcriptional factors, and kinases are known to be involved in this process [65–71]. It appears that ciliogenesis of motile cilia might be quite different from ciliogenesis of primary cilia: in *HFH-4* knockout mice classic 9 + 2 cilia are absent, but 9 + 0 cilia are present; and in cells possessing motile cilia, centriole migration and docking to the apical membrane is abnormal [64, 72].

Intraflagellar Transport (IFT)

The integrity and function of cilia completely depends on the anterograde and retrograde microtubule-based transport of molecules along the length of the axoneme, a process called intraflagellar transport (IFT). First described in *Chlamydomonas* [73, 74], IFT was later confirmed in other organisms including mammals [12, 75]. The IFT system consists of two types of motor proteins (i.e., kinesis and dynein) and 15–20 polypeptides assembled into large group of IFT particles. IFT particles are delivered from the base to the ciliary tip at a speed of 2.5 μm/s via kinesin-driven process (i.e., anterograde transport). Once the IFT particles reach the tip, they undergo modification that results in inactivation of the kinesin and facilitates their retrograde return (tip to base transport) to the cell interior at speed of 4 μm/s through a dynein-dependent mechanism [1, 34, 35, 76, 77]. IFT polypeptides are organized (based on their molecular weight) into two large complexes – A (comprising of 4–5 proteins of ~550 kDa) and B (comprising of 12 proteins of ~710 kDa) [1, 78, 79].

In *C. elegans* and *Chlamydomonas* mutants with mutation in A-complex proteins, cilia are shorter and the IFT particles are accumulated along the axoneme indicating the importance of A-complex proteins in a retrograde transport [12]. Mutations in genes that encode B-complex proteins also lead to shortened cilia but there is no particle accumulation along the ciliary axoneme, suggesting that B-complex proteins are involved in anterograde transport [74].

Before the IFT particles enter the cilium, they gathered to the distal end on the basal body near the ciliary base. Some IFT particles (i.e., IFT20) were also found at the lateral site of the daughter centriole [1, 80].

Ciliogenesis in Cholangiocytes

Recent data indicate that disruption of ciliary integrity (structural and/or functional) is a primary contributor to the PKD. Indeed, as we discussed above, cholangiocyte cilia in liver cysts of the PCK rat, an animal model of ARPKD, are ~2 times shorter and malformed [29, 81].

Since primary cilia extend from a single parental centriole, it may suggest that not only ciliary defects but also centrosomal abnormalities may contribute to disease pathogenesis. It has been shown that loss of polycystin-1 function is sufficient to produce centrosome amplification and multiple spindles in kidney epithelia [82]. Proper positioning of centrosomes also appears to be essential for cell development, polarization, and cell function. For example, centrosomes are required for axonal polarity of neurons – after the last mitosis, centrosomes are found in the area where the axon will form [83, 84].

In PCK rats, number of cholangiocytes with supernumerary centrosome is significantly increased compared to normal rats. In normal rat cholangiocytes, centrioles are usually positioned close to one of the cell lateral junction. In contrast, in PCK rats this distance is increased by ~50%, while the distance between two cellular lateral junctions did not differ in normal and cystic cholangiocytes. Centrioles in PCK rats are heterogeneous in length, whereas in normal animals the length of centrioles is more uniform. Several electrodense spots and extra appendages (all indicators of centrosomal abnormalities) are also observed in centrioles of PCK rats. In addition, growth of ciliary axoneme in cultured PCK cholangiocytes (i.e., the final step of ciliogenesis) is impaired. In sub-confluent and immediate post-confluent monolayers of normal and PCK cholangiocytes cilia are absent. They appear in both cell lines at day 3 of post-confluence and progressively grow over time (up to day 14). Cilia are at average ~2 times shorter and the number of ciliated cells is decreased in the PCK cultures. Taken together these data suggest that ciliary abnormalities observed in the PCK rat are linked to defects in centrosome morphology, position, and their number in cystic cholangiocytes.

FUNCTIONS OF CHOLANGIOCYTE CILIA

Cholangiocyte cilia, like primary cilia in other epithelial cells, are located on the apical plasma membrane domain and are the sensory organelles. They recognize the current state of bile, i.e., its flow, chemical composition, and osmolality, and transmit the related information into intracellular signaling, i.e., function as mechano-, chemo-, and osmosensors [19].

Cholangiocyte Cilia as Mechanosensors

For the first time, the mechanosensory function of primary cilia was demonstrated in renal epithelia. It has been shown that bending of a single cilium by a micropipette or by alterations in perfusate flow rates

increases intracellular Ca^{2+} ($[Ca^{2+}]_i$) in MDCK cells, a cultured cell line derived from the collecting duct of canine kidney [85–87], and in cultured mouse renal epithelial cells [88, 89]. An acute increase in fluid flow rates in microperfused cortical collecting ducts of the rabbit and mouse nephrons also lead to ciliary-dependent elevation of $[Ca^{2+}]_i$ in epithelial cells [90, 91]. The mechanosensory function of renal cilia is provided by a cell surface receptor, polycystin-1 (PC-1), and a Ca^{2+} channel, polycystin-2 (PC-2), which are the ciliary membrane proteins that may form a functional mechanosensory complex [92, 93]. Indeed, a flow-induced $[Ca^{2+}]_i$ signaling response in renal epithelial cells was not observed in mouse with mutated *Pkd1* and *Pkd2* genes, encoding PC-1 and PC-2, respectively, and in wild-type renal epithelial cells preincubated with antibodies against extracellular but not intracellular domains of PC-1 and PC-2 [88, 89].

Bending of cholangiocyte cilia by luminal fluid flow in microperfused intrahepatic bile ducts also induces an increase in $[Ca^{2+}]_i$ (Fig. 3.9), which in turn inhibits intracellular cAMP signaling [27]. The mechanosensory function of cholangiocyte cilia is provided by PC-1, PC-2, and Ca^{2+}-inhibitable adenylyl cyclase 6 (AC6) (Fig. 3.9). AC6 is known to be colocalized with Ca^{2+} entry channels in discrete domains of the plasma membrane (i.e., "lipid rafts") where its activity is inhibited by extracellular Ca^{2+} [94, 95]. Given colocalization of PC-2 and AC6 on cholangiocyte cilia, an analogous mechanism may exist in the ciliary membrane, i.e., the activity of AC6 is inhibited by extracellular Ca^{2+} entering the cell via ciliary PC-2 [27].

Cholangiocyte Cilia as Chemosensors

Primary cilia express numerous receptors, ion channels, components of the intracellular signaling pathways, i.e., proteins that may provide chemosensory functions of these organelles [19, 30, 96]. For example, specific G protein-coupled receptors (i.e., chemoreceptors) which are activated by attractants, repellents, and pheromones are expressed in primary cilia of the sensory neurons of *C. elegans* [97]. Activation of ciliary chemoreceptors increases cGMP levels, which in turn triggers the opening of cGMP-gated channels transducing chemostimuli into the neuronal activity via the cGMP-signaling pathway [97]. The chemosensory function of cholangiocyte cilia is provided by a purinergic receptor, $P2Y_{12}$, and components of the cAMP signaling cascade, which include adenylyl cyclases (AC4, AC6, and AC8), protein kinase A (PKA), and exchange protein directly activated by cAMP (EPAC) [32]. $P2Y_{12}$, is activated primarily by ADP and links to the cAMP signaling pathway via G_i, i.e., is involved in inhibition of cAMP signaling

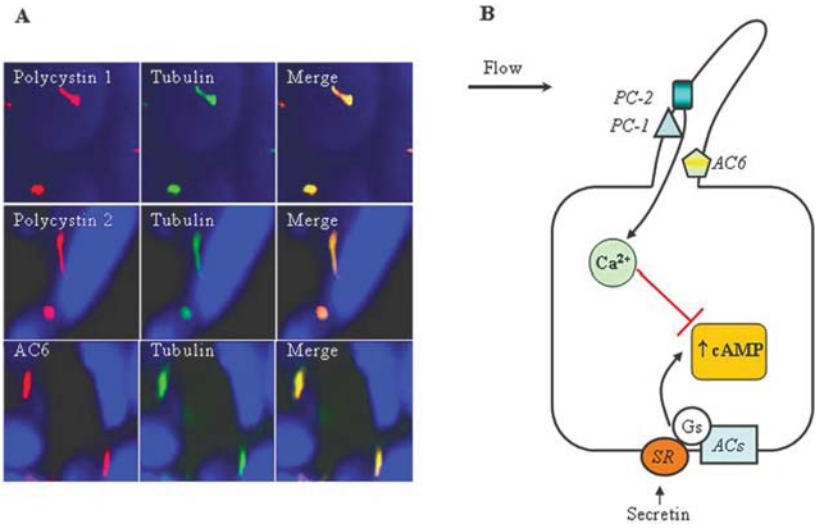

Fig. 3.9. Mechanosensory function of cholangiocyte cilia. (**a**) Cholangiocyte cilia (stained with an antibody to a ciliary marker, acetylated a-tubulin, *green*), express polycystin-1 (PC-1), polycystin-2 (PC-2), and adenylyl cyclase 6 (AC6, all in *red*). Nuclei are visualized by DAPI (*blue*). (**b**) In response to flow, PC-1 and PC-2 form functional complex providing entry of extracellular calcium into the cell which subsequently increases $[Ca2+]_i$. Elevated $[Ca2+]_i$ suppresses previously activated (e.g., by secretin) cAMP signaling pathway possibly with the involvement of AC6. Panel **a** reproduced from (Gastroenterology, 2006) with permission from Elsevier [27].

(Fig. 3.10). ADP perfused through the lumen of isolated rat intrahepatic bile ducts or applied to the apical surface of normal ciliated rat cholangiocytes in culture does not affect the basal cAMP levels but inhibits a forskolin-stimulated cAMP increase. This effect of ADP is abolished by pharmacological removal of cilia and by siRNAs to the gene encoding $P2Y_{12}$ suggesting that extracellular signaling provided by biliary nucleotides is transduced by cilia into intracellular cAMP signaling [32].

Cholangiocyte Cilia as Osmosensors

Primary cilia in *C. elegans* sensory neurons express OSM-9, a putative TRP (Transient Receptor Potential)-like channel protein that provides osmosensory functions of these organelles [98]. Recently, Transient Receptor Potential Vanilloid 4 (TRPV4) channel was identified as mammalian homologue of OSM-9 [99, 100]. TRPV4 is a nonselective cation channel highly permeable to Ca^{2+} and exquisitely sensitive

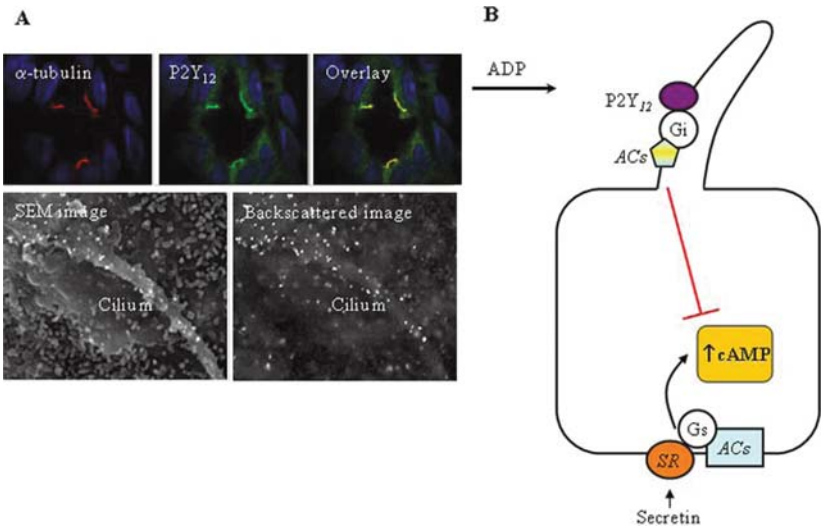

Fig. 3.10. Chemosensory function of cholangiocyte cilia. (**a**) Expression of $P2Y_{12}$ in cholangiocyte cilia is shown by immunofluorescent confocal microscopy (*top panels*) and by immunogold SEM (*bottom panels*). $P2Y_{12}$ (*green*) in cilia is visualized by acetylated a-tubulin (*red*); nuclei are stained with DAPI (*blue*). Conventional SEM and backscattered images demonstrate the ciliary expression of $P2Y_{12}$. (**b**) ADP present in bile activates $P2Y_{12}$ which is linked to ACs via G_i subunit. As a result, previously elevated (e.g., by secretin) cAMP levels are decreased. Panel **a** reproduced from (Am J Physiol Gastrointest Liver Physiol, 2008) with permission from the American Physiological Society/hotwire press [32].

to even minute changes in osmolality of extracellular milieu, being activated by extracellular hypotonicity and inhibited by extracellular hypertonicity [101]. Activation of TRPV4 results in an increase in $[Ca^{2+}]_i$ through influx of extracellular Ca^{2+} into the cell; however, the molecular mechanisms by which hypotonicity affects TRPV4 functions remains unknown [102].

In epithelial cells lining the oviduct, TRPV4 is localized to motile cilia, where it may perform multiple roles [26, 103]. TRPV4 is also expressed on cholangiocyte cilia providing their osmosensory function (Fig. 3.11) [25]. In cultured normal mouse cholangiocytes (NMC) and in isolated rat intrahepatic bile ducts, ciliary TRPV4 is involved in functional responses of biliary epithelia to changes in fluid (bile) osmolality. In microperfused rat intrahepatic bile ducts, hypotonicity induces an increase in $[Ca^{2+}]_i$ and in secretion of bicarbonate ions, which are the major contributor to ductal bile flow [25]. In NMC transfected with TRPV4 shRNA to suppress its expression, increase in Ca^{2+} induced

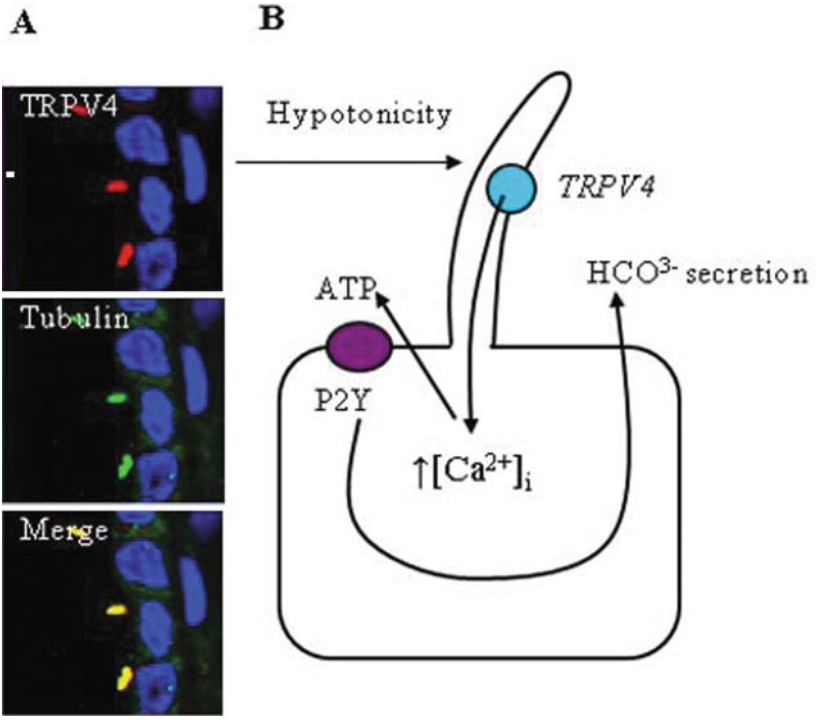

Fig. 3.11. Osmosensory function of cholangiocyte cilia. (**a**) TRPV4 is expressed in cholangiocyte cilia as detected by co-staining of rat liver sections with antibodies to TRPV4 (*red*) and acetylated a-tubulin (*green*); nuclei are in *blue*. (**b**) Hypotonicity of bile induces an increase in $[Ca^{2+}]_i$ with the involvement of TRPV4 followed by ATP release. ATP binds to P2Y receptors expressed on cholangiocyte apical plasma membrane and enhances bicarbonate secretion subsequently increasing bile flow. Panel **a** reproduced from (PNAS, 2007) with permission from National Academy of Sciences and Stanford University/highwire press [25].

by hypotonicity was completely abolished. Removal of cilia by chloral hydrate and suppression of TRPV4 by specific siRNAs in isolated rat intrahepatic bile ducts also abolished both an increase in $[Ca^{2+}]_i$ and bicarbonate secretion in response to hypotonicity. Taken together these observations suggest that ciliary TRPV4 may monitor changes in bile osmolality and induce cholangiocyte functional responses to such changes.

CILIARY HYPOTHESIS OF CYSTOGENESIS

The data presented above strongly suggest that cholangiocyte cilia play an important role in normal physiology of bile duct epithelial cells

regulating cAMP/$[Ca^{2+}]_i$ homeostasis. It has been shown in recent years that many proteins (e.g., PC-1 and PC-2, fibrocystin, inversin, polaris, cystin), mutations of which are linked to cyst formation and/or fibrotic changes in liver and kidney, are expressed in cilia. The connection between cilia and fibrocystic renal and hepatic disorders was demonstrated in several animal models including *orpk* [74, 104, 105], *cpk* [74], and *Kif3A* KO [106] mice and PCK rats [29]. In these models, the disruption of ciliary-associated proteins by spontaneous mutations or in response to experimental manipulation results in structural and/or functional abnormalities of cilia subsequently activating or shutting down many different cellular pathways, in particular, cAMP and $[Ca^{2+}]_i$.

An increase in cAMP levels is one of the common features of the renal cystic disease. Elevated cAMP was observed in polycystic kidneys in different animal models [107–109] and has been shown to play a significant role in renal cystogenesis [110, 111] while reduction in its levels in vivo attenuates disease progression [108]. $[Ca^{2+}]_i$ has been also implicated in renal cystogenesis. Indeed, in ARPKD patients $[Ca^{2+}]_i$ levels in kidney cysts are significantly low and in cultured normal kidney cells silencing of *Pkhd1* (gene which is mutated in ARPKD) decreases concentration of $[Ca^{2+}]_i$ resulting in cell hyperproliferation [112, 113].

The interconnection between $[Ca^{2+}]_i$ and cAMP homeostasis in cholangiocytes and its role in hepatic cystogenesis is supported by observations that cholangiocytes lining liver cysts in the PCK rat are characterized by increased cAMP concentrations [114] and decreased levels of $[Ca^{2+}]_i$ [115]. The changes in activity of two intracellular signaling pathways were associated with structurally abnormal and malformed cilia [29, 81, 114]. The elevated cAMP is responsible for hepatic cystogenesis in the PCK rat, while a decrease of cAMP content by octreotide halts cyst expansion in vitro and in vivo [114]. Moreover, elevation of $[Ca^{2+}]_i$ levels in PCK cholangiocytes significantly reduces the rate of cell proliferation and suppresses cyst growth in culture [115]. Thus, these data support the importance of ciliary integrity and the cAMP and $[Ca^{2+}]_i$ intracellular signaling pathways in hepatic disease progression.

CONCLUSION

Cholangiocyte primary cilia are unique organelles that perform numerous functions critically involved in normal physiology of biliary epithelia. On the other hand, defects in cholangiocyte cilia

structure and/or functions are likely the reasons of cholangiociliopathies. Cholangiocyte cilia, like primary cilia in other epithelial cells, extending for several micrometers from the apical plasma membrane into the ductal lumen, have strategic location, which may allow these organelles to sense the current state of bile, i.e., its flow, chemical composition, and osmolality, and transmit the corresponding information into the cell via the intracellular signaling pathways.

Colocalization of PC-1, PC-2, AC6, $P2Y_{12}$, and TRPV4 on cholangiocyte cilia and initial functional studies show that under normal conditions primary cilia in biliary epithelia possess multiple sensory functions coordinating activity of the cAMP and $[Ca^{2+}]_i$ signaling pathways. In contrast, under pathological conditions, ciliary structural (i.e., shorter and malformed cilia) and functional (mistargeting of proteins involved in mechano-, chemo-, and osmosensory functions of cilia) abnormalities might lead to discoordination of $[Ca^{2+}]_i$/cAMP homeostasis resulting in up-regulation of the cAMP and down-regulation of $[Ca^{2+}]_i$ signaling pathways subsequently leading to hepatic cystogenesis.

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4 Genetics of Fibrocystic Diseases of the Liver and Molecular Approaches to Therapy

Maya Tuchman, William A. Gahl, MD, PhD, and Meral Gunay-Aygun, MD

CONTENTS

Summary

Congenital hepatic fibrosis (CHF), Caroli's disease (CD), and polycystic liver disease (PLD) are the three major descriptive

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categories of fibrocystic liver disease. Caroli's syndrome (CS) and CD probably represent different presentations of the same continuum. CS refers to CD in association with CHF. CHF/CS and PLD are often part of multisystem disorders associated with fibrocystic renal involvement. These are collectively referred to as "ciliopathies," since the abnormal proteins involved function on the primary cilium or its basal body. The inheritance pattern of CHF/CS is autosomal recessive, with rare exceptions such as the CHF associated with X-linked oral-facial-digital syndrome type 1. The inheritance pattern of PLD is autosomal dominant; the majority of patients have autosomal dominant polycystic kidney disease (ADPKD) caused by mutations in the *PKD1* or *PKD2* genes. Autosomal dominant polycystic liver disease (ADPLD), in which PLD is not associated with renal cysts, refers to a genetically distinct entity caused by mutations in the *PRKCSH* or *SEC63* genes. CHF/CS most commonly presents in association with autosomal recessive polycystic kidney disease (ARPKD) caused by mutations in the *PKHD1* gene. Multisystem syndromes associated with CHF/CS include Meckel, Bardet–Biedl, and Joubert syndromes and related cerebello-hepatorenal syndromes, renal-hepatic-pancreatic-dysplasia, and ciliary skeletal dysplasias such as Jeune's chondrodysplasia. Many syndromic ciliopathies display marked genotypic heterogeneity with multiple different genes causing the same disease. This chapter will review the molecular genetic bases of these disorders and provide an overview of novel targeted therapies.

Key Words: Hepatorenal fibrocystic disease, Polycystic liver disease, Congenital hepatic fibrosis, Caroli's syndrome, Caroli's disease, Polycystic kidney disease, Primary cilia, Centrosome, Nephronophthisis, Joubert syndrome and related disorders, COACH, Meckel syndrome, Bardet–Biedl syndrome, Polycystin 1, Polycystin 2, Fibrocystin, Hepatocystin, Sec63, EGFR-TKI inhibitors, Src-inhibitors, Rapamycin, Somatostatin inhibitors

INTRODUCTION

Hepatic fibrocystic diseases can be grouped into three major descriptive categories: congenital hepatic fibrosis (CHF), Caroli's disease (CD), and polycystic liver disease (PLD). Caroli's syndrome (CS) refers to CD in association with CHF [1–3]; CD and CS probably represent different presentations of the same continuum because they may coexist in different members of the same family [2]. The liver cysts in CS are non-obstructive, saccular, or fusiform dilatations of the intrahepatic biliary tree; hence, they are continuous with the biliary system [2].

In contrast, the liver cysts in PLD are isolated cysts that originate from biliary microhamartomas (von Meyenburg complexes) embedded in fibrous tissue; they are not part of the intrahepatic biliary tree [4, 5]. The inheritance pattern of PLD is autosomal dominant in most families. The majority of PLD patients have autosomal dominant polycystic kidney disease (ADPKD). Autosomal dominant polycystic liver disease (ADPLD) refers to a genetically distinct entity in which PLD is not associated with kidney cysts [6].

The inheritance pattern of CHF/CS is autosomal recessive in most cases. Two rare exceptions are X-linked CHF/CS in association with oral-facial-digital syndrome type I (OFD1) [7] and autosomal dominant CHF/CS in rare atypical ADPKD families [8]. In the majority of patients, CHF/CS is part of a multisystem disorder (Fig. 4.1) commonly associated with renal involvement in the form of polycystic kidney disease (PKD), nephronophthisis (NPHP), chronic tubulointerstitial nephropathy, or an isolated urinary concentration defect [8]. CHF/CS can rarely appear as an isolated finding, but no gene causing isolated CHF/CS has been identified to date. CHF/CS most commonly presents as part of autosomal recessive polycystic kidney disease (ARPKD), the most common childhood ciliopathy with an estimated frequency of 1 in 20,000 live births [9–11]. Although all patients with

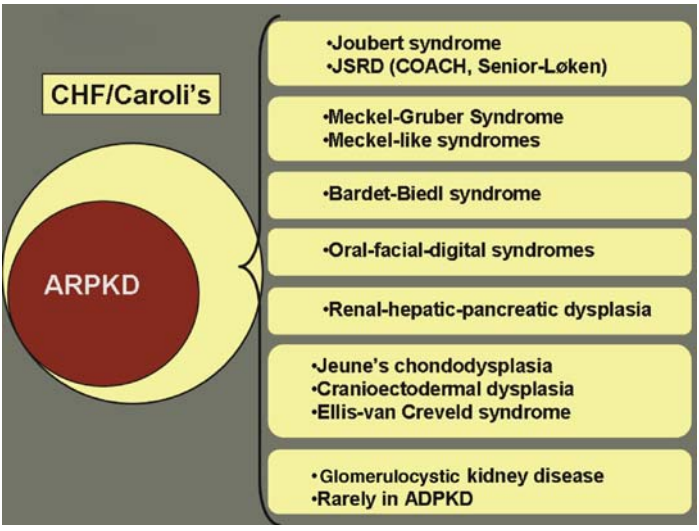

Fig. 4.1. CHF/CS is usually part of a multisystem disorder commonly associated with renal involvement. Many syndromes have CHF/CS included in their phenotypic expression.

ARPKD have CHF as a microscopic pathology at birth, abnormal liver echogenicity and splenomegaly may not be detectable during early childhood because periportal fibrosis and portal hypertension are time-dependent pathologies that progress with age. Other disorders in which CHF is a universal finding include Meckel syndrome (MKS) [12, 13], COACH syndrome (a subset of Joubert syndrome and related disorders (JSRD) with *c*erebellar vermis hypoplasia, *o*ligophrenia, *a*taxia, *c*oloboma, and *h*epatic fibrosis) [14, 15], and renal-hepatic-pancreatic-dysplasia (RHPD) [16–18]. Symptomatic CHF/CS, resulting in portal hypertension, cholangitis, or mildly elevated liver enzymes, occurs in a subset of patients with various other ciliopathies including JSRDs [19–21], Bardet–Biedl (BBS) [22, 23], oral-facial-digital (OFD) syndromes [7, 24–26], and ciliary skeletal dysplasias such as Jeune's chondrodysplasia [27–29]. Whether CHF as a microscopic pathology is a universal finding in these syndromes remains to be determined. Asymptomatic or mild to moderately symptomatic CHF/CS might be under-diagnosed in these syndromes, given that pathologies of other systems often become the focus of attention and life span can be limited.

Syndromic ciliopathies display marked genotypic heterogeneity with multiple different genes causing the same disease. Several ciliopathies especially JSRDs, MKS, and Bardet–Biedl syndromes (BBS) display significant phenotypic and genotypic overlap. Some ciliary genes such as *CEP290* and *MKS3* are associated with a wide spectrum of clinical phenotypes ranging from severe perinatally fatal MKS to the milder Joubert syndrome [8].

Most proteins encoded by genes mutated in hepatorenal fibrocystic disorders localize to the primary cilium or its basal body, suggesting cilia-associated disease mechanisms [30, 31]. Exceptions to this include ADPLD and congenital disorder of glycosylation (CDG) type Ib-associated CHF. Primary cilia are microtubule-based antenna-like sensory organelles that extend outward from the surface of almost all eukaryotic cells including renal and biliary epithelium [30]. Differing from respiratory cilia, primary cilia number one per cell (monocilia) and are non-motile (rigid). Similar to cilia of the renal tubular epithelium, cholangiocyte cilia express proteins required for chemosensory and mechanosensory functions such as detecting luminal fluid flow [32]. Mutations in genes encoding these cystoproteins affect the structure and/or function of cholangiocyte cilia and cause fibrocystic pathology in the liver (Table 4.1).

This chapter will review the molecular genetic bases of the inherited hepatic fibrocystic diseases and provide an overview of the novel targeted therapeutic approaches enabled by recent advances in our understanding of the cellular bases of these diseases.

Table 4.1
Summary of genes and proteins defective in fibrocystic liver disease

Gene	*Disease(s)*	*Protein*	*Localization*	*Probable function*
PKD1	ADPKD	Polycystin 1	Primary cilium, tight junctions, adherent junctions, desmosomes, focal adhesions	Fluid flow mechanosensor, cell signaling, cell–cell adhesion
PKD2		Polycystin 2	Primary cilium, centrosome, endoplasmic reticulum	Cation channel, cell signaling
PKHD1	ARPKD	Fibrocystin/ polyductin	Primary cilium, centrosome, plasma membrane	Signal transduction, cilia structure
PRKSCH	ADPLD	Glucosidase II β sub-unit/ hepatocystin	Endoplasmic reticulum	Essential for the maturation and conservation of GIIα, an ER-resident enzyme involved in processing, folding, and quality control of glycoproteins
SEC63		Sec63	Endoplasmic reticulum membrane	Part of the multicomponent translocon involved in protein processing, chaperone binding, and protein folding

Table 4.1
(continued)

Gene	*Disease(s)*	*Protein*	*Localization*	*Probable function*
MKS1	MKS	MKS1 protein	Centrosome	Ciliogenesis
CC2D2A		CC2D2A protein	Unknown	Ciliogenesis
MKS3	MKS, JSRD	Meckelin	Primary cilium, plasma membrane	Ciliogenesis
RPGRIP1L		RPGRIP1L protein	Primary cilium, centrosome	Interacts with nephrocystin-4 and -6
CEP290	MKS, JSRD, NPHP, BBS	Nephrocystin-6	Centrosome	Regulates ATF4 (transcription factor involved in renal cyst formation) activity, ciliogenesis
AHI1	JSRD	AHI1 protein	Primacy cilium, centrosome, adherens junctions	Interacts with nephrocystin-1
NPHP1	JSRD, NPHP	Nephrocystin-1	Primary cilium, centrosome, adherens junctions	Control of cell division, cell–cell matrix adhesion signaling
BBS1	BBS	BBS1 protein	Primary cilium, centrosome	BBSome
BBS2		BBS2 protein	Centrosome	BBSome

BBS3	ADP-ribosylation-like factor (ARL) protein 6	Primary cilium, centrosome	Regulates membrane-associated intracellular trafficking events and undergoes intraflagellar transport
BBS4	BBS4 protein	Centrosome	Facilitates cargo loading for microtubule-dependent intracellular transport within the cilium, BBSome
BBS5	BBS5 protein	Centrosome	BBSome
BBS6	BBS6 chaperonin	Centrosome, pericentriolar material	ATP-dependent nascent protein folding
BBS7	BBS7 protein	Primary cilium, centrosome	BBSome
BBS8	TTC8 protein	Centrosome	BBSome
BBS9	BBS9 protein	Unknown	BBSome
BBS10	BBS10 protein	Unknown	Unknown
BBS11	TRIM32/E3 ubiquitin ligase	Cytoskeleton	Cytoskeletal regulation

Table 4.1
(continued)

Gene	*Disease(s)*	*Protein*	*Localization*	*Probable function*
BBS12		BBS12 protein	Unknown	Unknown
JAG1	AGS	Jagged-1	Cell surface	Acts as a ligand for the Notch transmembrane receptors and contributes to the regulation of cell fate
NOTCH2		Notch family transmembrane receptor	Cell surface	Involved in development through control of cell fate decisions
NPHP2	NPHP	Inversin	Primary cilium	Interacts with nephrocystin-1 and with β-tubulin
NPHP3		Nephrocystin-3	Unknown	Unknown
NPHP4		Nephrocystin-4/nephroretinin	Primary cilium, centrosome	Interacts with proteins involved in cell adhesion and cytoskeleton organization
NPHP5	Nephrocystin-5	Primary cilium	Unknown	

IFT80	ATD1	Intraflagellar transport protein	Primary cilium	Component of intraflagellar transport (IFT) complex B which is essential for development and maintenance of motile and sensory cilia
OFD-1	OFD1	OFD-1 protein	Centrosome	Primary cilium assembly, left–right axis determination
MPI	CDGIB	Mannose-6-phosphate isomerase	Cytosol	Catalyzes the interconversion of fructose-6-phosphate and mannose-6-phosphate; plays a critical role in maintaining the supply of D-mannose derivatives

ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; ADPLD, autosomal dominant polycystic liver disease; MKS, Meckel syndrome; JSRD, Joubert syndrome related disorders; NPHP, nephronophthisis; BBS, Bardet–Biedl syndrome; AGS, Alagille syndrome; ATD1, Jeune asphyxiating thoracic dystrophy; OFD1, oral-facial-digital syndrome-l; CDGIB, congenital disorder of glycosylation type Ib.

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

Since ADPKD occurs in approximately 1 in every 1,000 individuals, and the majority of ADPKD patients develop PLD [33], ADPKD represents the most common cause of cystic liver disease. CS [34] and CHF complicated by portal hypertension are rarely associated with ADPKD [8].

ADPKD is genetically heterogeneous, with two genes identified to date: *PKD1* encoding polycystin-1 (PC1) and *PKD2* encoding polycystin-2 (PC2) [35–37]. A few families do not map to these loci, suggesting a third locus [35]. Approximately 85% of ADPKD is caused by *PKD1* and 15% by *PKD2* mutations. Patients with mutations in *PKD1* and *PKD2* have similar clinical manifestations, but with different severity and age of onset; the *PKD2* mutations cause milder symptoms with later onset of renal insufficiency [38]. PLD develops in both *PKD1*- and *PKD2*-related ADPKD [33]. No genotype–phenotype correlation exists for PLD. Most ADPKD patients have an affected parent, while approximately 10% of patients develop disease due to new mutations. Mosaicism for *PKD1* mutation has been reported [33]. The mutation detection rates for *PKD1* and *PKD2* are similar at approximately 85–90% [33]. Although *PKD1* and *PKD2* sequencing is available, the diagnosis of ADPKD is still made clinically, primarily based on imaging studies. Molecular genetic testing by sequence analysis is used when imaging results are not diagnostic or when a definitive diagnosis is required for a young adult family member, such as during evaluation to be a kidney donor.

The *PKD1* gene maps to chromosome 16p13.3 and spans 50 kb [39]. It has 46 exons encoding a large transcript of 12,909 bp [35, 36]. More than 300 different *PKD1* mutations scattered throughout the gene have been described. Most lead to truncated protein products; some are missense mutations resulting in single amino acid change. Approximately 4% are larger deletions or duplications that can be detected by a multiplex ligation-dependent probe amplification (MLPA) assay [33]. More than half of *PKD1* mutations are unique to a single family. Sequencing of the *PKD1* gene is complicated because of the presence of multiple pseudogenes on chromosome 16. ADPKD patients with truncating *PKD1* mutations do not have a more severe phenotype than those with missense mutations [40, 41]. However, mutations in the 5′ half of the *PKD1* gene are more likely to be associated with intracranial aneurysms than those in the 3′ half [42].

The *PKD2* gene spans approximately 70 kb of chromosome 4q21 [43]. Its 15 exons encode a transcript of 2,904 bp [37, 44]. More than 90

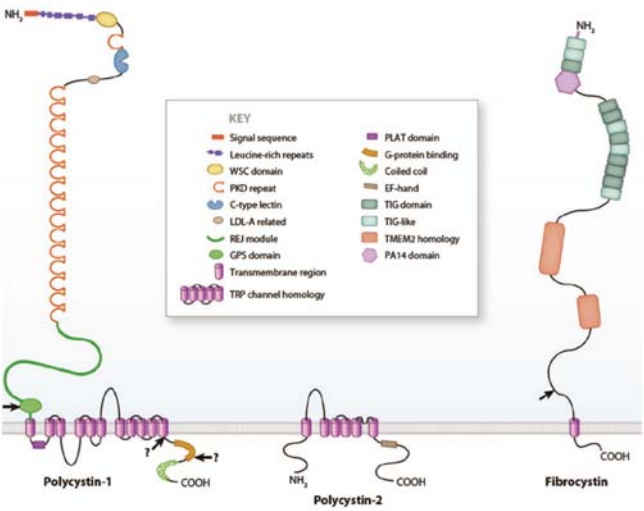

Fig. 4.2. Structures of the ADPKD proteins polycystin-1 and -2 and the ARPKD protein, fibrocystin. Details of domains found in these proteins are shown in the key. *Arrows* indicate places where the proteins are thought to be cleaved. (Used with permission from Harris and Torres [122]).

different mutations, most of which are truncating, are scattered throughout the gene (HGMD – ADPKD mutation database); most are unique to a single family [44].

PKD1 encodes PC1, a 4,303-amino acid, receptor-like transmembrane protein, of approximately 500 kD (Fig. 4.2). PC1 has 11 transmembrane domains, a large extracellular region, and a small intracytoplasmic tail. Its large extracellular N-terminal region has several identifiable motifs including multiple Ig-like domains that participate in protein–protein and protein–carbohydrate interactions [36, 45]. PC1 localizes to the plasma membrane of the primary cilium and at focal adhesions, desmosomes and adherens junctions.

PKD2 encodes PC2,a110kD protein with six transmembrane domains (Fig. 4.2). It is found in the plasma membrane, endoplasmic reticulum, and, like PC1, in primary cilia. PC2 is a nonselective cation channel capable of transporting Ca^{2+} and is a new member of the transient receptor potential (TRP) family [37]. PC1 and PC2 physically interact at their cytoplasmic domains and form the polycystin complex. PC1 is cleaved at its G protein-coupled receptor proteolytic site (GPS domain); the functional importance of this cleavage is not completely understood [46]. In addition, similar to notch signaling, the C-terminal tail of PC1 is cleaved and migrates to the nucleus [47]. In bile duct

or renal tubular epithelial cells, cilia project into the lumen, eliciting a Ca^{2+} influx in response to the flow of bile or urine. It has been hypothesized that PC1 acts as the sensor of flow-induced ciliary bending, while PC2 transduces the mechanical signal into an intracellular calcium response by transporting extracellular Ca^{2+} into the cell [48]. Epithelial cells in mouse models with defective PC1 (*Pkd1–/–*) have normal appearing cilia, but lack the fluid-induced Ca^{2+} response seen in normal cells, suggesting that an intact polycystin complex is required for the mechanosensory function of the primary cilia [49]. Changes in intracellular Ca^{2+} concentration result in multiple downstream consequences, including cell cycle regulation and differentiation.

AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE (ARPKD)

All patients with ARPKD, including perinatally symptomatic patients with predominantly renal symptoms and those who present in childhood or in adulthood with liver-related symptoms, have mutations in the *PKHD1* gene located on chromosome 6p12. *PKHD1* extends over a 0.5 Mb genomic region and has at least 86 exons, with multiple alternatively spliced transcripts [50, 51]. Its longest, uninterrupted, open reading frame contains 67 exons encoding a 16 kb mRNA [50, 52]. To date, more than 300 pathogenic mutations have been described in *PKHD1*. Many are unique to single families [53]. Truncating *PKHD1* mutations are associated with a more severe phenotype. ARPKD patients who survive the neonatal period have at least one missense mutation [54].

PKHD1 encodes fibrocystin/polyductin (FC), a receptor-like, membrane-associated protein of approximately 450 kD. FC contains a single transmembrane domain, a short C-terminal intracellular tail, and a large extracellular N-terminal region. Its extracellular portion contains several immunoglobulin-like plexin-transcription factor domains and parallel β-helix 1 repeats [50, 51].

FC is expressed in most tissues, especially the kidney. On a subcellular level, FC is expressed in the basolateral membrane, cytoplasm, and, like PC1 and PC2, in the primary cilium. Cholangiocytes isolated from the PCK rat, the rat model of ARPKD, develop undersized and dysmorphic cilia, suggesting an important role for FC in ciliogenesis [55].

FC colocalizes with PC2 at the basal bodies of primary cilia, implying a close functioning relationship between the two. A ciliary protein, kinesin-2, acts as a linker between FC and PC2, allowing for stimulation of PC2 cation channel activity [56]. The functional link between

FPC and PC2 suggests that the mechanisms of cytogenesis in ARPKD and ADPKD have a common pathway [56].

AUTOSOMAL DOMINANT POLYCYSTIC LIVER DISEASE (ADPLD)

ADPLD is a genetically heterogeneous disease [57]. To date, two genes have been identified as mutated in ADPLD patients: *PRKCSH* and *SEC63*, encoding the proteins hepatocystin and Sec63 [58–61]. Unlike the proteins implicated in ADPKD and ARPKD, hepatocystin and Sec63 are not ciliary proteins but are involved in the ER processing of proteins [6]. These two proteins account for approximately one third of the isolated PLD patients.

The *PRKCSH* gene resides on chromosome region 19p13.2–13.1 [57]. Before it was identified as one of the ADPLD genes, *PRKCSH* was known to encode a previously described human protein called "protein kinase C substrate 80 K-H" or the non-catalytic "beta-subunit of glucosidase II" (GIIβ). GIIβ itself does not have enzymatic activity but is essential for the maturation and conservation of GIIα in the endoplasmic reticulum. Glucosidase II is an ER-resident enzyme that plays a vital role in carbohydrate processing, accurate folding, and quality control of newly synthesized glycoproteins (Fig. 4.3) [62]. GIIβ has since been referred to as "hepatocystin." Hepatocystin is highly conserved and localizes to the lumen of the ER. It contains a low-density lipoprotein receptor domain class A and two EF-hand domains, which are both potential Ca^{2+} interacting motifs, suggesting that Ca^{2+} plays a role in its function. ADPLD patient mutations in *PRKCSH* are all predicted to cause premature chain termination leading to the loss of the highly conserved terminal four amino acids, His-Asp-Glu-Leu, which form an ER luminal retention sequence [59]. The loss of this sequence results in failure to retain hepatocystin in the ER. Mutant hepatocystin is then directed to the secretory pathway and trafficked out of the cell [63, 64].

SEC6, the second gene associated with ADPLD, is located on chromosome 6 and encodes a highly conserved integral membrane protein of the ER. Sec63 is an 83 kD protein predicted to span the ER membrane three times. It contains a luminal N-terminus, a cytoplasmic C-terminus with a coiled-coil region, and a luminal DnaJ domain between the second and the third transmembrane span [60]. Sec63 is part of the multicomponent translocon for integral membrane and secreted proteins. It is involved in both the posttranslational and the cotranslational pathways. Cotranslational maturation events include transmembrane domain integration, signal peptide cleavage, disulfide

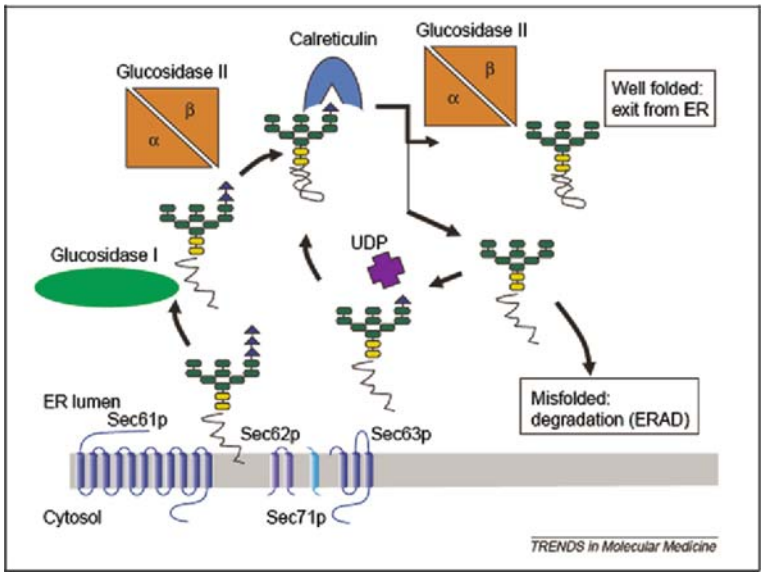

Fig. 4.3. The role of Sec63 and hepatocystin in glycoprotein handling in the endoplasmic reticulum (ER). Protein translocation into the ER is facilitated by the translocon. The Sec61 translocon associates with a second oligomeric membrane protein complex, the Sec62–Sec63 complex. This complex contains a cytoplasmic signal sequence receptor site that binds to newly synthesized glycoproteins. The luminal J-domain of Sec63 facilitates recognition by ER chaperones at the luminal exit site of the translocon. After transfer of a glycoprotein into the ER lumen through the translocon, three glucoses (*blue triangles*) are trimmed away by glucosidase I and II, respectively, and terminal mannoses (*green hexagons*) by one or more different ER mannosidases (not shown). Glucosidase II consists of a catalytic a-subunit and a non-catalytic b-subunit (hepatocystin). This generates a monoglucosylated glycoprotein that binds to calnexin and/or calreticulin, two ER chaperones that are specific for monoglucosylated core oligosaccharides (note that only calreticulin is shown). The protein is thereby exposed to another folding factor, ERp57, a thiol oxidoreductase, and associates with both chaperones. Cleavage of the remaining glucose by glucosidase II terminates the interaction with calnexin and calreticulin. On their subsequent release, correctly folded glycoproteins can exit the ER. If the glycoprotein is not folded at this time, the oligosaccharides are reglucosylated by UDP-glucose–glycoprotein glucosyltransferase (depicted as UDP), which places a single glucose back onto the glycan and thereby promotes a renewed association with calnexin and calreticulin. If the protein is permanently misfolded, they are recognized by a quality control receptor and targeted for re-translocation through the translocon (ERAD). (Used with permission from Drenth [6]).

bond formation, trimming and transfer of N-linked glycans, chaperone binding, and protein folding. N-linked glycans are vital for proper protein folding. Chaperone proteins in the ER bind to the three glucose residues on the core N-linked glycan and assist in the folding of the protein portion of the glycoprotein. Both hepatocystin and Sec63p are localized in the ER fraction of human liver [65]. Hepatocystin is involved in the trimming and transfer of N-glycans, and, therefore, the proper folding of glycoproteins. This is a process that occurs immediately downstream of the Sec translocon, suggesting a functional link between PRKCSH and SEC63 (Fig. 4.3) [6]. It is possible that several glycosylated ciliary cystoproteins such as PC1, PC2, and FC are proteins processed by Sec63 and heptocystin. Loss of function of Sec63 or heptocystin may lead to abnormal processing, less efficient folding, and subsequent degradation of ciliary cystoproteins. The absence of kidney involvement in ADPLD can be due to the presence of an alternative pathway for the maturation of glycoproteins in kidney [59].

MECKEL (GRUBER) SYNDROME (MKS)

Meckel syndrome (MKS) is a genetically heterogeneous disease. To date five MKS causative genes, *MKS1*, *MKS3, CEP290, RPGRIP1L,* and *CC2D2A,* have been identified (Table 4.1) [66–71]. *MKS1* is localized to chromosome 17q21–q24 and encodes a 559-amino acid polypeptide that contains a conserved B9 domain [67]. MKS1 protein localizes to basal bodies and the B9 domain is a typical component of proteins involved in the flagellar apparatus and basal body proteome associated with ciliary function. siRNA-mediated silencing of *MKS1* blocks centriole migration to the apical membrane and formation of the primary cilium [72].

The *MKS3 (TMEM67)* gene is localized on chromosome 8q24; it encodes a 995-amino acid transmembrane protein, meckelin [66]. Meckelin contains a signal peptide, a cysteine-rich domain, a 490-amino acid extracellular region with four sites for N-glycosylation, seven transmembrane domains, and a 30-amino acid cytoplasmic tail. Consistent with the MKS phenotype, *MKS3* is expressed in the kidneys, liver, and digits. On a subcellular level, meckelin localizes both to the primary cilia and to the plasma membrane. The MKS1 and meckelin proteins physically interact; they are required for the migration of the basal body toward the apical membrane during primary cilia formation [72, 73].

The *MKS3* gene displays frameshift, missense, and splice site mutations in several classical MKS patients who died in the perinatal period with occipital encephalocele, cystic dysplastic kidneys, and postaxial

polydactyly [66]. Compared with patients having *MKS1* mutations, those with *MKS3* mutations are less likely to have polydactyly and encephalocele and more likely to have milder central nervous system (CNS) phenotypes [74–76].

MKS3 splice site and missense mutations have been identified in individuals with Joubert syndrome. These mutations are presumed to represent hypomorphic alleles, creating milder phenotypes in comparison to the more lethal mutations in *MKS3* that cause Meckel syndrome [66, 75].

The *CC2D2A* gene is located on chromosome 4p15. It has 38 exons; its cDNA is 5,067 bp and encodes a 1,620-amino acid polypeptide [70]. Its calcium binding domain is thought to function in calcium-dependent phospholipid binding and in membrane-targeting processes. *CC2D2A*-mutated patient fibroblasts demonstrate that the cells lack cilia, suggesting a critical role for CC2D2A in cilia formation.

The *CEP290* and *RPGRIP1L* genes are reviewed under Joubert syndrome.

Another locus for MKS has been mapped to chromosome 11q13; the gene has not yet been identified [77].

JOUBERT SYNDROME

Joubert syndrome is also genetically heterogeneous. To date, eight chromosomal loci have been mapped and mutations in *AHI1, NPHP1, CEP290, MKS3 (TMEM67), RPGRIP1L*, and *ARL13B* genes have been documented in patients with Joubert syndrome [19, 78].

The *AHI* (Abelson helper integration site 1) gene maps to chromosome 6q23.3 [79, 80]. It consists of 31 exons and encodes Jouberin, a 1196-amino acid protein, which contains a coiled-coil domain, an SH3 domain, and six WD40 repeats. These WD40 repeats are thought to contribute to a variety of important functions, such as RNA processing, vesicular trafficking, and signal transduction. The identification of several missense mutations in the WD40 domains suggests that they play an important role in AHI1 function [81].

NPHP1 gene is localized on chromosome 2q13 and it has 20 exons. It is located in a region flanked by two large inverted repeat elements. An approximately 290 kb homozygous deletion of *NPHP1,* originally associated only with juvenile nephronophthisis, has been identified in some individuals with Joubert syndrome. This homozygous deletion results in an absence of nephrocystin-1 protein. The detection rate for this mutation in Joubert patients is estimated to be 1–2% [82]. Sporadic point mutations have also been identified in NPHP1 in patients with Joubert syndrome [83]. Nephrocystin-1 is a 733-amino acid protein localized to

the primary cilium of the cell as well as to cell–cell adherens junctions. It contains an SH3 domain that may mediate interactions with other proteins such as the products of the *INVS*, *NPHP3*, and *NPHP4* genes mutated in other forms of nephronophthisis [82].

CEP290, also referred to as *NPHP,* is a 55-exon gene on chromosome 12q21.3. *CEP290* accounts for about 10–15% of Joubert syndrome cases [84]. *CEP290* encodes a 290 kD centrosomal protein, nephrocystin-6, comprised of 2,479-amino acids and contains 13 coiled-coil domains, a nuclear localization signal, six RepA/Rep + protein KID motifs, three tropomyosin homology domains, and an ATP/GTP-binding site motif. Nephrocystin-6 modulates the activity of ATF4/CREB2, a transcription factor involved in cAMP-dependent renal cyst formation [84]. Nephrocystin-6 also interacts with a centriolar satellite protein, PCM-1, that is involved in microtubule organization. Depletion of CEP290 therefore disrupts the cytoplasmic microtubule network. CEP290 and PCM-1 are intrinsically involved in ciliogenesis by virtue of their roles in ciliary targeting of Rab8, a small GTPase that interacts with the BBS protein complex (see below) to promote ciliogenesis [85].

The gene *RPGRIP1L* is located on chromosome 16q12.2. Exon 15 appears to be a mutational hot spot, since approximately 50% of all reported mutations are clustered within this exon. Both truncating and missense mutations have been identified in *RPGRIP1L* among JSRD patients, while MKS patients with mutations in this gene exhibit more severe truncating mutations [78]. *RPGRIP1L* encodes a 1,315-amino acid protein containing five coiled-coil domains and two central protein kinase C conserved regions, both necessary for protein–protein interactions. The protein localizes to the basal body centrosome and ciliary axoneme and colocalizes with the NPHP proteins NPHP4 and NPHP6 (CEP290) [86].

BARDET–BIEDL SYNDROME (BBS)

Bardet–Biedl syndrome (BBS) is another genetically heterogeneous ciliopathy with 12 genes identified to date [87]. BBS patients display similar clinical features independent of which gene is mutated. Approximately 20–30% of BBS patients have no mutations in any of the 12 known BBS genes, suggesting the existence of other BBS genes. Several of the BBS proteins localize to the centrosome and basal body. BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9 are part of a stable multiprotein complex referred to as the BBSome [88]. The BBSome complex localizes to non-membranous centriolar satellites in the cytoplasm and to the membrane of the cilium. Since cilia lack ribosomes, all

proteins involved in their construction and function must first be docked to the basal body and then shuttled along the ciliary axoneme by a raft of proteins. The BBSome interacts with Rab8 GDP/GTP exchange factor, which localizes to the basal body and promotes extension of the ciliary membrane and transport of proteins to the cilia [73, 88]. These proteins form a complex with PCM1 that is involved in ciliogenesis by recruiting proteins necessary for centrosome replication and in organizing or anchoring microtubules [89].

BBS1 is located on chromosome 11q13 and is composed of 17 exons [90]. It encodes a 593-amino acid protein that contains a predicted beta-propeller domain also found in BBS2 and BBS7 proteins. In *Caenorhabditis elegans*, the beta-propeller domain is expressed exclusively in ciliated cells and localizes to transition zones and the ciliary axoneme [91]. *BBS1* mutations are responsible for approximately 20–25% of cases of BBS. *BBS2* is located on chromosome 16q21 and is composed of 17 exons. *BBS2* mutations are responsible for approximately 8% of all BBS patients. It encodes a 721-amino acid protein that, like BBS1, contains a beta-propeller domain [91, 92]. *BBS3* (ARL6) is located on chromosome 3p12–q13 and is composed of 9 exons. It encodes a 186-amino acid ADP-ribosylation-like factor (ARL) protein that belongs to the Ras superfamily of small GTP-binding proteins. This family of proteins plays an important role in membrane-associated intracellular trafficking and in microtubule assembly [93, 94].

BBS4 is located on chromosome 15q22.3–q23 and is composed of 16 exons. It encodes a 519-amino acid protein that includes at least 10 TPR domains predicted to be involved in protein–protein interactions. The BBS4 protein localizes to the basal body and centrosome and is suspected to function as an adaptor protein that facilitates cargo loading for microtubule-dependent intracellular transport within the cilium [95, 96]. The *BBS5* gene, located on chromosome 2q31, encodes a 342-amino acid protein. In the mouse, the BBS5 protein localizes to the basal body as well as to the ciliary axoneme in the ependymal cells lining the ventricles of the brain [97]. *BBS6* (MKKS) is located on chromosome 20p12, is composed of six exons, and causes approximately 6% of BBS [98]. It encodes a 570-amino acid protein with homology to eukaryotic T-complex-related proteins (TCPs) and to archeobacterial chaperonins which belong to the type II class of chaperonins. These groups of proteins are involved in ATP-dependent nascent protein folding. The BBS6 protein localizes to the pericentriolar material and, during mitosis, to the intracellular bridges. Silencing of BBS6 in cultured cells leads to cytokinesis defects such as multinucleation and multicentrosomalization [99]. The *BBS7* gene, located on chromosome 4q27, is composed of 19 exons and encodes a 672-amino

acid protein [100]. Like BBS1 and BBS2, BBS7 contains a predicted beta-propeller domain near the N-terminal, is expressed exclusively in ciliated cells, and localizes to the transition zones adjacent to the basal bodies and the ciliary axoneme in *C. elegans.* Mutations in *BBS7* cause structural and functional ciliary defects as well as impaired intraflagellar transport in *C. elegans* [91]. *BBS8* (TTC8), located on chromosome 14q32.1, is composed of 16 exons and encodes a 531-amino acid protein with homology to the BBS4 protein. It contains eight TPR domains, which are typically involved in protein–protein interactions. BBS8 also localizes to the centrosome and basal body of ciliated cells [101], and its mutations cause structural and functional ciliary and intraflagellar transport defects [91]. *BBS9*, located on chromosome 7p14, was originally identified as the parathyroid hormone response gene B1. The BBS9 protein is widely expressed and shares no similarity to the other BBS proteins. Its specific function is not yet known [102]. Mutations in the *BBS10* gene located on chromosome 12q are responsible for approximately 20% of individuals with Bardet–Biedl syndrome. *BBS10* encodes a 723-amino acid protein containing three functional, conserved, chaperonin domains [103]. Like BBS6, it contains a flexible protrusion region specific to group II chaperonins. The *BBS11* (TRIM32) gene, located on chromosome 9q31–q34.1, encodes a 652-amino acid ubiquitin ligase protein that is a member of the TRIM family and contains the characteristic ring finger, B-box, and coiled–coiled motifs. BBS11 is thought to have E3 ubiquitin ligase activity; it binds to myosin and ubiquitinates actin, suggesting a role in cytoskeletal regulation [104]. *BBS12* encodes a chaperonin-like protein of 710-amino acids. Mutations in *BBS12* are responsible for approximately 5% of Bardet–Biedl patients. The BBS12 protein, like BBS6 and BBS10, is related to group II chaperonins and contains vertebrate-specific chaperonin-like sequences.

ALAGILLE SYNDROME

Two genes causing AGS have been identified [105, 106]. *JAG1*, located on chromosome 20p12, is composed of 26 exons and is mutated in approximately 90% of AGS patients [106]. Missense, nonsense, splice site, and frameshift mutations have been reported with no mutational hot spot identified. In approximately 7% of AGS patients, deletion of the entire *JAG1* gene has been documented using fluorescence in situ hybridization (FISH) [107]. *JAG1* encodes Jagged-1, a cell surface protein that acts as a ligand for the NOTCH transmembrane receptors.

The *NOTCH2* gene, located on chromosome 1p13–p11, is composed of 34 exons. Fewer than 1% of patients with Alagille syndrome have

NOTCH2 mutations. To date, only two AGS causing mutations have been identified in *NOTCH2*. One is a splice site mutation removing exon 33, while the other is a missense mutation that results in the loss of a cysteine residue in one of the EGF-like repeats of the extracellular domain [105]. *NOTCH2* encodes a notch family transmembrane receptor. It consists of multiple epidermal growth factor-like (EGF) repeats and an intracellular domain with seven ankyrin repeats known to function in protein–protein interactions. NOTCH proteins are involved in development through their control of cell fate decisions. They are part of a conserved intercellular signaling pathway regulating cell–cell interactions [105]. *JAG1*'s and *NOTCH2*'s involvement in AGS suggests that the NOTCH signaling pathway is key in the development of the organs affected in AGS, such as the heart, biliary system, kidney, skeleton, and eyes.

NEPHRONOPHTHISIS (NPHP)

NPHP is inherited in an autosomal recessive fashion. In a subset of patients, NPHP may be associated with extrarenal manifestations including congenital hepatic fibrosis, retinal degeneration (Senior–Løken syndrome), and cerebellar vermis aplasia (JBTS) [108]. To date, six novel genes have been identified as causing *NPHP: NPHP1, NPHP2/inversin, NPHP3, NPHP4, NPHP5,* and *NPHP6* [108]. Only a subset of NPHP patients have mutations in these genes. Mutations in *NPHP1* are responsible for approximately 25% of juvenile NPHP. Other NPHP genes are mutated in fewer than 2% of all NPHP patients. CHF is known to be associated with *NPHP2* and *NPHP3* mutations. Whether all other NPHP genes will be associated with CHF is yet to be determined. *NPHP1* encodes nephrocystin-1, a protein that localizes to adherens junctions and focal adhesions involved in cell–cell and cell–basement membrane communications, respectively. Nephrocystin interacts with other NPHP proteins, including nephrocystin-2/inversin, nephrocystin-3, and nephrocystin-4, and with cell–cell and cell–matrix signaling components such as focal adhesion kinase 2, tensin, and filamin A and B [109–111].

The *NPHP2 (INVS)* gene located on chromosome 9q21–q31, encodes inversin. Mutations in *NPHP2* cause infantile NPHP, leading to end-stage kidney disease before 3 years of age. Inversin localizes to the primary cilium and interacts with nephrocystin-1 and with β-tubulin, which makes up the microtubule axoneme of primary cilia [112]. Mutations in *NPHP3*, encoding nephrocystin-3, cause adolescent NPHP [113]. *NPHP4* encodes nephrocystin-4 (nephroretinin), a protein in a complex with other proteins involved in cell adhesion and actin

cytoskeleton organization, such as nephrocystin-1, tensin, filamin, and α-tubulin [114]. The *NPHP5* gene encodes nephrocystin-5, a protein that localizes to the primary cilia of epithelial cells and to connecting cilia of photoreceptors, consistent with the early-onset retinitis pigmentosa seen in all type 5 NPHP patients [115]. *NPHP6/CEP290*, which encodes a centrosomal protein, is the cause of NPHP type 6 and JBTS type 5 (discussed under Joubert syndrome). *AHI1* mutations have been detected in patients with JBTS with and without NPHP.

JEUNE ASPHYXIATING THORACIC DYSTROPHY

A subset of patients with Jeune asphyxiating thoracic dystrophy (JATD) have mutations in the *IFT80* gene [116]. IFT80 is a component of intraflagellar transport (IFT) complex B, which is essential for the development and maintenance of motile and sensory cilia. Knockdown of ITF80 in zebrafish causes cystic kidneys, and knockdown in tetrahymena thermophila resulted in shortened or absent cilia [116].

ORAL-FACIAL-DIGITAL SYNDROME

OFD-1 is a male-lethal X-linked dominant multisystem disorder caused by mutations in the *OFD1* gene [7]. *OFD1* encodes a centrosome/basal body protein required for primary cilium assembly and for left–right axis determination. Mutations in *OFD1* were found in approximately 80% of OFD-1 patients. Genomic deletions of OFD1 account for approximately 25% of patients with negative mutation screening by DNA sequencing [117]. No genotype–phenotype correlation for polycystic kidney disease or CHF/CS exists at this time.

MOLECULAR APPROACHES TO THERAPY

Better understanding of the molecular mechanisms underlying the hepatorenal fibrocystic diseases has stimulated the development of novel targeted therapies (Fig. 4.4) [48, 118–122]. Most of the initial proof of principal animal studies and human safety and efficacy trials are focused on the effects of these treatments in kidneys. However, some promising medications also ameliorate the severity and retard the progression of rate of liver disease.

Renal cyst epithelium in various rodent models of ARPKD and ADPKD exhibits certain common characteristics. Differing from the normal epithelium that is well differentiated, absorptive, and polarized,

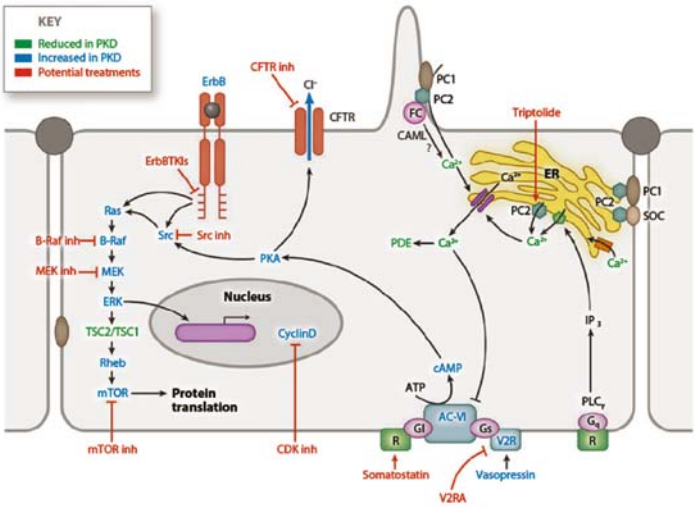

Fig. 4.4. Cellular changes associated with polycystic kidney disease (PKD). Components and pathways that are downregulated and upregulated in PKD are indicated. Potential treatments that target these defective pathways are shown in red. (Used with permission from Harris and Torres [122]).

with low rates of proliferation and apoptosis, cystic epithelium is characterized by de-differentiation, defective epithelial polarity, increased proliferation and apoptosis, abnormal cell–matrix interactions, and a secretory phenotype [52, 123, 124]. Many proteins, such as epithelial growth factor receptors (EGFR) and water channels (aquoporins), are mislocalized and/or overexpressed in cystic epithelium. PKD mutations induce complex changes in renal epithelial cell phenotype associated with activation in cAMP/Ras/Raf/ERK signaling, downstream to which mammalian target of rapamycin (mTOR) is activated and probably contributes to the excessive tubular epithelial cell proliferation. Two main abnormalities, common to both renal and biliary cystic epithelia, are defective calcium homeostasis and elevated cAMP (Fig. 4.2) [52, 124, 125]. In addition, the cyst epithelium shows an abnormal proliferative response to elevated cAMP, in contrast to normal cells that are growth inhibited by cAMP through inhibition of the Ras/Raf/MEK/ERK pathway [126]. This switch from inhibitory to proliferative response to cAMP is causally related to the disturbed intracellular calcium homeostasis that is secondary to the presence of defective PKD proteins (PC1, PC2, and FC). Other intracellular abnormalities

due to disturbance of multiple downstream pathways include an overactive EGFR axis, abnormal WNT signaling, and defects in cell–matrix interaction. EGFR is overexpressed and mislocalized to the apical membrane of cystic renal epithelial cells in the collecting tubules [127, 128]. Similarly, Na/K-ATPase is mislocalized to the apical membrane of cystic renal tubular cells in a mouse model of ARPKD [129]. In normal kidneys, canonical beta-catenin-dependent WNT signaling is active during kidney development, but only during times of injury repair after maturation is complete. When urine flow starts in the embryo, flow-induced ciliary signaling switches the WNT pathway from the canonical beta-catenin-dependent to the non-canonical beta-catenin-independent state through increased expression of inversin, the protein mutated in infantile nephronophthisis [130]. Abnormal activation of beta-catenin-dependent WNT signaling in mature tubules results in cyst formation. This process involves abnormal planar cell polarity and disturbed mitotic spindle orientation.

Although most of the data on the characteristics of cystic epithelium were generated using kidney tissue, recent work on cholangiocytes of the PCK rat by Masyuk et al. shows similar findings [32, 131]. Cholangiocyte cilia function as mechanosensors, osmosensors, and chemosensors. In microperfused intrahepatic bile ducts, bending of cholangiocyte cilia by luminal fluid flow induces an increase in intracellular calcium. PC1 and PC2 are required for this response [132]. Within the cholangiocyte, cilia signaling occurs through cAMP with the involvement of calcium and inhibitable adenylyl cyclase 6 (AC6). Transient Receptor Potential Vanilloid 4 (TRPV4), a mammalian homolog of OSM-9 localized to cholangiocyte primary cilia, is involved in the functional responses of the cholangiocytes to changes in bile osmolality and tonicity and regulates cilia-dependent bicarbonate secretion [32]. P2Y12, a receptor located on cholangiocyte cilia that is activated by biliary nucleotides, causes changes in intracellular cAMP levels [133]. Cholangiocyte cilia also contain the components of the AKAP (A-kinase anchoring protein) signaling complex, which include one isoform of EPAC, an exchange protein activated directly by cAMP (i.e., EPAC2) [32]. In normal cholangiocytes, AQP1, CFTR, and AE2 proteins are targeted to the apical membrane in response to secretin and cAMP stimulation [134, 135]. Cholangiocytes of the PCK rat (the homologue of human ARPKD) overexpress AQP1, CFTR, and AE2 at the basolateral membrane. Secretin-stimulated expansion of PCK cysts is inhibited by the basolateral application of CFTR and AE2 inhibitors. Elevated expression and altered topography of AQP1, CFTR, and AE2 are associated with hepatic cyst expansion in ARPKD.

Modulation of cAMP signaling via vasopressin 2 (V2) receptor antagonists results in a beneficial effect in rodent models of cystic kidney disease. However, cholangiocytes do not express V2 receptors. Another means to regulate cellular cAMP levels is through somatostatin's action on SST2 receptors present in the liver and kidney. cAMP levels are markedly elevated in bile ducts and serum of the PCK rat compared with normal rats [136]. A somatostatin analogue, octreotide, decreases cAMP levels in cholangiocytes and decreases mitotic indices in hepatic and renal epithelia, suppressing hepatic cyst growth and reducing hepatic fibrosis in the PCK rat model of ARPKD [136]. A phase 2–3 clinical trial (NCT00426153) of octreotide using liver volume as the primary outcome parameter is underway at the Mayo Clinic [122]. Other human trials of somatostatin inhibitors are ongoing in the Netherlands and Italy [137].

Sweeney et al. showed that c-Src inhibitor SKI-606 ameliorates both renal and hepatic disease in the PCK rat, with a significant reduction in biliary ductal cyst development and fibrosis [121]. Treatment with the EGF tyrosine kinase inhibitor, gefitinib inhibited the cystic dilatation, and improved the liver fibrosis of the intrahepatic bile ducts of PCK rats, without any beneficial affect on renal cyst development [138].

Sirolimus (rapamycin) inhibits cell growth and proliferation and promotes apoptosis by inhibiting mTOR-mediated signaling. Treatment of the bpk and orpk mouse models with the mTOR inhibitor rapamycin showed effective inhibition of PKD [139, 140]. Compared with normal biliary epithelia and noncystic areas of PLD, PLD cyst lining epithelia express high levels of activated mTOR and its downstream effector, p-S6rp [141]. Qian et al. retrospectively measured the volumes of polycystic livers and kidneys in ADPKD patients who had received kidney transplants and participated in a prospective randomized trial that compared sirolimus-containing immunosuppression to a non-sirolimus regimen. The patient group treated with sirolimus for an average of 19.4 months had an $11.9 \pm 0.03\%$ reduction in polycystic liver volume, whereas the group treated with tacrolimus for a comparable duration had a $14.1 \pm 0.09\%$ increase. Currently, a prospective clinical trial of Sirolimus is underway in Switzerland [137].

In summary, several classes of new or existing drugs may be beneficial in the treatment of CHF/CS or PLD. Several of these agents are currently under phase II or III clinical trials [122]. Although most of these trials are primarily focused on the kidney outcome, future studies designed for measuring outcome of the liver disease are likely in view of the recent promising results.

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II Diagnosis

5 Radiologic Findings in the Fibrocystic Diseases

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CONTENTS

Summary

Noninvasive evaluation of patients with suspected or known fibrocystic liver diseases is widely available using cross-sectional imaging techniques. The various types of fibrocystic diseases, as well as important differential diagnostic entities, can be distinguished from each other confidently using morphologic and functional criteria. Biliary atresia, for example, may not only be suspected

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by morphologic ultrasound appearance of the liver in combination with the appropriate clinical parameters but also be proven by cholangiogram.

When specific questions, such as communication between the biliary system and the small intrahepatic cysts, need to be answered to find the correct diagnosis, endoscopic retrograde cholangiography (ERC) can provide the best spatial resolution as well as functional information. ERC is considered the gold standard for evaluation of the biliary tree and, in selected cases, may be the only modality to demonstrate key features differentiating biliary hamartomas, polycystic liver disease, and Caroli disease. However, magnetic resonance cholangiography (MRC) has reached a level of spatial resolution that frequently allows noninvasive delineation of these small structures confidently in many patients.

Choledochal cysts are often diagnosed on computed tomography, magnetic resonance tomography, or endoscopic ultrasound incidentally or when evaluating a complication such as cholangitis. ERC may be indicated to clarify the type of choledochal cyst or treat complications such as choledocholithiasis. More invasive methods, such as percutaneous transhepatic cholangiography or postoperative T-tube cholangiography, are predominantly performed as an interventional procedure for the treatment of complications of fibrocystic hepatic diseases.

Key Words: Fibrocystic liver disease, Biliary atresia, Congenital hepatic fibrosis, Hamartoma, Choledochal cyst, Autosomal recessive polycystic liver disease, Caroli disease, Ultrasound, Computed tomography, Magnetic resonance cholangiopancreatography, Endoscopic retrograde cholangiopancreatography

BILIARY ATRESIA

No single procedure is consistently 100% accurate in the neonate and young infant in diagnosing biliary atresia and excluding other potential causes of conjugated hyperbilirubinemia, such as choledochocele and neonatal hepatitis. Since rapid diagnosis and treatment are essential to ensure the best clinical outcome, radiographic tests may not obviate liver biopsy and intraoperative cholangiography to establish a diagnosis. However, radiological tests often can provide useful information in the young child with obstructive jaundice, and sometimes can establish a diagnosis. The various common radiological modalities (ultrasound, hepatic scintigraphy, and magnetic resonance imaging) will be reviewed with attention to their potential strengths and weaknesses, and an imaging approach to the neonate with obstructive jaundice will be presented.

Ultrasound (US) evaluation of the liver and biliary tree is best accomplished in the fasting infant (4 h) using a 7.5 MHz curvilinear transducer and a 13.5 MHz (high frequency) linear array transducer. The exam consists of systematic evaluation of the liver parenchyma, biliary system, gallbladder, pancreas, spleen, and hepatoportal vasculature. While there are several sonographic findings that have been associated with biliary atresia, no single finding is specific. Humphrey et al., in a study of 90 infants with conjugated hyperbilirubinemia (30 with biliary atresia), found a diagnostic accuracy of 98% by US if a constellation of findings are discovered [1]. The criteria include the "triangle cord sign" (Fig. 5.1a), which is an echogenic region along the portal vein corresponding to fibrous tissue [2], gall bladder absence or abnormal gall bladder size and morphology (Fig. 5.1b), increased hepatic artery diameter, and absence of the common bile duct. They also stressed the importance of evaluating other potential signs of syndromic biliary atresia, which include polysplenia (Fig. 5.2) and an interrupted inferior vena cava [1]. In addition, findings that may influence the surgical approach to patients with biliary atresia, such as the presence of a preduodenal portal vein (Fig. 5.3), can be identified at US and CT (Fig. 5.3b) and other potential causes of obstructive jaundice, particularly choledochal cyst, may be excluded.

There are multiple potential pitfalls when utilizing US in this setting. First, US is extremely operator dependent, and important findings may be overlooked by inexperienced operators. Second, the individual US findings associated with biliary atresia, in and of themselves, lack both diagnostic sensitivity and specificity. For example, the common bile duct is often not visualized in normal neonates and also may be visualized in patients with certain types of biliary atresia. The triangle cord sign has a reported 84% sensitivity and 98% specificity for biliary atresia [3] but has also been a reported finding in patients with neonatal hepatitis and advanced cirrhosis and periportal cirrhosis [4]. A more recent report found that the triangle cord sign was absent in 6 of 20 patients with biliary atresia [5]. In addition, 25% of patients with biliary atresia may have a gall bladder that is normal in size and morphology, and conversely, an abnormal gall bladder morphology and size also may be seen in neonatal hepatitis.

Hepatobiliary scintigraphy (HIDA) relies on the uptake of a technetium 99m (TC-99m) radiolabeled agent by the hepatocytes (hepatocyte clearance) and subsequent excretion into the biliary system (hepatobiliary transit time). Images are obtained at various intervals, up to 24 h after administration of the radiotracer. The evolution of radiopharmaceuticals has resulted in the development of agents that work well (with reported extraction of up to 70%) in patients with

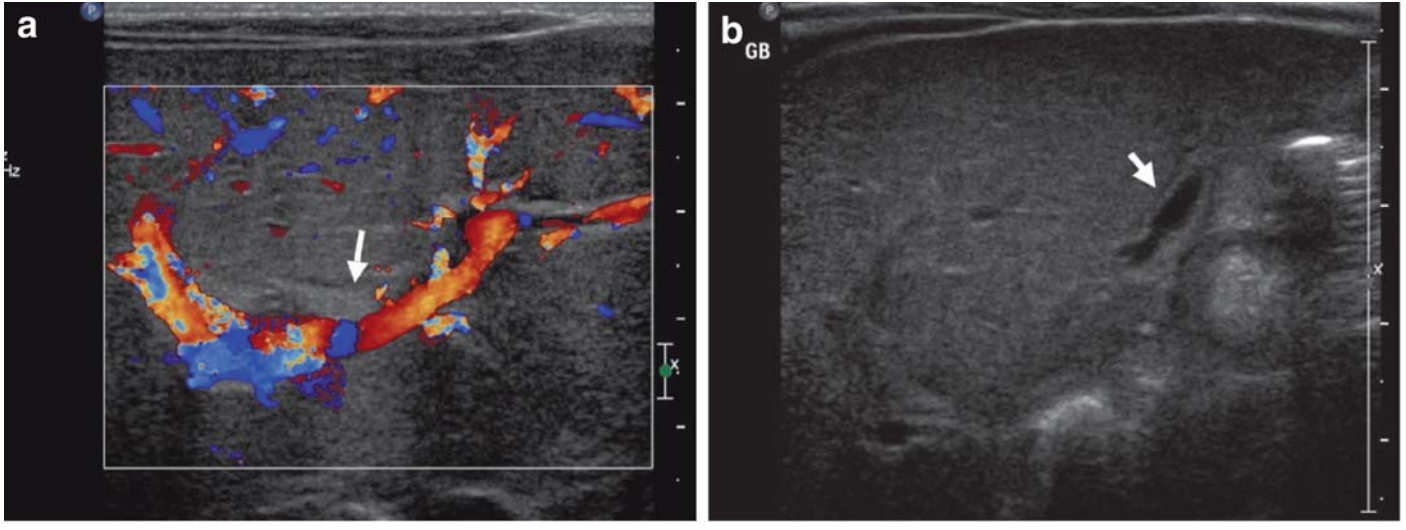

Fig. 5.1. (**a**) Color Doppler ultrasound of an 8-week-old infant with biliary atresia demonstrates the "triangular cord sign," described as an area of increased echogenicity (*arrow*) along the anterior wall of the portal vein. (**b**) Gray scale sonography of the same patient demonstrates a small, contracted gallbladder (*arrow*). The common bile duct was not visualized.

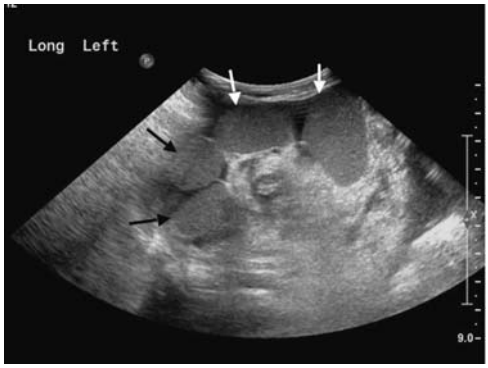

Fig. 5.2. Ultrasound evaluation of a patient with syndromic biliary atresia demonstrates multiple small spleens, or polysplenia (*white and black arrows*), within the left upper quadrant.

serum bilirubin levels up to 20 mg/dL [6]. Pretreatment with phenobarbital augments hepatic function, and thus, uptake of radiotracer, and is therefore generally recommended. In patients with biliary atresia, no radiotracer activity will be detected within the bowel (Fig. 5.4). If radioactivity is detected within the bowel, biliary atresia is excluded from the differential diagnosis in a neonate or young infant with jaundice (Fig. 5.5).

A potential limitation of hepatobiliary scintigraphy is that hepatocyte function must be sufficient to clear the radiotracer from the blood pool. If hepatocyte clearance is insufficient, a false-positive examination mimicking biliary atresia may result [7].

The use of MRC in jaundiced infants was first described by Guibaud in 1998 [8]. The technique relies on the fluid signal from bile or mucous within the bile ducts for visualization of the biliary tree. Guibaud reported a sensitivity of 96–97% in diagnosing biliary atresia using MRC [8]. Follow-up studies by different authors show mixed results ranging from an accuracy of 82 to 98%, sensitivity of 90 to 100%, and specificity of 77 to 96% [9,10]. Of note, these studies involved relatively small patient populations ranging from 24 to 26 patients. False positives were reported in patients who had decreased bile secretion due to a variety of reasons, including metabolic disorders and severe cholestasis. False negatives were seen in biliary atresia patients in whom the disease primarily affected the intrahepatic ducts [9].

A recent report in 23 patients using the hepatic-specific MRC contrast agent Mangafodipir has shown promising results with 100% accuracy, specificity, and sensitivity in diagnosing biliary atresia [11].

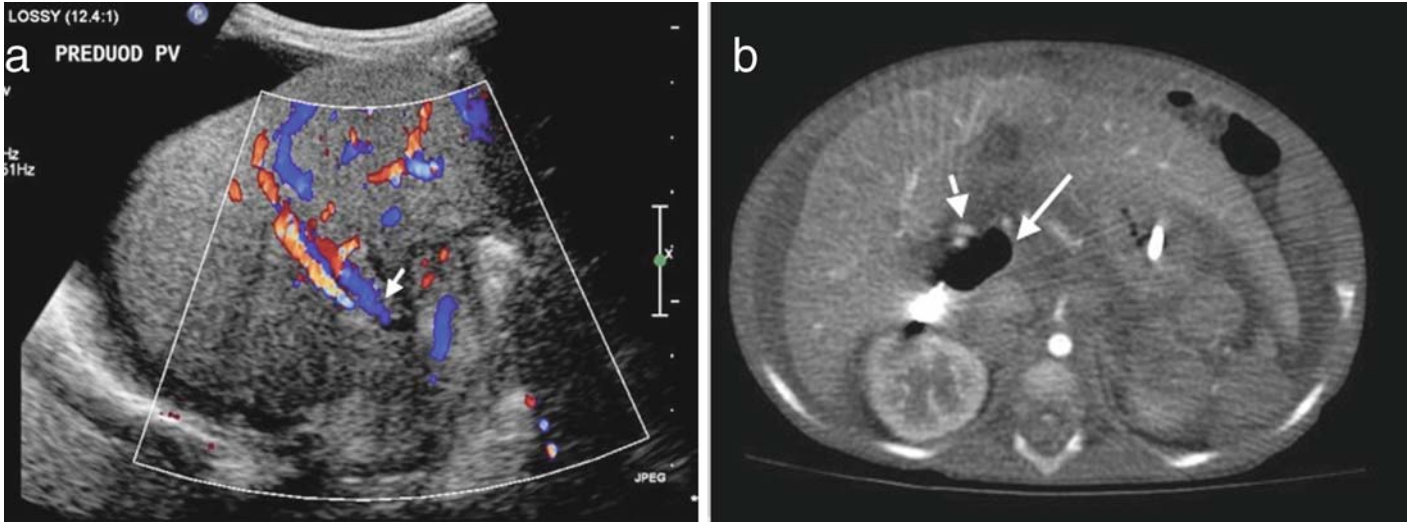

Fig. 5.3. (**a**) Color Doppler evaluation of the infant shown in Fig. 5.2 with syndromic biliary atresia demonstrates a preduodenal portal vein (*arrow*). (**b**) The preduodenal portal vein (*short arrow*) was later confirmed by CT, located anterior to the duodenum (*long arrow*).

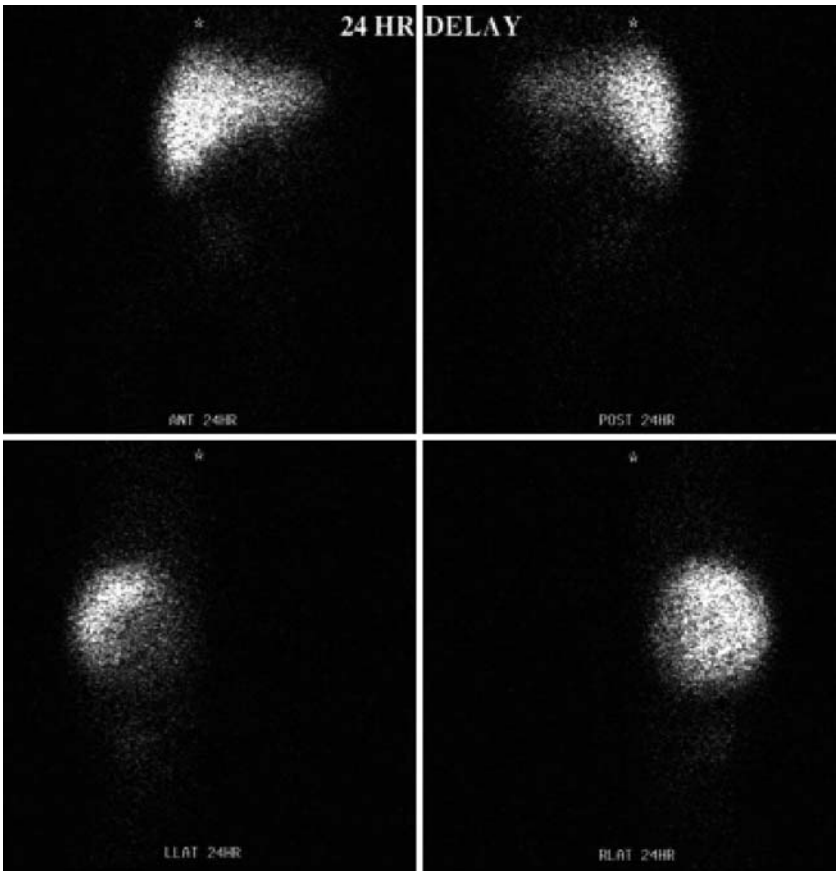

Fig. 5.4. Hepatoscintigraphy of the same 8-week-old infant with biliary atresia displayed in Fig. 5.1 demonstrates retention of radiotracer within the liver, and no excretion into the bowel at 24 h.

Conventional MRC relies solely on the fluid signal from bile ducts for their visualization, whereas Mangafodipir is taken up by hepatocytes and then excreted into the bile, which then improves its visualization. Mangafodipir-enhanced MRC is able to visualize both the biliary system and the secreted contrast within the intestines in patients with patent biliary ducts. Thus, both anatomical and functional information can be obtained using this technique, although further studies are warranted owing to the small patient population of this study. Furthermore, both conventional MRC and contrast-enhanced MRC require that the patient fast (4 h), often require sedation, and may be limited by respiratory or bowel motion. Mangafodipir is not currently available in

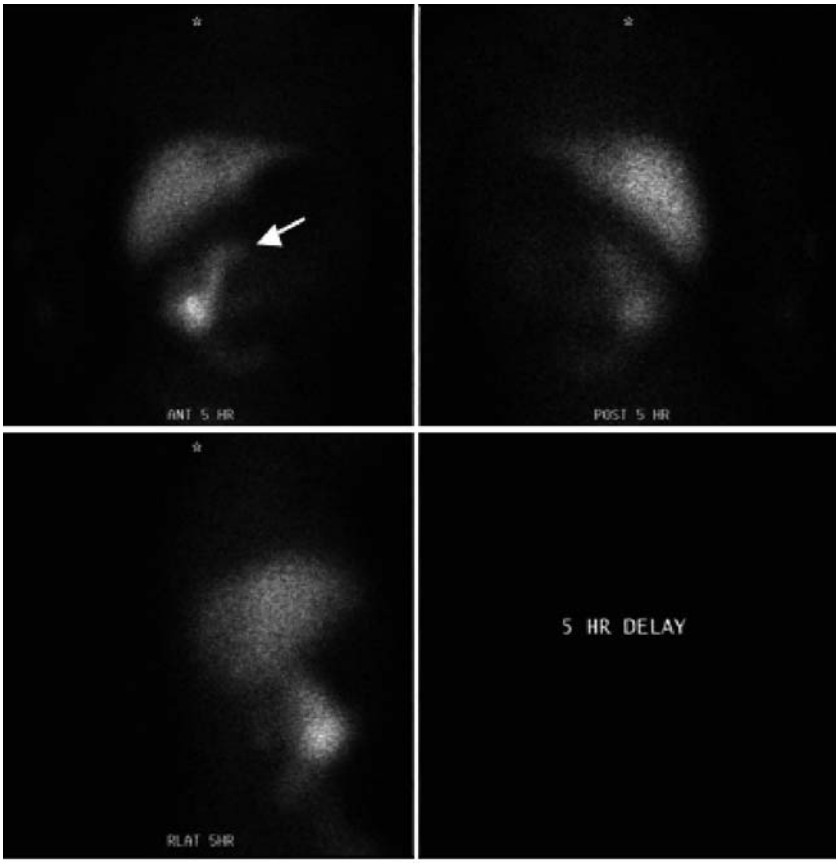

Fig. 5.5. Hepatoscintigraphy of an infant with neonatal hepatitis demonstrates a patent biliary system with radioactivity detected within the bowel (*arrow*) at 5 h.

the United States. Gadoxetate disodium (Gd-EOB-DTPA), another hepatospecific contrast agent which is also excreted in the bile, is now available in the USA [12–14]. Its FDA approved indication, however, is detection and characterization of hepatic lesions; its value in the evaluation of the biliary pathology has yet to be confirmed.

Given the strengths and limitations of current radiological techniques for evaluating the neonate or young infant that presents with conjugated hyperbilirubinemia, abdominal ultrasound followed by hepatobiliary scintigraphy (ideally after pretreatment with Phenobarbital) is recommended as the initial imaging workup. Ultrasound is useful in evaluating for choledochocele as a potential cause of obstructive jaundice and may detect some of the syndromic features that can

be seen with biliary atresia. Hepatobiliary scintigraphy can exclude biliary atresia from the differential diagnosis if intestinal activity is detected. Finally, MRC, particularly contrast-enhanced MRC with Mangafodipir, shows promise in evaluating both anatomy and function, although larger studies are needed to verify its accuracy, sensitivity, and specificity.

AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE AND CONGENITAL HEPATIC FIBROSIS

The spectrum of radiographic findings associated with autosomal recessive polycystic kidney disease/congenital hepatic fibrosis (ARPKD/CHF) mirrors the phenotypic variability (see Chapter 4 and Chapter 14). Of note, the radiographic findings of CHF can also be seen in Meckel syndrome, tuberous sclerosis, and, rarely, autosomal dominant polycystic kidney disease (ADPKD), among other conditions. The following discussion of ARPKD/CHF will emphasize the radiographic findings within the liver and present recommendations for an imaging algorithm.

Severe renal involvement and minimal hepatic disease, which is typically seen by ultrasound as enlarged, echogenic kidneys and normal hepatic parenchyma, represents one end of the spectrum of ARPKD/CHF. CHF and polycystic liver disease are characterized by US as increased echogenicity of the hepatic parenchyma, particularly along the portal tracts, which has been attributed to periportal fibrosis (Fig. 5.6) [15]. The left lobe of the liver, both medial and lateral segments, may be hypertrophied, a finding demonstrated by US or CT

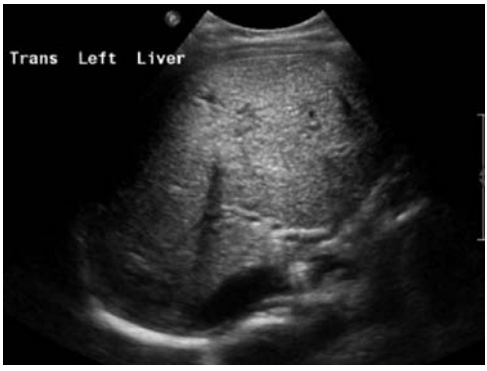

Fig. 5.6. Ultrasound image from a patient with ARPKD demonstrates heterogeneously increased echotexture of hepatic parenchyma.

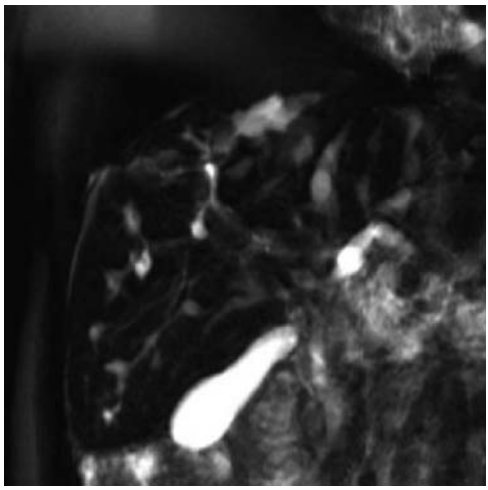

Fig. 5.7. MRCP from the patient shown in Fig. 5.6 demonstrates diffuse intrahepatic biliary ductal dilatation.

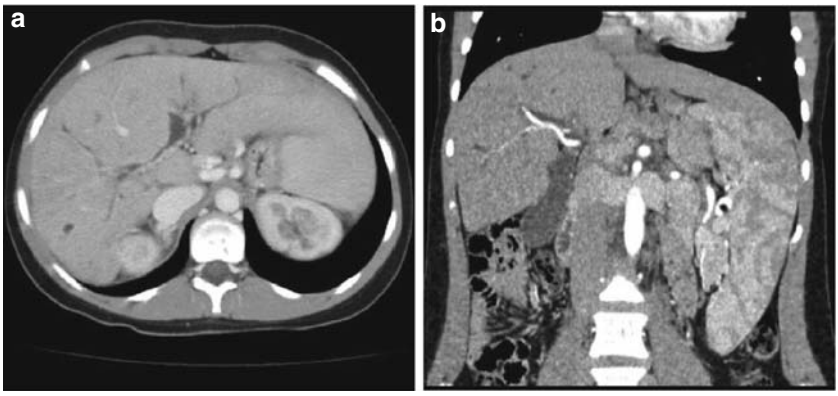

Fig. 5.8. Contrast-enhanced CT also from the patient shown in Fig. 5.6 showing mild to moderately dilated bile ducts: (**a**) axial and (**b**) coronal reconstruction.

[16]. There also may be biliary ductal dilatation, which can be more readily apparent by MRC than by US or CT (Figs. 5.7 and 5.8a, b) [17,18]. It is important to note that the radiographic constellation of findings of periportal fibrosis and biliary ductal dilatation is identical to that of Caroli syndrome. As the degree of periportal fibrosis progresses, radiographic signs of portal hypertension may be seen, such as splenomegaly and varices.

In a child with suspected ARPKD/CHF, ultrasound is a reasonable screening tool to assess renal morphology and hepatic parenchyma. If portal hypertension is suspected, US can also be used to evaluate for splenomegaly, varices, and Doppler findings of portal hypertension. US is favored over CT as a screening tool since it does not use ionizing radiation or intravenous contrast. However, if specific assessment of the biliary tree is desired in a patient with ARPKD/CHF, then MRC should be considered as it has proven to be a low risk and more effective method than US or CT to assess the biliary tree in children and adults with ARPKD/CHF [17,18].

SIMPLE HEPATIC CYSTS AND AUTOSOMAL DOMINANT POLYCYSTIC LIVER DISEASE

Simple hepatic cysts are the second most common focal lesion of the liver after cavernous hemangioma (see Chapter 18). They occur in 2.5–4.7% of the general population and 11% in a hospital population when examined with ultrasound [19–22]. Their solitary occurrence is random and not associated with other hepatic findings. Once their number exceeds 10, however, another form of fibrocystic liver diseases, such as biliary hamartomas or autosomal dominant polycystic liver disease (ADPLD), should be considered. Also, if the lesion is greater than 1 cm in diameter, other diagnostic considerations include parasitic diseases such as hydatid disease, abscesses, and neoplasms such as cystadenomas; choledochal cysts must also be considered.

Incidentally discovered hepatic cysts are often 1 cm or smaller in size. Subcentimeter cysts are often indistinguishable from small metastases or abscesses by CT, MR, and positron emission tomography [9,23]. Hepatic cysts are usually round or oval, and sometimes lobulated. They may be unilocular or septated. They are smoothly defined with a thin or absent wall, without mural nodularity [24]. Sonographically they show an anechoic content and posterior acoustic enhancement (Figs. 5.9a and 5.10a). They may contain debris, which may be mobile and settle during the examination.

US, however, is not absolutely specific, and small malignancies such as necrotic or cystic metastases, biliary cystadenocarcinomas, and lymphoma may have a simple cystic appearance. Even though most simple cysts are derived from small bile ducts, a spatial relationship to the portal triad is rarely seen. In fact, the typical location of a hepatic cyst is beneath the liver capsule. If the lesions are 1 cm or smaller, further characterization by other modalities utilizing intravenously administered contrast or radiotracers may not be helpful.

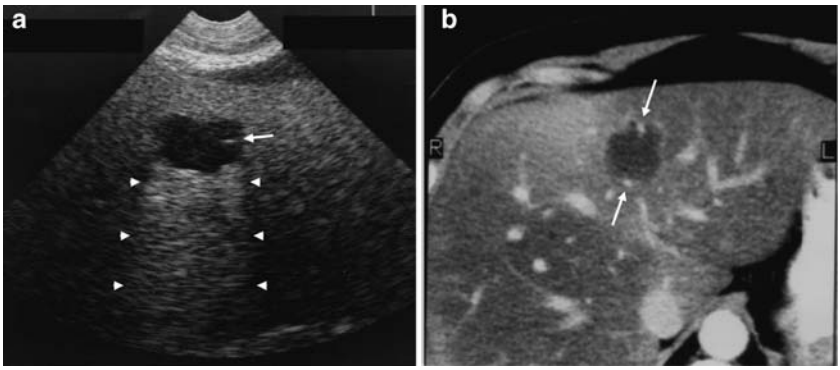

Fig. 5.9. (**a**) Ultrasound: smooth delineation, anechoic content, and posterior acoustic enhancement are typical sonographic characteristics of the simple cyst. Note small echogenic focus at the lateral wall of the cystic wall (*arrow*) representing a small vessel. Hyperechoic fatty parenchyma increases the conspicuity of anechoic cyst. (**b**) Contrast-enhanced CT of the same patient demonstrates lobulated low attenuation cyst with water density. No nodularity of the wall or solid components is detected. There are two small vessels at the periphery of the cyst that should not be confused with nodular components of the wall (*arrows*, one corresponding to vessel seen on ultrasound). Note hypodense appearance of the surrounding liver parenchyma, representing fatty transformation (Courtesy of Dr. Patrick C. Freeny, University of Washington Medical Center, Department of Radiology).

In addition to ultrasound, CT is the most common modality by which small simple cysts are detected incidentally. The finding usually consists of a small round, oval, or lobulated, homogeneously hypodense lesion, which does not enhance after contrast administration (Figs. 5.9b and 5.10b, c). The accuracy of the quantification of density with or without contrast enhancement in CT is strongly dependent on the size of the lesion and on the reconstructed slice thickness. If the slice thickness is too thick in relation to the size of the target, incorrectly high attenuation coefficients are measured leading to the assumption that the lesion is not cystic. However, if intravenous contrast is administered following a non-enhanced exam, contrast enhancement of up to 20 Hounsfield units may be seen in a simple cyst. This enhancement is often called "pseudo-enhancement," since it does not reflect true contrast accumulation through vessels, but rather, densitometrically higher values based on volume averaging, resulting from the proximity of normally enhancing adjacent liver parenchyma. CT is one of the most commonly used diagnostic tools to detect simple cysts and its sensitivity has increased substantially for small cysts with the use of thinner slices

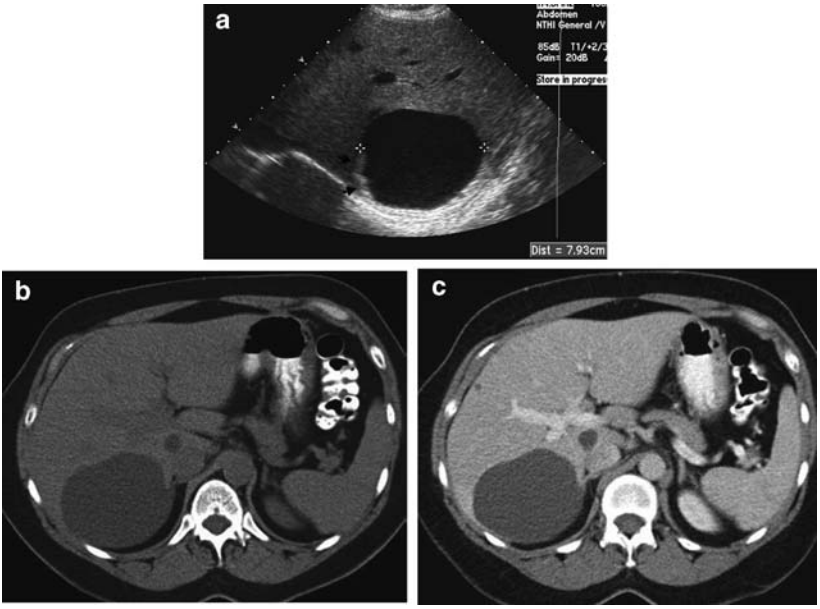

Fig. 5.10. Large simple cyst in segment six of the liver. (**a**) Ultrasound: note that increased through transmission can also be seen in this subcapsular location, where the posterior acoustic amplification occurs over other than hepatic parenchyma (*black arrows* delineate a hyperechoic artifact). (**b**) CT of the same patient: non-contrast-enhanced CT demonstrates large homogeneously hypodense cyst in the right lobe of the liver without perceptible wall. Note presence of other smaller cysts. (**c**) absent enhancement of cyst wall or contents characterizes this lesion as a simple cyst (Courtesy of Dr. Carlos Cuevas, University of Washington Medical Center, Department of Radiology).

utilizing multi-channel CT scanners. However, CT also has the lowest specificity, since density and shape are its only diagnostic parameters.

MR offers several techniques in addition to intravenous contrast administration; this may be helpful, although not always definitive, in the distinction between a simple cyst versus a complex cyst or solid mass. T1-weighted imaging techniques allow identification of components such as hemorrhage, iron, fat, or calcium. T2-weighted imaging generates fluid-specific signal characteristics that distinguish simple cysts from solid non-enhancing masses (Fig.5.11).

Imaging modalities utilizing radioactively labeled tracer show simple cysts as photopenic spots. This nonspecific finding in isolation often does not allow differentiation of cysts from other lesions and requires further correlation with other cross-sectional imaging.

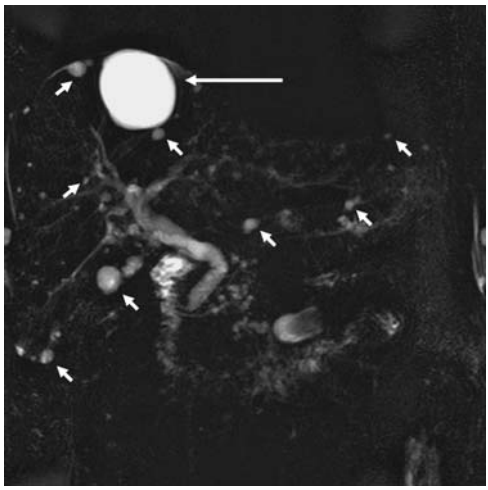

Fig. 5.11. MRC of simple cysts: three-dimensional MRCP (single shot technique with fat suppression) demonstrates all cysts as homogeneously hyperintense well-defined structures, not related to the bile duct: large simple cyst in the dome of the liver (*long arrow*) has similar signal characteristics compared to numerous small cysts (*short arrows*). Note mild extrahepatic bile duct dilatation is secondary to removed gall bladder, no intrahepatic bile duct dilatation is seen.

It is, therefore, of paramount importance to interpret the finding of a focal cystic liver lesion in the context of the patient's clinical situation and, if available, to compare the imaging findings with previous imaging. However, simple cysts may change their size, and variability of cyst size over time does not exclude a hepatic cyst. Other imaging modalities that may complement each other, such as a combination of CT and US or US and MR, are often necessary.

The radiographic similarity of simple cysts to cystic malignancies, such as metastases from adenocarcinomas, sarcomas, gastrointestinal stroma tumors (GIST), and lymphomas, may be problematic (Fig. 5.12). Simple cysts may also mimic cystic degeneration of solid neoplasms after chemotherapy or embolization, as well as liquefied hematomas, and infections, particularly fungal and bacterial abscesses. Cystic lesions in the liver are usually well seen with US, CT, or MR and are therefore amenable to image-guided aspiration or biopsy, if tissue sampling is needed to establish a definitive diagnosis.

Autosomal dominant polycystic liver disease or adult polycystic liver disease (PLD) is characterized by the presence of innumerable well-defined cysts within both lobes of the liver. Their size varies considerably between 1 mm and greater than 12 cm, and this variability of

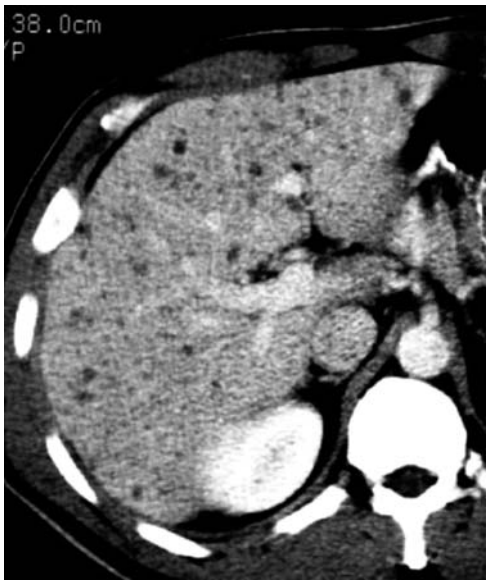

Fig. 5.12. Contrast-enhanced CT: Incidental finding of multiple small focal hypodense cysts in both lobes of the liver with variable density and size, indistinguishable from small hypovascular metastases that may have a similar appearance (Courtesy of Dr. Patrick C. Freeny, University of Washington Medical Center, Department of Radiology).

size within one patient is a diagnostic criterion to distinguish ADPLD from other cystic entities of the liver such as hamartomas or Caroli disease [25]. Similar to biliary hamartomas, the cysts do not communicate with the bile ducts, since ADPLD is thought to result from progressive dilatation of the abnormal bile ducts in microhamartomas [26]. Most cysts have imaging features of simple fluid, but hemorrhagic cysts are encountered not infrequently and fluid–fluid levels may be observed.

The cystic walls are smooth and usually show no septations or nodularity. However, thin calcifications of the wall may be seen, which is considered to be a sequela of chronic or remote hemorrhage or inflammation (Fig. 5.13a, b) [26]. Walls of the cysts do not enhance after administration of intravenous contrast.

The overall size of the liver is often grossly enlarged by the number and size of the cysts; the hepatic parenchyma, however, may be significantly compressed or replaced to such a degree that it comprises only a small portion of the overall liver volume.

Ultrasound, CT, or MR may all show the variable degrees of involvement. Also, the characteristics of simple versus complex contents and

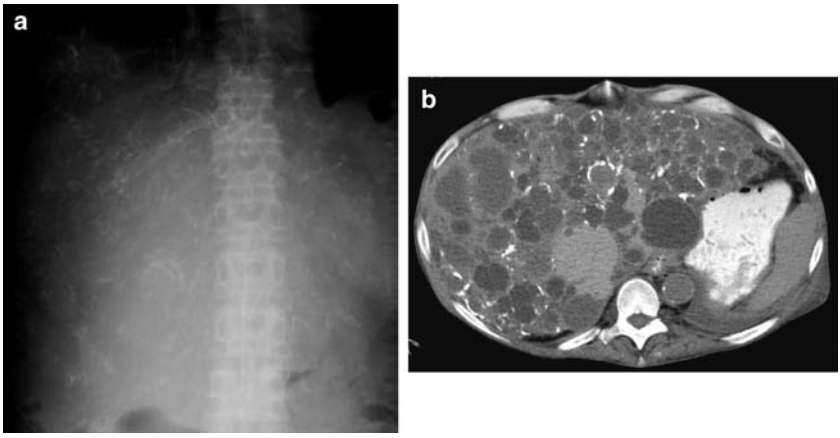

Fig. 5.13. Polycystic liver disease with numerous partially calcified cysts. (**a**) plain film of the abdomen demonstrates multiple thin calcifications, barely suggesting cystic etiology of the calcifications (**b**) Axial CT of the same patient demonstrates numerous cysts, many of them calcified. Note most calcifications are not completely circular.

calcified walls may be demonstrated by all of these modalities. CT and US, however, are most sensitive for the detection of thin calcifications. Technetium-99m (Tc-99m) DIHIDA scintigraphy may show largely photopenic areas in the liver representing the cysts and may allow distinction from Caroli disease, but since more specific findings as described above are necessary to diagnose ADPLD, US, CT, or MR are the preferred imaging modalities.

The complications of ADPLD are well identified by US, CT, or MR. These include symptomatic mass effect on the stomach or duodenum by peripherally located cysts, intrahepatic mass effect on bile ducts and portal veins, which may result in portal vein thrombosis and portal hypertension, and hemorrhage, infection, rupture of cysts, and Budd–Chiari syndrome in advanced stage.

Polycystic kidneys may coexist in 40% and multiple cysts in the pancreas in 9% of the autosomal dominant form of polycystic liver disease (Fig. 5.14a, b) [27].

MICROHAMARTOMAS

Microhamartomas (also called biliary hamartomas or von Meyenburg complexes) radiographically are also sometimes called simple hepatic cysts that arise from the biliary ductal system. Rarely they are solely fibrous in nature. Consequently, they often have the same imaging

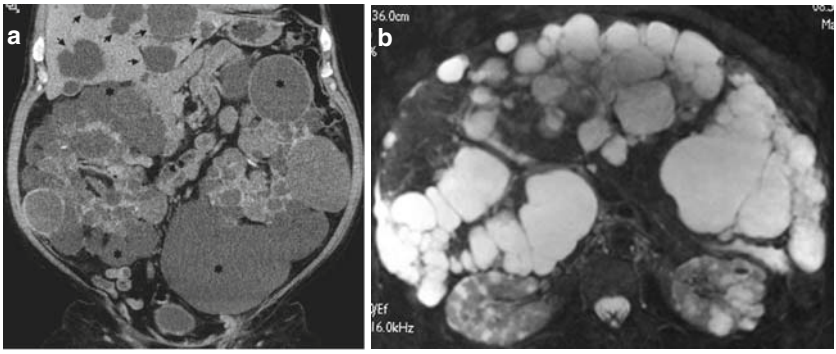

Fig. 5.14. ADPLD of two different patients demonstrating variable degree of hepatic and renal involvement of ADPLD: (**a**) coronal non-enhanced CT demonstrates only moderate amount of intrahepatic cysts (*arrows*) and massive enlargement of both kidneys secondary to innumerable cysts of variable size. Asterices delineate superior-most and inferior-most cysts within each kidney. Note that left kidney is even larger than the liver. (**b**) T2-weighted axial MR of another patient with liver dominant polycystic disease replacing the majority of hepatic parenchyma. Note numerous but relatively small cysts in both kidneys indistinguishable from renal tubular ectasia, with substantial amount of renal parenchyma left intact.

appearance as simple cysts. They are only distinguished from simple cysts by their distribution, their uniformly small size, and typically large number. Hamartomas are usually multiple simple cysts that measure 1.5 cm or smaller, located in both lobes of the liver (Fig. 5.15a). A communication with bile ducts is not seen [25]. The biliary ductal system is usually normal and not ectatic or irregular. Since hamartomas are mostly cystic in nature, they typically show no contrast enhancement. However, it has been reported that the fibrous stroma that may surround the cystic portion of the hamartoma may show enhancement [28]. Autosomal dominant polycystic liver disease is usually associated with a more variable cyst size and with cystic findings in the pancreas or kidneys. However, biliary hamartomas and ADPLD may coexist.

Since von Meyenburg complexes are not connected to the biliary tree, imaging may demonstrate the absence of a connection, therefore helping to distinguish multiple hamartomas from Caroli disease, where a small communication between the bile duct system and the cyst is considered diagnostic (see below).

Magnetic resonance cholangiography is the most widely accepted noninvasive modality used to characterize multiple cystic liver lesions. If the number and distribution of small cystic lesions are typical, and

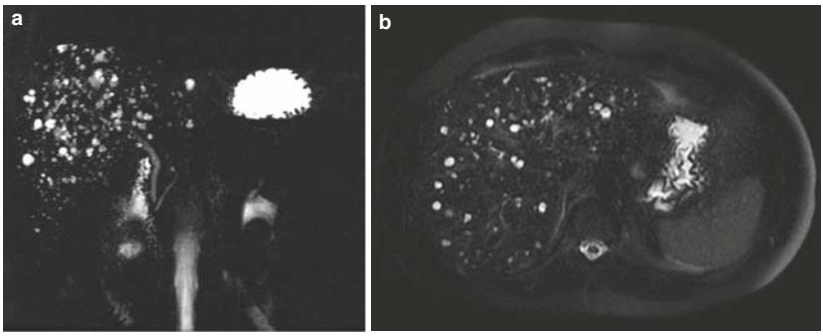

Fig. 5.15. Microhamartomas: (**a**) three-dimensional single shot heavily T2-weighted MR demonstrating good overview of bilobar multiple cysts in only one acquisition. Note normal caliber of CBD and intrahepatic bile ducts. (**b**) Axial thin slice heavily T2-weighted MR of the same patient accentuating hyperintense cystic character of multiple small lesions and demonstrating absence of communication to small intrahepatic bile ducts, typical for hamartomas.

no communication between the cysts and the adjacent bile duct is seen, hamartomas are favored. If a small communication between the ducts and the cysts is seen, Caroli disease (i.e., type V choledochal cyst by Todani's classification) or Caroli syndrome is likely [29]. It is important to adjust the parameters of the MR sequences so that only fluid containing lesions are seen and thin slices are displayed; 1.5–2 mm slice thickness with overlapping reconstruction is the most useful in order to confidently exclude the presence of small thin communicating bile ducts in heavily T2-weighted images (Fig. 5.15b). If uncertainty persists, more invasive endoscopic retrograde cholangiography (ERC), which offers the best spatial resolution available below 1 mm, may be performed to exclude the presence of communication with the bile ducts [30].

Radionuclide imaging does not have sufficient resolution to offer any additional information to the evaluation. However, hepatobiliary scintigraphy can be used to exclude or confirm other fibrocystic entities, such as biliary atresia (see above).

The most important differential diagnosis of biliary hamartomas is cystic metastases, which may have an identical appearance (Fig. 5.12). Occasionally, image-guided biopsy may be indicated for definitive diagnosis. Superinfection of biliary hamartomas or malignant transformation into cholangiocarcinoma has been reported but are extremely rare [31]. Both may be detected by contrast-enhanced cross-sectional imaging modalities.

CHOLEDOCHAL CYSTS

Choledochal cysts are part of a spectrum of biliary pathology and have been categorized by the Todani classification system [29]. The following section first outlines the various advantages and disadvantages of the different imaging options for diagnosing choledochal cysts. Next, the imaging appearance of the different types of choledochal cysts is discussed and recommendations for specific imaging studies are given based on type of cyst.

While choledochal cysts may be incidentally visualized by CT or MR, the current standard of care for diagnosis and classification of choledochal cysts is MRC [32]. MRC allows a noninvasive depiction of very small fluid containing structures within or outside the liver without the use of intravenous contrast. The distinction between extrahepatic and intrahepatic biliary cysts or a combination of the two can be made confidently with MRC. Multi-detector computed tomography (MRCT), with its capability to allow isotropic reformations in any imaging planes, also is capable of demonstrating with similar confidence the spatial relationship between the biliary cysts and the extrahepatic ducts or the liver parenchyma. However, the inferior contrast resolution of MDCT as compared to MRC can limit its ability to identify a potential communication between bile ducts and intrahepatic cysts, and thus hampers the differentiation of non-communicating biliary hamartomas from type IV and type V choledochal cysts, the latter of which is equivalent to Caroli disease [29]. MRC is also preferred over Tc-99m hepatobiliary scintigraphy, which was used in the past to identify biliary communications, because of its better spatial resolution, ease of use, faster image acquisition, and lack of the use of ionizing radiation or an intravascular contrast agent.

Of all of the imaging modalities, the best spatial resolution is provided by conventional ERC or equivalent intraluminal contrast studies such as T-tube cholangiogram or percutaneous transhepatic cholangiography (PTC). ERC is still considered the gold standard in diagnosing bile duct-related abnormalities but is not always indicated as a first diagnostic tool given its somewhat invasive character compared to MRCP. An additional advantage of ERC is that it allows tissue sampling via biopsy or brushing of ductal segments, if cholangiocarcinoma is suspected.

The fusiform type I cyst of the common bile duct is typically easily appreciated by MR in a coronal two-dimensional reformation or in a three-dimensional display mode. Three-dimensional MRC thick slab technique gives the best initial overview of the extent of the cyst, which is actually an ectatic ductal segment rather than a true cyst

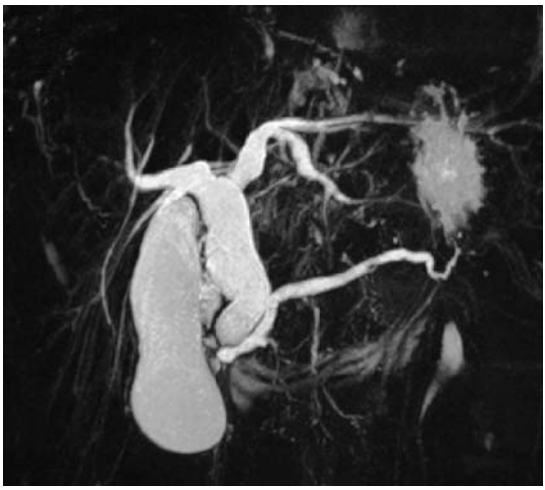

Fig. 5.16. Choledochal cyst type I, confirmed by surgery: MRC (three-dimensional technique, coronal view) demonstrates diffusely and fusiformly dilated CBD with mild transition of dilatation into the right and left hepatic duct, but no separate intrahepatic dilatation. Note mass effect on main pancreatic duct, which is also mildly dilated (courtesy Dr. Carlos Cuevas, University of Washington Medical Center, Department of Radiology).

(Fig. 5.16). Thinner two-dimensional slices allow a better analysis of potential intraluminal filling defects or irregularities of the wall that may help distinguish a type I cyst from obstructive causalities associated with extrahepatic bile duct dilatation. Axial images demonstrate a well-defined cystic round or oval structure in the anatomic location of the common bile duct.

Ultrasound also can show type I cysts, if an acoustic window allows visualization of the entire common bile duct (CBD). If the pancreatic segment is not seen, the etiology of a fusiform-dilated CBD remains unclear, as an obstructing mass or stone causing extrahepatic bile duct dilatation cannot be excluded. However, in the absence of intrahepatic bile duct dilatation, the presence of a fusiform-dilated CBD is suggestive of a type I choledochal cyst. It should also be noted that in patients who are postcholecystectomy, the CBD may also be dilated up to 1.5 cm with no or only subtle central intrahepatic bile duct dilatation.

Type II cysts are saccular ectasias of the common hepatic or common bile duct that are usually 2–3 cm in diameter, but can also be gigantic, holding up to 8 L of bile. Such large cysts typically are symptomatic [33]. Rarely, it can be a challenge to distinguish fusiform type I cysts from saccular type II cysts of the extrahepatic bile ducts (Fig. 5.17a, b).

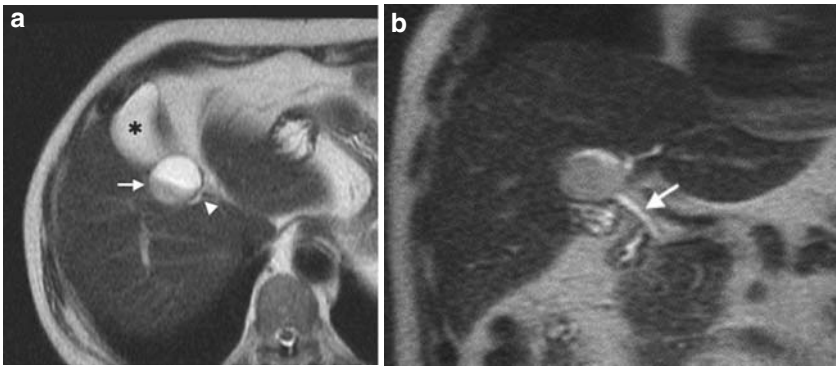

Fig. 5.17. Choledochal cyst type II of the common hepatic duct: T2-weighted single shot fast spin echo MR of the liver in an asymptomatic patient with prostate cancer, incidental finding on surveillance MR (**a**) axial images at the level of the gallbladder (*) demonstrates well-defined cystic structure with debris/fluid level (*arrow*) anterior to the non-dilated common hepatic duct. (**b**) Coronal image: note non-dilated CBD (*arrowhead*).

Type III cysts (choledochoceles) are ectatic ampullary segments of the CDB, frequently associated with mild bile duct dilatation. They may protrude into the duodenum or may be located in the pancreatic head without causing any mass effect (Fig. 5.18). They may be detected by MRCP or CT. Since they can be small and the pancreatic head cannot always be seen, ultrasound is less useful. However, endoscopic ultrasound offers excellent spatial resolution and does not suffer from lack of an acoustic window provided normal duodenal anatomy is present (Fig. 5.18). CT and MRCP, or at least T2-weighted MR, are quite sensitive to the detection of choledochoceles and extrahepatic bile duct dilatation. A subtle or moderately well-defined ectatic portion of the ampullary segment of the CBD associated with mild at least extrahepatic bile duct dilatation is the main finding on cross-sectional imaging modalities (Fig. 5.18).

Type IV choledochal cysts are a combination of extrahepatic and intrahepatic segmental ectasias of bile ducts. Since intrahepatic short segmental bile duct dilation might be difficult to see on ultrasound and CT, MRC is preferred to display this type of cyst. ERC, however, is considered the gold standard and offers immediate opportunity for diagnostic tissue sampling or therapeutic intervention (Fig. 5.19). Various forms of synchronous intra- and extrahepatic choledochal cysts exist, mostly categorized as type IVa and IVb [34].

Because of the distinct imaging appearance and clinical association with other entities, such as congenital hepatic fibrosis, the intrahepatic type V choledochal cysts, which is identical to Caroli disease, stands out

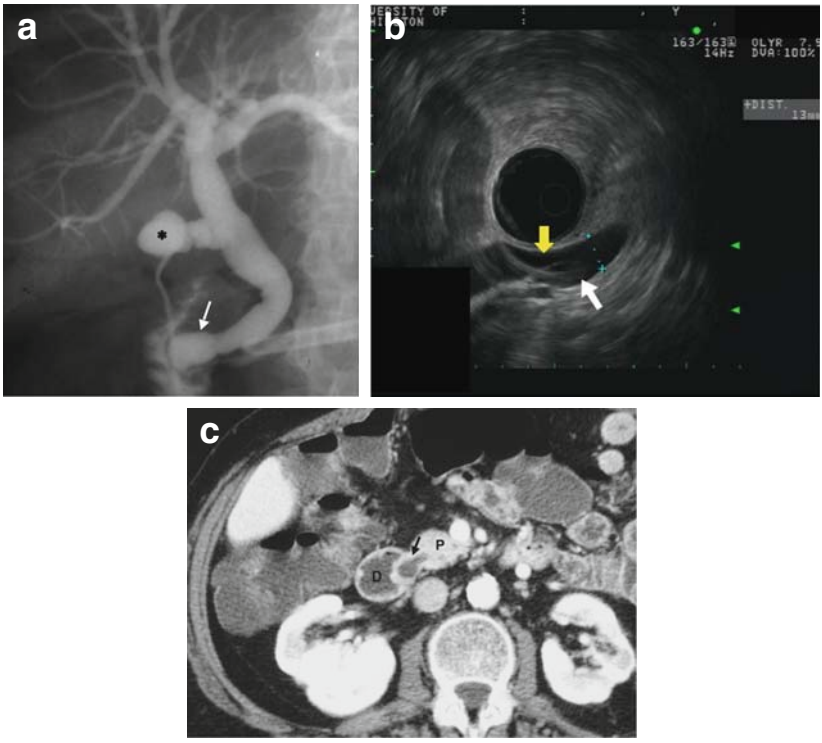

Fig. 5.18. Choledochocele (**a**) tube cholangiogram through the stump (*) of the cystic duct following cholecystectomy: incidental finding of a small choledochocele (*arrow*) with no to minimal mass effect on the duodenal wall, outlined by adjacent contrast. Subtle dilatation of the CBD might also be compensatory effect secondary to cholecystectomy (**b**) different patient than on (**a**): 74-year-old female with an asymptomatic choledochocele located within the head of the pancreas in the region of the ampulla. Endoscopic ultrasound (EUS) examination demonstrates communication between this choledochocele (*white arrow*) and the common bile duct (*yellow arrow*), Courtesy Dr. Joo Ha Hwang, University of Washington Medical Center, Department of Medicine. (**c**) Contrast-enhanced CT demonstrates small choledochocele (*black arrow*) protruding into the fluid-filled duodenum (D), P = pancreatic head (different patient). Bile duct dilatation is not depicted (Figures (**a**) and (**c**) are courtesy of Dr. Patrick C. Freeny, University of Washington Medical Center, Department of Radiology).

from other forms of biliary ductal cysts and will be discussed separately below.

Imaging also has a role in the detection of associated complications. These are mainly the sequelae of mass effect by the dilated extrahepatic bile ducts and include cholestasis and cholelithiasis,

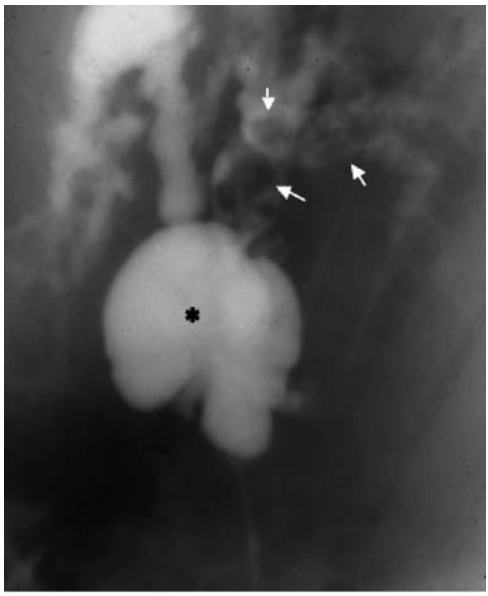

Fig. 5.19. Choledochal cyst type IV: ERC demonstrates large cyst type I of the CBD associated with massive bile duct dilatation of the entire intrahepatic bile duct system. Note contrast filling defects in the left ductal system (*arrows*) representing accumulation of debris, preventing exclusion of small obstructing cholangiocarcinoma without performing brushing or biopsy sampling (courtesy of Dr. Patrick C. Freeny, University of Washington Medical Center, Department of Radiology).

cholangitis, pancreatitis, duodenal obstruction, bile leak, biliary cirrhosis, and portal hypertension. Detection of the rare development of cholangiocarcinoma within the dilated ductal segments of any form of ectasia is one of the most important and challenging tasks of imaging.

CAROLI DISEASE

Caroli disease represents the type V choledochal cyst and manifests as intrahepatic biliary ductal ectasias. Two forms of Caroli disease have been described [35]: a non-hereditary form, which involves one hepatic lobe only, usually the left, sometimes confined to a single segment, and a hereditary form, which is the most commonly encountered, involving both lobes with multiple biliary fusiform or saccular dilatations [36]. In both forms, the retained communication between the bile ducts and the sacculations distinguishes Caroli disease from all other forms of cysts

related to fibrocystic diseases of the liver. Consequently, the demonstration of a persisting communication between bile ducts and "cysts" is one of two main diagnostic criteria. MRC is capable of demonstrating not only segmentally dilated small bile ducts or small cysts in close proximity to bile ducts but also the diagnostically important identification of a communication between bile ducts and cysts (Fig. 5.20). If doubt of a communication between bile ducts and "cysts" persists, ERC may be indicated (Fig. 5.21). ERC was the gold standard to distinguish biliary sacculations from non-communicating cysts until MRC was introduced.

Cross-sectional imaging modalities show biliary sacculations as multiple segmental or bilobar simple cysts, often indistinguishable from multiple simple cysts and biliary hamartomas. For better distinction, the "central dot sign" has been established for Caroli disease as a second characteristic diagnostic criterion [37,38]. The central dot sign represents the fibrovascular stalk of the portal triad surrounded by the adjacent dilated segment of bile duct and is best seen on contrast-enhanced CT or MR (Fig. 5.22). However, ultrasound and non-enhanced MR can also show this feature [26,38]. Biliary cysts and hamartomas have lost their communication to the bile ducts and are detached from the portal triad. Thus, they do not surround the portal triads and thus do not produce the central dot sign.

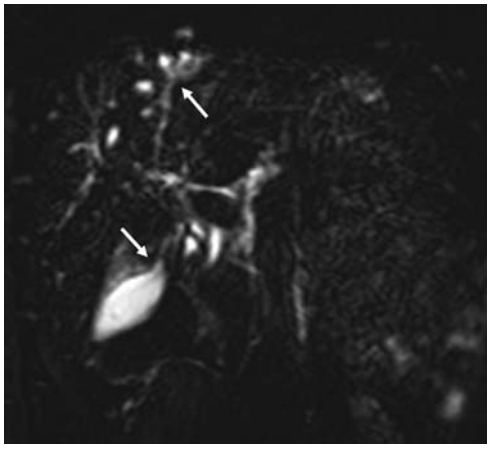

Fig. 5.20. Caroli disease: three-dimensional fat suppressed single shot MRCP demonstrates normal central bile ducts and clear communication (*arrows*) between peripheral small dilated bile ducts and globular ectasias ("cysts").

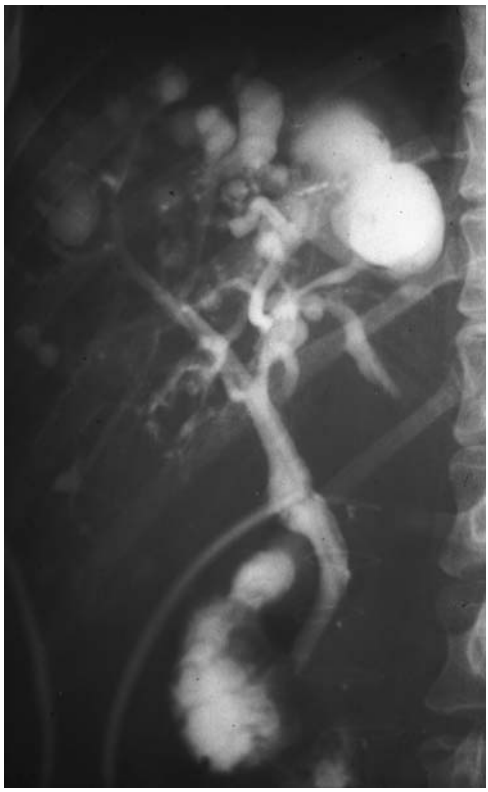

Fig. 5.21. Caroli disease: T-tube cholangiogram in a patient following cholecystectomy demonstrates non-dilated central intra- and extrahepatic bile ducts, but massively ectatic terminal bile ducts peripherally within both lobes, proving communication to "cysts."

Caroli syndrome, defined as the combination of intrahepatic biliary ductal ectasia and congenital hepatic fibrosis, may be detected by US, CT, or MR. However, again, MRC is the most sensitive and specific at identifying the communication between bile ducts and "cysts."

Complications of Caroli disease include demonstration of cholangitis, abscess formation, hepatolithiasis, and portal hypertension, the latter particularly in the presence of hepatic fibrosis. Also, the detection of cholangiocarcinoma, which is reported to have a prevalence of 7% in patients with Caroli disease or syndrome, is an important imaging contribution to the workup of these patients [39].

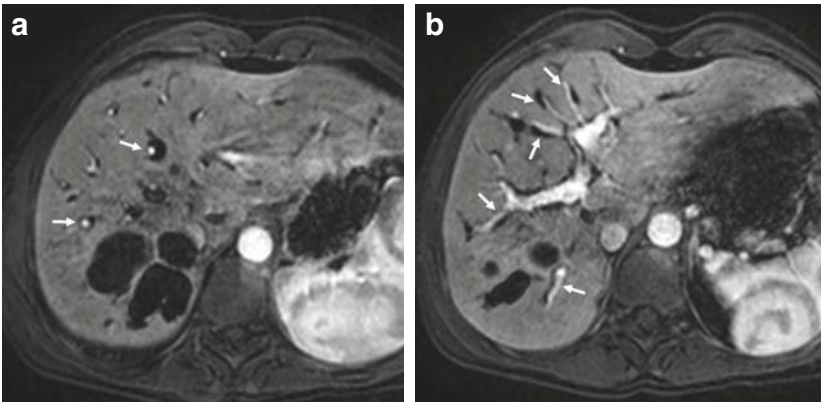

Fig. 5.22. Caroli disease – central dot sign: (**a**) Contrast-enhanced fat suppressed axial gradient echo T1-weighted MR in late arterial phase of same patient as in Fig. 5.20 demonstrates dilated bile ducts surrounding a central vessel (*arrows*), representing the portal vein. (**b**) Coronal image of the same patient using the same technique demonstrates close spatial relationship between portal vessels and dilated bile ducts (*arrows*) in a long orientation.

SUMMARY

Radiological imaging has a pivotal role in the diagnosis of fibrocystic diseases. Functional imaging modalities, such as Tc-99 DIHIDA scintigraphy, can demonstrate absence of bile ducts. Other cross-sectional methods, such as US, CT, or MR, are indispensable tools to identify and characterize cystic and fibrocystic abnormalities. These modern imaging techniques, including MRC, allow noninvasive distinction between isolated intrahepatic cysts, biliary irregularities, and communicating ductal sacculations. The radiological detection of often severe complications of fibrocystic diseases, such as abscess formation, portal hypertension, and cholangiocarcinoma, contributes significantly to the management and thus the clinical outcome of these patients. When differential diagnostic entities, such as cystic metastases or complications such as cholangiocarcinoma cannot be excluded confidently, image-guided tissue biopsy offers a relatively safe and reliable alternative.

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6 Pathology of Fibrocystic Diseases of the Liver

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CONTENTS

Summary

Fibrocystic liver diseases may occur in isolation or present as autosomal dominant or recessive polycystic disease. Abnormal embryologic development of the ductal plates is thought to be the common underlying cause of this diverse group of hepatic and biliary tract lesions. They also share common histopathological features including intrahepatic bile ductal dilatation and hepatic fibrosis of various degrees. These lesions can be clinically silent or lead to clinically

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obvious symptoms and signs. The pathology of these lesions is discussed in this chapter.

Key Words: Fibrocystic disease, Polycystic liver, Ductal plate malformation

INTRODUCTION

Fibrocystic diseases of the liver constitute a spectrum of heterogeneous groups of related hepatic and biliary tract lesions. These lesions share two common histopathological characteristics: dilatation of the intrahepatic bile ducts and variable degrees of hepatic fibrosis. They can occur alone or in association with abnormalities of other organs. Abnormal embryologic development of the ductal plates is thought to be the underlying etiology. These diseases may be asymptomatic or may cause various signs and symptoms, including portal hypertension, gastrointestinal bleeding and infection. Each of these lesions has unique pathologic features but may overlap to some degree in the gross or microscopic findings (Tables 6.1 and 6.2). The following is a concise review of the pathology of these diseases.

JAG1/NOTCH SEQUENCE AND ALAGILLE SYNDROME

Alagille syndrome, also known as arteriohepatic dysplasia, is a rare autosomal dominant inherited disease. It occurs in 1 in 70,000 live births. The disorder is associated with several developmental abnormalities including cholestatic liver disease, congenital heart disease, peculiar facial appearance, posterior embryotoxon in the eye, and butterfly-shaped vertebral arch deficits [1–4].

Mutations of the Jagged1 (*JAG1*) gene on chromosome 20p12 and mutations of the Notch-2 receptor have been reported to cause Alagille syndrome [5]. JAG1 is a ligand of the Notch receptors. The Notch signaling pathway plays a vital role in the regulation of cell differentiation, thereby controlling neurogenesis, hematopoiesis, myogenesis, somitogenesis, endocrinogenesis, and adipogenesis. Activation of the Notch signaling pathway requires the binding of *JAG1* to the Notch receptors. Disruption of the JAG1/Notch sequence thus leads to embryonic developmental abnormalities of many organs, including the liver. Notch signaling has been shown to be essential in the development of the biliary tree during ductal plate remodeling. Hepatoblast and mature hepatocyte transdifferentiation into cholangiocytes is controlled by the Notch signaling pathway. It has been shown that mutation in the *JAG1* gene leads to overexpression of the hepatocyte growth factor (HGF)

Table 6.1
Comparison of pathologic features of fibrocystic liver diseases

Disease	*Gross appearance*	*Fibrosis*	*Cirrhosis*	*Cholestasis*	*Bile ducts*	*Inflammation*	*Ductular reaction*	*Other features*
Alagille syndrome		Rare	Rare	Degree varies	Absent number of interlobular bile ducts	Portal	Yes	Giant cell transformation, pseudoxanthomatous change, copper accumulation in periportal hepatocytes
Biliary atresia	Enlarged, dark green liver	Periportal and bridging	Early stage: no Late stage: diffuse	Dark green and black inspissated bile	Numbers significantly reduced by 5 months of age; bile ducts dilated with thickened walls	Portal neutrophilic infiltration	Yes prominent	Giant cell transformation in some cases

Disease	*Gross appearance*	*Fibrosis*	*Cirrhosis*	*Cholestasis*	*Bile ducts*	*Inflammation*	*Ductular reaction*	*Other features*
ARPKD in infancy	Enlarged liver	Yes Features of CHF and Caroli disease	No	Usually not	Dilated		Yes	Hypoplasia of portal vein branches leading to portal hypertension
Congenital hepatic fibrosis	Enlarged, extremely firm liver; fibrotic cut surfaces	Diffuse; periportal; "jigsaw pattern"	No	Usually not	Numerous; interrupted circular arrangement (feature of ductal plate malformation)	"Portal hypertensive form": little or no inflammation "Cholangitic form": marked neutrophilic inflammation involving bile ducts and fibrotic tissue bands, can have microabscess	Yes	Bile ducts can also contain traces of mucin

Caroli disease and Caroli syndrome	Liver with cystic cavities arising from dilated intrahepatic bile ducts	CD: no CHF CS: associated CHF, diffuse, "jigsaw pattern"	No	Usually not	Numerous; dilated, thickened walls; ductal fibrosis (degree varies); ductal plate malformation may be present	Severe ductal chronic inflammation; can see acute inflammation within dilated bile ducts	CD: mucous glands may be present within dilated bile ducts CS: bilirubin calculi can be present within lumen of cystic cavities

ARPKD, autosomal recessive polycystic kidney disease; CHF, congenital hepatic fibrosis; CD, Caroli disease; CS, Caroli syndrome

Table 6.2
Comparison of pathologic features of cystic components

Disease	*Gross*	*Cyst content*	*Wall*	*Lining*	*Stroma*	*Other features*
Simple hepatic cyst	Well-demarcated cyst. Usually less than 4 cm in diameter	Clear serous fluid. Hemorrhage can be seen	Thin	Cuboidal or low columnar biliary epithelial cells		Complications: intracystic hemorrhage, infection. Squamous cell carcinoma and adenocarcinoma have been reported
Choledochol cyst	Multiple cysts. Size varies	Up to 10 L of bile	Thickened, fibrotic cyst wall. Bile may be present within wall	Columnar epithelium (usually denuded). Goblet and Paneth cells		Risk of developing cholangiocarcinoma. Squamous cell carcinomas, anaplastic carcinomas, and adenocarcinomas with sarcomatous features have been reported

ADPKD and PLD	Multiple cysts. Size varies (<1 mm–12 cm)	Serous fluid Exudate and abscess when infected and ruptured	Collapsed cyst has corrugated wall	Cuboidal or columnar epithelium. Flat epithelium in larger cysts	Scanty stroma. Dense and/or hyalinized in area with von Meyenburg complexes. Mild chronic inflammatory cell infiltrate may be present	Cysts have no communication with biliary tract Von Meyenburg complex is a frequent feature Associated cholangiocarcinoma, congenital hepatic fibrosis, mitral valve prolapsed have been reported
Microhamartoma (von Meyenburg complexes)	Usually multiple, small, less than 5–10 mm in diameter	Bile	Very thin wall. Bile ducts are dilated, branching, irregular	Cuboidal epithelium	Dense fibrous stroma	

ADPKD, autosomal dominant polycystic kidney disease; PLD, polycystic liver disease

gene which has been demonstrated to be the most critical protein in liver development. The overexpression of HGF is thought to result in increased differentiation of the hepatic stem cells to hepatocytes and less to biliary cells, thus causing bile duct paucity. Of note, alteration in the expression of *JAG1* and Notch has also been reported in the course of chronic liver diseases [1,2,4,6,7].

Cholestatic liver disease in Alagille syndrome is due to the paucity of the intrahepatic bile ducts. The predominant histological finding in the liver is the absence of bile ducts within the portal areas (Fig. 6.1). The ratio of interlobular bile ducts to the number of portal areas is between 0.9 and 1.8 in normal children, whereas in Alagille syndrome, this ratio is significantly reduced to between 0.0 and 0.4. Bile duct loss is progressive due to chronic, continuous damage to the biliary epithelium. Cholangiodestructive lesions characterized by proliferation of reactive ductules accompanied by portal inflammation and fibrosis are seen in early infancy. Rare cases of extensive fibrosis and progression to cirrhosis and hepatocellular carcinoma have been reported [8]. The degree of cholestasis varies, ranging from mild to progressive liver failure, and is particularly prominent during the first year of life. Other histological findings include giant cell transformation, patchy pseudoxanthomatous change, and copper accumulation in periportal hepatocytes [7].

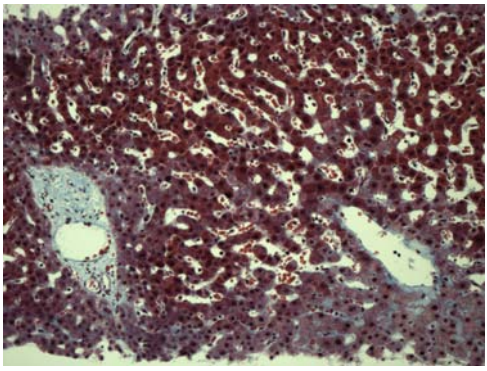

Fig. 6.1. A portal tract shows the absence of interlobular bile ducts in Alagille syndrome (Masson trichrome stain, courtesy of Dr. Dhanpat Jain, Department of Pathology, Yale University).

BILIARY ATRESIA

Biliary atresia is the most common cause of neonatal cholestasis and the main indication for liver transplantation in young pediatric patients. This congenital disorder occurs in approximately 0.5–3.2 of 10,000 live births. It is a progressive inflammatory fibrosing obliterative disorder

of the biliary tract of unknown etiology [9,10]. A single segment or the entire extrahepatic biliary tree is completely fibrotic leading ultimately to ductopenia, and eventual biliary cirrhosis. In the majority of the patients, the bile ductal malformation is an isolated entity; however, other abnormalities which have been designated as the biliary atresia splenic malformation syndrome (BASM) (i.e., situs inversus, polysplenia, absence of inferior vena cava) are seen in approximately 10% of affected infants [11].

Biliary atresia is classified into two forms. The "early" or embryonic/fetal form is seen in 15–35% of cases and the "late" or perinatal/acquired form affects 65–85% of cases [12]. It is also divided into two anatomical subtypes: correctable and uncorrectable [13]. Chromosomal abnormalities such as trisomy 18, trisomy 21, or Turner syndrome have been associated with the embryonic form [14].

The gross appearance of the liver changes as the disease progresses from the early to the late stage. Initially, the liver appears enlarged and dark green without regenerative nodules. As fibrosis progresses and cirrhosis develops, the liver becomes finely nodular and subsequently diffusely cirrhotic (Figs. 6.2 and 6.3). The macronodular cirrhosis with peripheral micronodular fibrotic areas can grossly resemble focal nodular hyperplasia. The bile ducts are dilated, sometimes with thickened walls and are filled with dark green/black inspissated bile. Microscopically, there are cholestasis and portal tract changes similar to those seen in adult large bile duct obstruction. The portal tracts are expanded by edema, neutrophilic infiltrate, and a prominent periportal ductular reaction. Inspissated bile may be present within the ductular

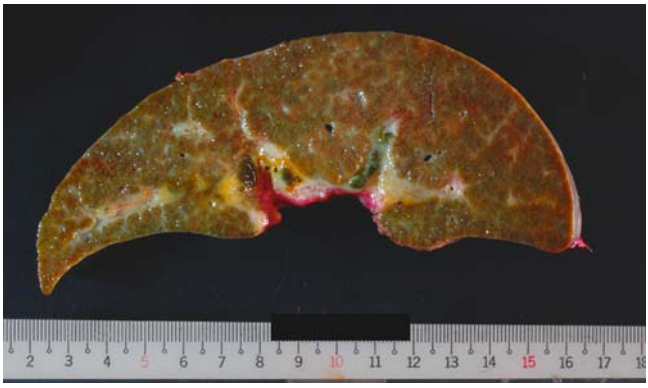

Fig. 6.2. Gross photo of an explant with biliary atresia-induced cirrhosis, characterized by the *greenish* nodules. Courtesy of Dr. Chao-Cheng Huang, Department of Pathology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

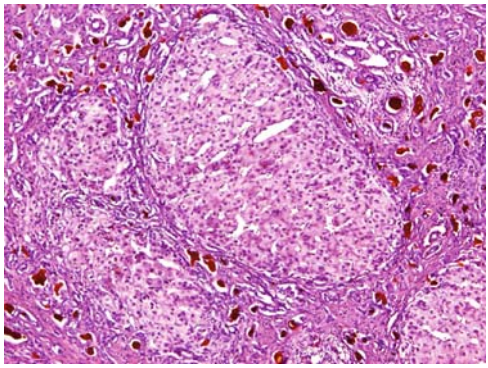

Fig. 6.3. Cirrhosis due to biliary atresia. The photomicrograph shows cirrhotic nodules of variable sizes. Inspissated bile is present within the ductal structures (hematoxylin and eosin stain, 100× magnification). Courtesy of Dr. Chao-Cheng Huang, Department of Pathology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

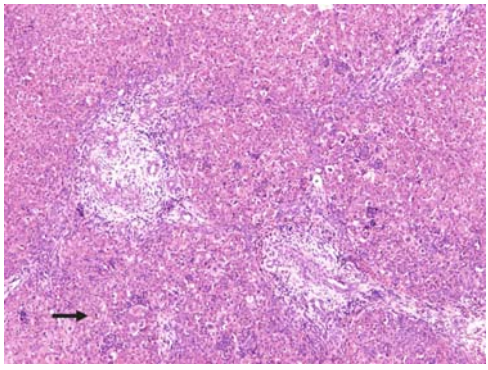

Fig. 6.4. Early biliary atresia. The photomicrograph shows cholestasis and portal tract changes similar to those seen in adult large bile duct obstruction. Portal tracts are expanded by edema, inflammatory infiltrate, and prominent periportal ductular reaction. There is fibrous portal expansion. Inspissated bile is also present within the ductular structures (*arrow*) (hematoxylin and eosin stain, 100× magnification). Courtesy of Dr. Chao-Cheng Huang, Department of Pathology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

structures (Fig. 6.4). Periportal fibrosis with bridging fibrosis may be seen. Portal fibrosis is progressive and the severity depends on the age at diagnosis and the duration of ductal obstruction. By 5 months of age, the number of interlobular bile ducts is significantly reduced. About 15% of cases show giant cell transformation of hepatocytes [14,15].

POLYCYSTIC KIDNEY DISEASE

Autosomal Recessive Polycystic Kidney Disease (ARPKD)

Polycystic kidney disease can be transmitted as either an autosomal dominant or an autosomal recessive trait. Autosomal recessive polycystic kidney disease (ARPKD), with an estimated incidence of 1 in 20,000, occurs less frequently than autosomal dominant polycystic kidney disease (ADPKD). The clinical characteristics of ARPKD include cystic dilatation of collecting ducts of the kidney and hepatic abnormalities (bile duct dysgenesis and periportal fibrosis). Mutations in a single gene, polycystic kidney and hepatic disease 1 (*PKHD1*), located on chromosome 6p21, have been identified as the cause of ARPKD [16–18].

ARPKD is generally considered to be a disorder of infants. Detection of greatly enlarged echogenic kidneys typically occurs in utero or during the neonatal period in the majority of patients. In approximately 50% of cases, associated hepatic abnormalities develop, including hepatomegaly, increased hepatic echogenicity on ultrasound, and dilated intrahepatic bile ducts. A small number of cases are diagnosed in older children or teenagers. These older patients general present with the manifestations of portal hypertension. Rarely, ARPKD is diagnosed in adults [19, 20].

Individuals who survive beyond the neonatal period and into adulthood often have complications associated with both renal and hepatic abnormalities. The kidneys are massively enlarged but usually still maintain the normal shape. Numerous subcapsular microcysts less than 3 mm in diameter, running perpendicularly to the cortex, are grossly visible. Microscopically, the nephrons show cystic dilatations, specifically limited to the collecting tubules with a few cysts in other parts of the nephrons. The cysts are lined by flattened epithelium. The severity of the renal disease correlates with the number of affected nephrons [21].

The hepatic lesions in ARPKD seldom give rise to grossly visible cysts. The liver shows portal enlargement, periportal fibrosis, numerous dilated bile duct profiles (ductal plate malformation), and hypoplasia of portal vein branches leading to portal hypertension. Congenital hepatic fibrosis and/or Caroli disease is often seen in ARPKD. It has been unclear whether isolated congenital hepatic fibrosis and Caroli disease are separate disease entities or part of the spectrum of ARPKD. A recent study has suggested that congenital hepatic fibrosis and Caroli disease are indeed part of the ARPKD spectrum [22, 23].

Autosomal Dominant Polycystic Kidney Disease and Polycystic Liver Disease

Polycystic liver disease (PLD) is a benign, autosomal dominant congenital disorder, existing either as an isolated condition or in association with autosomal dominant polycystic kidney disease (ADPKD) [24]. PLD is rare, usually asymptomatic and incidentally diagnosed. Autopsy series show a prevalence of 0.13–0.6%. Approximately 30% of cases of PLD are associated with ADPKD [25]. Increasing age, severity of renal cystic disease, severity of renal dysfunction, and exogenous female steroid hormone ingestion are risk factors for hepatic cyst development and progression. While the lifetime risk of PLD is equal among men and women, greater numbers and larger sizes of hepatic cysts are seen in women [26].

PLD has been linked to mutations in three distinct genes: *PKD1*, *PKD2*, and *PRKCSH* (protein kinase C substrate 80 K-H). *PKD1* mutations are more prevalent and lead to greater disease severity compared to *PKD2* mutations. Patients with *PKD2* mutations develop later onset of disease and have an increased life expectancy of approximately 16 years. Mutations in *PKD1* and *PKD2* are characteristically seen in patients with concomitant renal and liver cystic disease. Patients with isolated liver cystic disease typically have mutations in the *PRKCSH* gene [24, 26].

In contrast to the renal function loss often seen in renal cystic disease, liver function is usually not diminished and liver failure rarely occurs even with widespread hepatic disease. Symptoms can include abdominal pain, early satiety, and shortness of breath secondary to abdominal distention. Complications include infection, cyst rupture, and hemorrhage [24, 27].

The development of multiple cysts within the liver is gradual and spread widely throughout both lobes. The cysts vary in number and size, ranging from <0.1 to 12 cm or greater in diameter (Fig. 6.5). They contain serous fluid and have no communication with the biliary tract. They are lined by cuboidal or columnar epithelium. However, larger cysts are lined by flat epithelium (Fig. 6.6). When collapsed, the cyst wall appears corrugated and loose connective tissue is seen within the lumen. Pus and cholangitic abscess are seen when the cysts are infected. Von Meyenburg complexes (a cluster of irregularly shaped, duct-like structures embedded in a fibrous stroma) are a frequent feature (Fig. 6.7) and are occasionally associated with cavernous hemangioma. The supporting stroma is dense and/or hyalinized in the area of the von Meyenburg complexes, otherwise it is scanty [26]. A mild chronic inflammatory cell infiltrate may be seen within

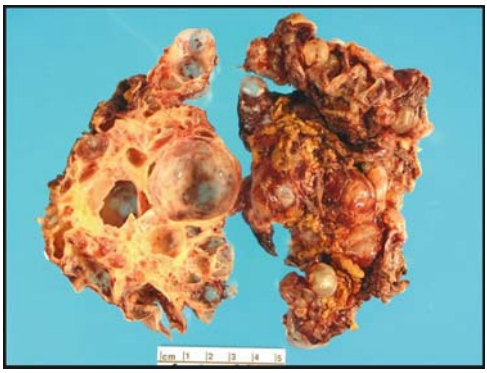

Fig. 6.5. A gross photo showing an autosomal dominant polycystic kidney disease (ADPKD)-associated polycystic liver with many variable-sized cysts that contain serous fluid.

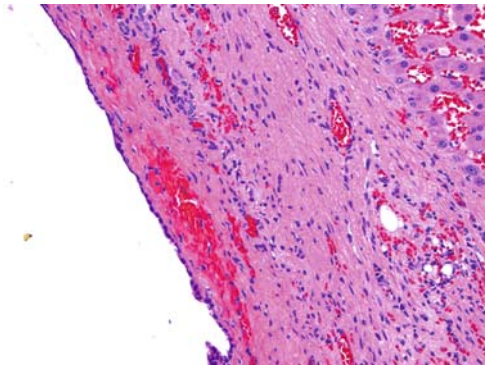

Fig. 6.6. The photomicrograph shows polycystic liver with the cystic cavity lined by cuboidal, columnar to flat epithelium (hematoxylin and eosin stain, 200× magnification).

the stroma. Cholangiocarcinoma, congenital hepatic fibrosis, and mitral valve prolapse have been reported to be associated with polycystic liver disease [28–31].

CAROLI DISEASE AND CAROLI SYNDROME

Caroli disease (CD) is a congenital condition in which the larger (segmental) intrahepatic bile ducts are dilated. Patients present with recurrent episodes of fever and right upper quadrant pain [32]. Jaundice and elevated liver tests occur when there is blockage of the common bile duct [33–36]. Complications include abscess formation,

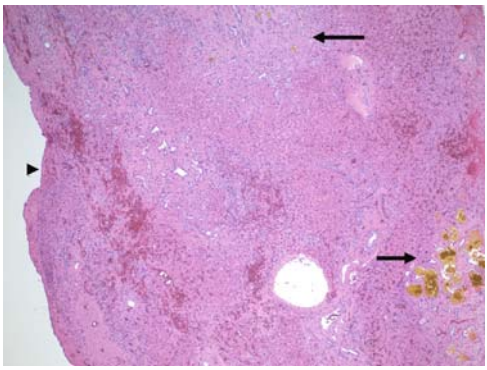

Fig. 6.7. Von Meyenburg complex. This is a frequent feature associated with polycystic liver disease. The photomicrograph shows the presence of polycystic liver (*arrowhead*) along with a von Meyenburg complex (*arrows*, cluster of irregularly shaped, duct-like structures embedded in the fibrous stroma) (hematoxylin and eosin stain, 40× magnification).

cholangitis, septicemia, intrahepatic lithiasis, and amyloidosis [37–39]. Spontaneous rupture of a bile duct, adenocarcinoma, and hepatocellular carcinoma have also been reported [39, 40].

The pathogenesis of CD is believed to be partial or complete arrest of remodeling of the ductal plate of the large intrahepatic bile ducts [41]. Caroli disease when associated with congenital hepatic fibrosis is referred to as Caroli syndrome (CS). Caroli disease has also been seen to be associated with infantile polycystic disease, adult polycystic disease [42, 43], and choledochal cysts [44].

The large, extensively dilated bile ducts appear grossly as cystic cavities that may be moniliform or saccular with fibrous cords extending across. Intraductal polypoid projections containing small portal vein branches indicative of the ductal plate malformation may be present. Normal bile ducts may be seen in continuity with and between the dilated ducts. Bile staining can often be seen lining the bile ducts. Bilirubin calculi may be present in the lumen of the cavities.

Histologically, the liver shows dilated intrahepatic bile ducts with thickened walls and severe chronic inflammation. Acute inflammation, varying degrees of fibrosis, and mucous glands may be present within the dilated ducts. The ductal epithelium consists of cuboidal to tall columnar cells with focal hyperplasia and focal or diffuse ulceration. Rarely, focal severe epithelial dysplasia may be seen.

Histomorphological features of congenital hepatic fibrosis are also seen in Caroli syndrome. The liver shows diffuse periportal fibrosis. The fibrous bands vary in thickness and often surround a single hepatic

lobule or groups of lobules giving rise to the "jigsaw" pattern. Within the fibrous septa are numerous small round or slightly dilated, irregular bile duct lined by cuboidal or low columnar epithelial cells. The ducts are usually arranged in an interrupted circular configuration, a feature of ductal plate malformation.

Renal involvement in Caroli syndrome includes renal tubular ectasia (medullary sponge kidney, cortical cyst), lesions of adult recessive or autosomal dominant polycystic kidney disease [45].

CONGENITAL HEPATIC FIBROSIS

Congenital hepatic fibrosis is a developmental malformation and a component of the fibropolycystic liver disorders. It can occur alone but almost always occurs in association with autosomal recessive polycystic kidney disease [46]. Congenital hepatic fibrosis associated with Caroli disease (dilatation of intrahepatic bile ducts) is termed Caroli syndrome (see above) [47]. The signs and symptoms of congenital hepatic fibrosis are generally nonspecific, with complications of portal hypertension being the most common presentation. Other clinical manifestations include hepatosplenomegaly, esophagogastric varices, and spontaneous gastrointestinal bleeding [48]. The age of onset ranges from early childhood to the fifth and sixth decades of life [46]. Four clinical forms of congenital hepatic fibrosis have been described: portal hypertensive, cholangitic, mixed portal hypertensive-cholangitic, and latent forms [49].

Grossly, the liver is generally enlarged and extremely firm with fibrotic cut surfaces. The entire liver is usually involved; however, involvement of only one or two lobes is occasionally seen. Diffuse periportal fibrosis is a common histologic feature. Microscopically, islands of unremarkable liver parenchyma are separated by bands of fibrous tissue of varying thickness, giving rise to the classic "jigsaw pattern" (Fig. 6.8). The fibrous bands contain numerous small, uniform bile ducts, some of which can be dilated, irregularly shaped, and contain bile and traces of mucin. The ductal lining consists of cuboidal or low columnar epithelial cells. The bile ducts often have an interrupted circular arrangement, a feature of ductal plate malformation. In the portal hypertensive form, little or no inflammatory activity is seen within the fibrotic areas. In contrast, marked neutrophilic infiltration involving the bile ducts and fibrous bands is a feature of the cholangitic form. Microabscess formation resulting from duct rupture can occur in the cholangitic form which may lead to difficulty in differentiating this form of congenital hepatic fibrosis from extrahepatic biliary obstruction with ascending infection.

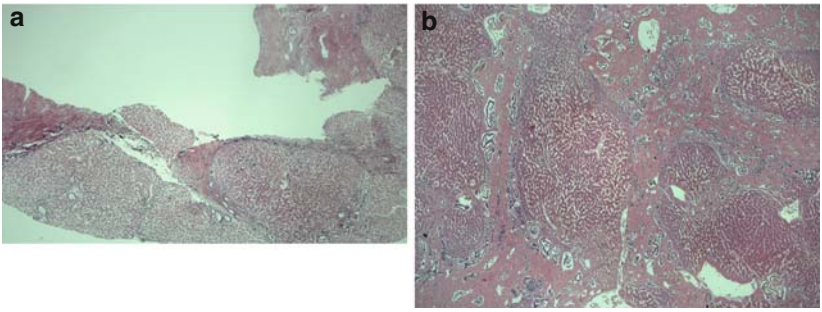

Fig. 6.8. Congenital hepatic fibrosis. **a**. At low-power magnification, islands of unremarkable liver parenchyma are separated by bands of fibrous tissue of varying thickness. **b**. At higher magnification, the fibrous bands contain numerous small, uniform bile ducts, some of which can be dilated, irregularly shaped, and contain bile and traces of mucin. The ductal lining consists of cuboidal or low columnar epithelial cells (courtesy of Dr. Dhanpat Jain, Department of Pathology, Yale University).

SIMPLE HEPATIC CYST

Simple hepatic cysts, also known as benign liver cysts or non-parasitic hepatic cysts, are a congenital disorder occurring in 2.5–7% of the population [50]. The majority of simple hepatic cysts are asymptomatic and require no treatment. Cysts that are larger than 4 cm in diameter may be followed for stability. The most common complications are intracystic hemorrhage and infection. Rare complications include torsion, compression of adjacent organs, rupture, and pulmonary embolism [51]. Rare cases of squamous cell carcinoma and adenocarcinoma arising from non-parasitic hepatic cyst have also been reported [52, 53].

Simple cysts are well demarcated and have thin wall [54] in contrast to the typically thickened wall seen in choledochal cysts. The cyst wall lining consists of a single layer of cuboidal to low columnar biliary epithelial cells. The cyst usually contains clear serous fluid [55], although hemorrhage can sometimes be seen within the cyst.

CHOLEDOCHAL CYST

Choledochal cysts are a congenital dilatation of the extrahepatic and/or intrahepatic bile ducts. It is more commonly seen in East Asian populations, with a female to male ratio of approximately 3.5:1 [56]. The majority of patients (60%) are diagnosed before the age of 10 but the disease can be diagnosed at any age [57]. The clinical features

include right upper quadrant mass, jaundice (more commonly seen in children), and signs and symptoms of cholangitis (more commonly seen in adults). Affected individuals have an increased risk of developing cholangiocarcinoma.

Choledochal cysts are classified into five types based on the Todani modification of the Alonso-Lej classification. Type I is the most common type comprising 80–90% of all choledochal cysts and consists of a solitary extrahepatic cyst. Type II includes diverticulum of the common bile duct or the gallbladder. Type III, an intraduodenal cyst, is also known as choledochocele. Type IV consists of intrahepatic and extrahepatic cysts. Type V consists of multiple intrahepatic cysts and is also known as Caroli disease [58, 59].

Choledochal cysts vary in size and may contain as much as 10 L of bile (Fig. 6.9). Microscopically, the cyst wall is fibrotic, thickened (in contrast to simple hepatic cyst which has a thin wall), and chronically inflamed. Bile can be seen within the wall. The epithelial lining of the cyst is usually denuded. When preserved, the lining consists of columnar epithelial cells (Fig. 6.10). Intestinal metaplasia with abundant mucinous glands is a feature which is seen in almost all patients older than 15 years. Paneth cells as well as neuroendocrine differentiation have also been noted in some choledochal cysts. Postganglionic neural dysfunction has been suggested to be the etiology of choledochal cysts due to the absence of ganglion cells in the narrow portion of a choledochal cyst [60–64].

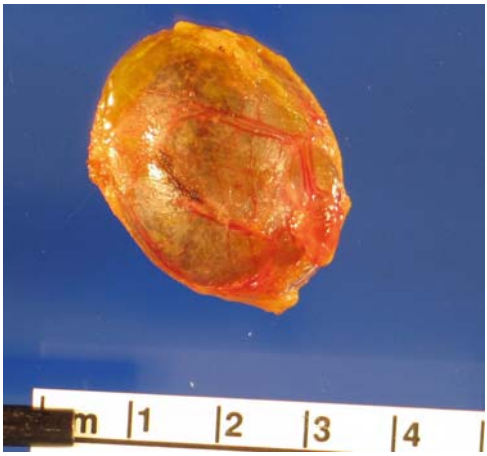

Fig. 6.9. A gross photo of a choledochal cyst. There are numerous blood vessels overlying the surface.

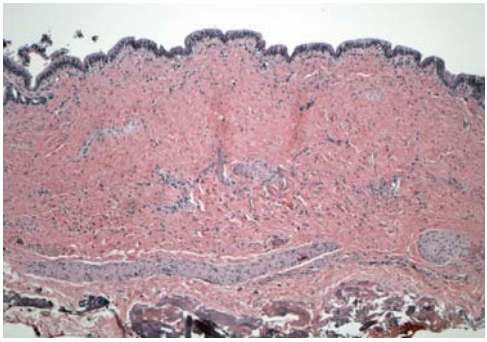

Fig. 6.10. The cyst wall of choledochal cyst is fibrotic, thickened, and chronically inflamed. The epithelial lining of the cyst consists of columnar epithelial cells (courtesy of Dr. Dhanpat Jain, Department of Pathology, Yale University).

Patients have a risk of developing cholangiocarcinoma, which increases with age. While adenocarcinoma is the most commonly encountered tumor resulting from choledochal cysts, squamous cell carcinomas, anaplastic carcinomas, and adenocarcinoma exhibiting sarcomatous features have also been reported [65, 66].

VON MEYENBURG COMPLEXES (BILE DUCT HAMARTOMA)

Von Meyenburg complexes, also known as biliary microhamartomas or bile duct hamartomas, are benign hepatic lesions resulting from developmental malformation of the ductal plate [67]. They are seen in 5.6% of adult and 0.9% of children at autopsy and often are associated with polycystic kidney and liver disease [68].

Von Meyenburg complexes are relatively small cystic lesions, ranging in size from less than 5 to 10 mm in diameter [69]. Microscopically, they consist of irregularly shaped, dilated, branching bile ducts embedded in a dense fibrous stroma (Fig. 6.11). The ducts have a cuboidal epithelial lining and can contain bile [70].

Von Meyenburg complexes are considered part of the spectrum of adult polycystic disease [71]. Both solitary bile duct cysts and the cysts of polycystic liver disease are thought to derive from dilatation of von Meyenburg complexes [23]. There have been rare reports of malignant transformation to cholangiocarcinoma [72–75]. Rare association with hepatocellular carcinoma and esophageal carcinoma has also been reported [71, 76].

The various lesions described in this chapter represent a spectrum of the hepatic fibrocystic diseases. While some may be incidental

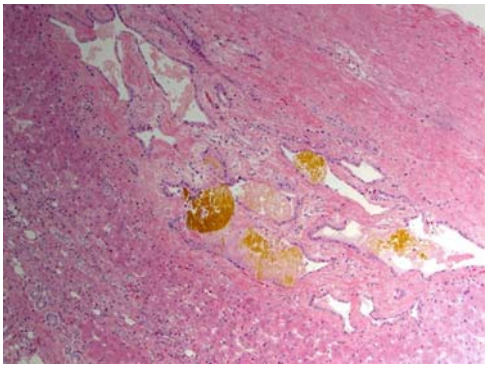

Fig. 6.11. Bile duct hamartoma (von Meyenburg complex). Histologically, it is composed of a variable number of ductal structures embedded in a hyalinized stroma. The ductal structures are variably dilated and may have microcystic dilatation with bile in the ductal lumens. The ductal lumens are lined by a flattened or a cuboidal epithelium (hematoxylin and eosin stain, 100× magnification).

findings and may not cause clinically significant diseases, some may have dismal outcome with ultimate loss of the bile ducts such as Alagille syndrome or biliary atresia that require liver transplantation. Therefore close clinical, radiographic, and pathologic correlations are essential to differentiate these lesions and hence to facilitate appropriate managements.

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III Disease States

7 Alagille Syndrome and *JAGGED1/NOTCH* Sequence

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CONTENTS

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Summary

Alagille syndrome (AGS) is a highly variable, autosomal dominant disorder that affects the liver, heart, eyes, face, and skeleton. AGS is caused by mutations in *JAGGED1*, a ligand in the Notch signaling pathway. This pathway is evolutionarily conserved and is involved in cell fate determination. *JAGGED1* mutations are identified in more than 90% of clinically diagnosed probands. The majority of the mutations are predicted to result in premature termination of the protein in the extracellular domain. The finding that mutations in *JAGGED1* and *NOTCH2* cause AGS indicates that Notch signaling is important in the development of the organ systems affected. *JAGGED1* expression during normal embryogenesis is widespread in different organ systems and overlaps with the clinical manifestations of AGS. In situ hybridization studies have revealed *JAGGED1* expression in the cardiovascular system, renal tubules, eyes, inner ear, pharyngeal arches, mesenchyme of limb buds, and developing nervous system. The study of AGS, in particular in mutant mouse models, has shed enormous light on Jagged–Notch signaling and this in turn has led to further insights into bile duct and cardiac development.

Key Words: Jagged1, Notch, Alagille, Alagille Syndrome

INTRODUCTION

Alagille syndrome (AGS) is a highly variable, autosomal dominant disorder that affects the liver, heart, eyes, face, and skeleton [1–3]. The kidneys, pancreas, and the vascular system are also involved in many cases, though these are not currently defining criteria [4, 5]. There is significant variability in the extent to which each of these systems is affected in an individual, if at all [6–8]. AGS has traditionally been diagnosed based on the presence of intrahepatic bile duct paucity on liver biopsy in association with at least three of the five major clinical features: chronic cholestasis, cardiac disease (typically right-sided lesions and most often peripheral pulmonary stenosis), skeletal abnormalities (usually butterfly vertebrae), ocular abnormalities (most likely posterior embryotoxon), and characteristic facial features [4]. The textbook frequency has been reported as 1 in 70,000 live births, though the advent of molecular testing and the subsequent identification of mildly affected individuals suggest that this is an underestimate [8].

AGS is caused by mutations in *JAGGED1*, a ligand in the Notch signaling pathway (Fig. 7.1) [9, 10]. This pathway is evolutionarily conserved and is involved in cell fate determination. *JAGGED1 (JAG1)* mutations are identified in more than 90% of clinically diagnosed

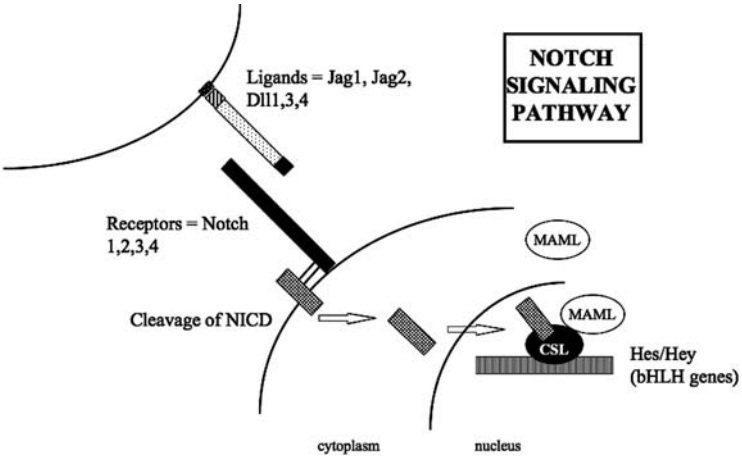

Fig. 7.1. Schematic representation of Notch signaling pathway. NICD, Notch intracellular domain; MAML, co-activator Mastermind-like; CSL, DNA-binding protein; bHLH, basic helix–loop–helix genes.

probands [11]. Recently mutations in *NOTCH2* have also been identified in a few patients with AGS who do not have *JAG1* mutations [12]. The study of AGS has shed enormous light on Jagged–Notch signaling and this in turn has led to further insights into bile duct and cardiac development.

IDENTIFICATION OF *JAGGED1*

AGS was recognized to have an autosomal dominant mode of inheritance in the first reports by Alagille [1] and Watson and Miller [3]. Based on the clues provided by cytogenetic analysis of families carrying visible deletions on the short arm of chromosome 20 [13–16], in 1997, two groups identified mutations in *JAG1* as the cause of AGS [9, 10]. Jagged1 is a single-pass transmembrane protein, with an extracellular and an intracellular domain (Fig. 7.2). The extracellular domain contains a region conserved among all Notch ligands called the DSL region (for ligands Delta and Serrate from *Drosophila*, and Lag-2 from *C*aenorhabditis *elegans*), 16 epidermal growth factor-like repeats, and a cysteine-rich region. There is a transmembrane domain and a small intracellular region. The genomic sequence is composed of 26 exons, and the standard strategy to screen for *JAG1* mutations is to analyze the coding regions of each of the exons, in addition to about 20 intronic bases surrounding each exon in order to identify potential splice-site mutations.

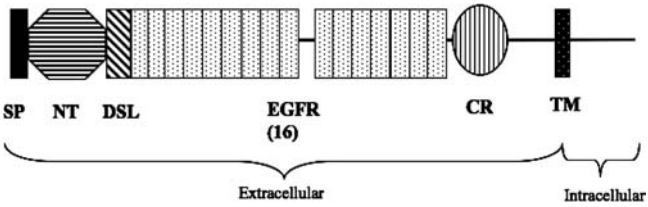

Fig. 7.2. Schematic representation of *JAG1* structure. SP, signal peptide; NT, N terminus; DSL, Delta, Serrate, Lag-2 region; EGFR, epidermal growth factor-like repeats; CR, cysteine-rich domain; TM, transmembrane domain.

JAGGED1 MUTATIONS IN ALAGILLE SYNDROME

To date, over 430 *JAG1* mutations have been identified in patients with AGS. The frequency of mutation detection has been around 60–70% in older studies [6, 17–26]. However, recently, a cohort of patients were exhaustively studied, with sequencing of the genomic coding region and cDNA, and the mutation rate was found to be 94% [11]. The frequency of sporadic mutations (i.e., new in the proband) is approximately 56–70%. Of affected parents with identified mutations, half were male and half were female.

The *JAG1* mutations identified in patients with AGS have been found distributed across the entire coding region, with no real hotspots (Fig. 7.3) [25]. The majority of the mutations are predicted to result in premature termination of the protein in the extracellular domain. Approximately 70% of AGS patients have protein-truncating (frameshift or nonsense) mutations [6, 11, 25]. Approximately 7% have

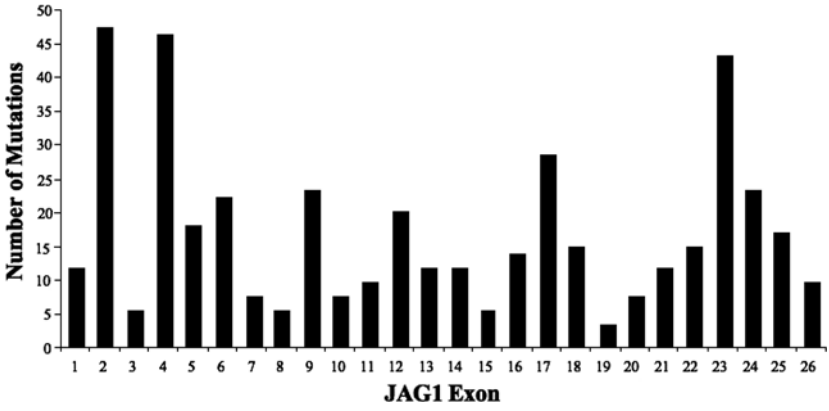

Fig. 7.3. Distribution of *JAG1* mutations by exon.

gene deletions, 9% have splice-site mutations, and 9% have missense mutations. Haploinsufficiency, a decrease in the amount of the normal protein, is hypothesized to be the mechanism causing AGS. However, there is evidence to support the role of other potential mechanisms, such as the dominant-negative effect of mutant transcripts [7, 27].

NOTCH SIGNALING PATHWAY

The Notch signaling pathway is a short-range intercellular signaling system involved in the regulation of cell fate determination (Fig. 7.1). It is evolutionarily conserved and functions in many different cell types throughout development to regulate cell fate decisions [28]. The name "Notch" derives from the characteristic notched wing found in flies carrying only one functioning copy of the gene. Homozygous mutations in Notch are lethal, and affected flies show hypertrophy of the nervous system, indicating the inability of appropriate cells to adopt an alternative cell fate. In mammals the key components of the pathway are five ligands (Jagged1, Jagged2, Delta-like 1, 3, and 4) that signal to four Notch receptors (Notch 1–4). Jagged1 is a single-pass transmembrane ligand belonging to the Delta, Serrate, Lag-2 (DSL) family. Notch signaling is initiated by contact between a cell surface ligand on one cell and a Notch receptor on an adjacent cell. Since both receptors and ligands in the pathway are anchored to the cell surface, signal transmission is restricted to cells that are physically adjacent. The Notch receptors appear on the cell surface as two associated peptides, one large extracellular domain that consists of primarily epidermal growth factor (EGF)-like repeats and the other consisting of the transmembrane segment and the intracellular domain [29]. Binding of a ligand (such as Jagged1) promotes two proteolytic cleavage events in the Notch receptor. The first cleavage is mediated by ADAM-family metalloproteases and the second by γ-secretase. The second cleavage releases the Notch intracellular domain (NICD), which translocates into the nucleus where it forms an active transcriptional tricomplex with the DNA-binding protein CSL (also known as CBF1) and the co-activator Mastermind. The assembly of this activator complex promotes transcription of downstream genes, in particular the basic helix–loop–helix genes *Hes* (*H*airy and *e*nhancer of *s*plit) and *Hey* family genes. These are transcriptional repressors that mediate many of the downstream responses of Notch signaling, usually to inhibit cellular differentiation through lateral inhibition [29, 30].

The Delta ligands themselves also undergo cleavage by ADAM proteases and γ-secretase, resulting in the release of extracellular and intracellular fragments. The physiologic function of these fragments is

unclear. Jagged1 is also proteolytically cleaved at the cell surface by metalloproteinase activity but shedding of the extracellular domain is not yet well understood [31].

The finding that mutations in *JAG1* and *NOTCH2* cause AGS indicates that Notch signaling is important in the development of the organ systems affected, i.e., the liver, heart, skeleton, eye, face, and kidney. Studies of the pattern of expression of the various Notch receptors and ligands confirm that Jagged1 is expressed in the locations and at the times expected for a gene that contributes to the normal development of the organs affected in AGS (heart, liver, skeleton, kidney) [32–36]. This is also supported by studies in the mouse, designed to test the consequences of loss of *Jag1* or *Notch2* [37–39]. To date, mutations in seven other Notch signaling pathway genes have been found to cause human disease (Table 7.1). Mutations in *NOTCH1* are associated with congenital cardiac disease [40] and T-cell neoplasms [41]. Mutations in *NOTCH2* have recently been demonstrated in two families with clinical features of AGS [12]. In addition, *NOTCH3* mutations cause CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), which is characterized by stroke and early dementia, with onset in fourth or fifth decade of life [42]. The autosomal recessive disorder spondylocostal dysostosis has been shown to be caused by three different genes in the Notch signaling pathway: Delta-like 3 (*DLL3*) [43], mesoderm posterior 2 (*MESP2*) [44], and lunatic fringe (*LFNG*) [45].

Table 7.1
***NOTCH* signaling mutations in human (constitutional) disease**

Presenilin	Alzheimer's disease
NOTCH1	Aortic valve disease, T-cell neoplasms
NOTCH2	Alagille syndrome
NOTCH3	CADASIL[a]
DLL3, *MESP2*, *LFNG*	Spondylocostal dysostosis
JAG1	Alagille syndrome

[a]CADASIL, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

GENOTYPE–PHENOTYPE CORRELATIONS

Although the AGS phenotype is highly variable, there is no apparent correlation with *JAG1* genotype in the majority of patients. There is

extreme variability of AGS phenotype within families, suggesting that other genetic or environmental factors significantly contribute to the clinical manifestations of the disease. A study of 53 *JAG1* mutation-positive relatives of a cohort of AGS probands demonstrated that only 53% met the clinical criteria for a diagnosis of AGS [8]. This included 11 of 53 with clinical features that would have led to a diagnosis of AGS and 17 of 53 (32%) who had mild features that would have been apparent only on targeted evaluation following the diagnosis of a proband in their family (i.e., discovery of elevation of liver enzymes or posterior embryotoxon in an asymptomatic individual). This study was done in an attempt to select a group of mutation carriers based on criteria other than a diagnosis of AGS and the frequency of clinical findings stands in contrast to those reported in patients with a clinical diagnosis of AGS, where clinical features are apparent, by definition [8].

The observation that relatives of probands with AGS often have only a partial manifestation of AGS led to the hypothesis that some patients with a *JAG1* mutation will present with apparently isolated heart disease, in the absence of a family history of AGS. Multiple patients that fit these criteria have now been reported [46–48]. In each of these reports, mutations in the gene were identified that included gene deletion and protein-truncating mutations [46], and missense mutations [47, 48]. These patients most likely represent one part of the spectrum for clinical consequences of *JAG1* mutations. One exception to this pattern is a relatively large family with cardiac disease in 14 individuals, which segregated with a *JAG1* missense mutation (G274D) [47]. This family is remarkable in that none of the mutation-positive individuals was reported to have hepatic features of AGS. Previously reported expression and functional studies of this mutant have demonstrated that some of the G274D protein molecules are normally processed and transported to the cell surface where they function appropriately, while some of them are incorrectly processed and transported [49, 50]. The missense mutation C234Y was also found to be segregating with congenital heart defects (tetralogy of Fallot, ventricular septal defects, and peripheral pulmonic stenosis), deafness, and posterior embryotoxon in a single family with eight affected individuals [48]. None of the affected individuals in this family had any evidence of hepatic dysfunction. Results of functional and expression studies have not yet been reported for this mutation. These results suggest that while haploinsufficiency of *JAG1* is associated with the well-characterized phenotype of AGS, the "leaky" G274D mutant, which allows more Jagged1 protein to reach the cell surface, is associated with a cardiac-specific phenotype. This is the first *JAG1* mutation identified with a phenotypic correlation; however, no additional studies have substantiated this correlation and there

is little other evidence to support genotype–phenotype correlations in AGS.

DISEASE MECHANISM IN ALAGILLE SYNDROME

Most *JAG1* mutations (70%) lead to a premature termination codon and two pathogenic mechanisms have been proposed to induce disease in AGS – haploinsufficiency or a dominant-negative effect of putative truncated proteins. Under a model of haploinsufficiency, an alteration in one of the *JAG1* genes leads to a complete lack of product or a severely defective product, resulting in insufficient protein. This mechanism is supported by several observations. The fact that large deletions of 20p12, which include the entire *JAG1* gene, cause AGS is good evidence that some cases of AGS are caused by haploinsufficiency of *JAG1*. In addition, there are no genotype–phenotype correlations in AGS and patients with a deletion of *JAG1* are indistinguishable from those with a protein-truncating or missense mutation. Studies of certain *JAG1* missense mutations (R184H, L37S, and G274D) reveal that they generate proteins which are abnormally glycosylated and do not reach the cell surface [49, 50]. Finally, in patients with nonsense or frameshift mutations, most of the transcripts containing premature termination codons may be degraded by the nonsense-mediated mRNA decay pathway.

A dominant-negative effect is also a potential mechanism for a dominant disorder. In this case, mutant protein antagonizes the activity of the remaining wild-type protein so that normal function of the gene is obliterated. It has been shown that some mutant transcripts can escape nonsense-mediated decay and then lead to the synthesis of truncated mutant proteins [27]. Furthermore, recent in vitro cell culture studies reveal that abnormal Jagged1 proteins (resulting from missense or premature termination codons) can be translated, and, following cleavage, the extracellular domain shed exerts biologic function by inhibiting Notch signaling [31]. It should be noted, however, that wild-type Jagged1 also had similar inhibitory effect, leading to speculation that this may represent an innate regulatory mechanism rather than a true dominant-negative effect.

In summary, most cases of AGS result from premature termination codons and there is substantial evidence to support a haploinsufficiency mechanism in most cases. However, it is apparent from in vitro studies that AGS mutations can encode mutant proteins, which have biologic activity, thereby supporting a dominant-negative mechanism in some cases, though further studies are required to confirm this.

JAGGED1 EXPRESSION IN ALAGILLE SYNDROME

JAG1 expression during normal embryogenesis is widespread in different organ systems and overlaps with the clinical manifestations of AGS. In situ hybridization studies have revealed *JAG1* expression in the cardiovascular system, renal tubules, eyes, the inner ear, pharyngeal arches, the mesenchyme of limb buds, and the developing nervous system (Figs. 7.4 and 7.5) [32, 33, 35]. The main site of *JAG1* expression is the cardiovascular system, specifically arterial structures and the developing cardiac outflow tracts. Of interest, *JAG1* expression in the liver also occurs in developing blood vessels, in the vascular endothelium of the hepatic arteries and portal veins [32, 33, 36, 51]. This has led to the speculation that the paucity of interlobular bile ducts in AGS may be secondary to underlying vasculopathy. However, immunohistochemical techniques have also demonstrated hepatic *JAG1* expression in the ductal plate [52]. Several studies have also shown that *Jag1* is expressed in portal mesenchyme adjacent to the ductal plate [39, 53]. The patterns of *Jag1* expression in the developing liver have been studied extensively, but are still somewhat controversial. The cell types involved in Notch pathway interactions in the developing liver are as yet unclear. In the adult liver, in contrast to embryonic stages, *Jag1* is expressed in bile ducts, blood vessels, and hepatocytes [51, 54], indicating that the role of *Jag1* may change during adulthood.

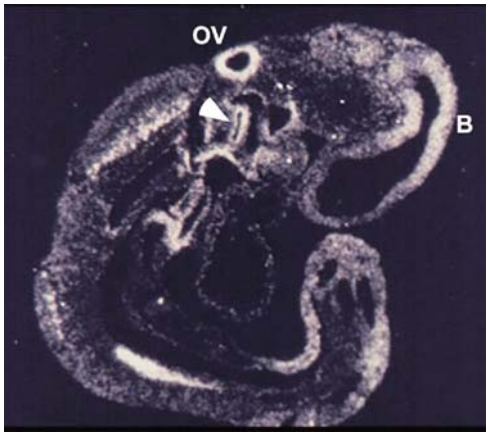

Fig. 7.4. *Jag1* expression in a mouse embryo at E9.5. In situ hybridization shows widespread expression of *Jag1* at E9.5 in a mouse embryo. Specifically, *Jag1* is strongly expressed in the otic vesicle (OV), the brain (B), and branchial arches (*arrowhead*).

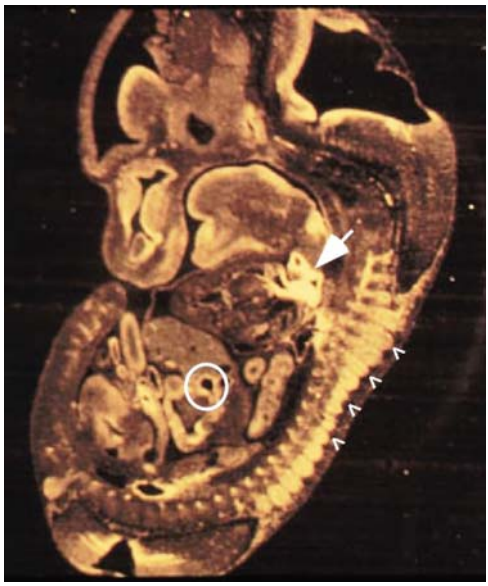

Fig. 7.5. *Jag1* expression in a mouse embryo at E12.5. In situ hybridization shows widespread expression of *Jag1* at E12.5 in a mouse embryo. Specifically, *Jag1* is strongly expressed in the cardiac outflow tract (*arrow*), the vertebral column (*small arrowheads*), and hepatic blood vessels (*circle*), correlating with the clinical phenotype in Alagille syndrome.

IDENTIFICATION OF *NOTCH2*

As described above, mutations in *JAG1* are identified in 94% of individuals with clinically defined AGS and *JAG1* haploinsufficiency is the likely primary mechanism of disease [11]. Total gene deletions account for 3–5% of these cases [15, 22]. The homozygote *Jag1* knockout mouse is embryonically lethal, and surprisingly, the *Jag1* knockout heterozygote mouse does not phenocopy AGS. The *Jag1* heterozygous mouse exhibits eye defects (isolated iris coloboma) and inner ear defects but no other phenotypic features of AGS (Table 7.2). Interestingly, a *Jag1/Notch2* double heterozygote (*Jag1* null allele and a *Notch2* hypomorphic allele) was found to have liver, cardiac, ocular, and renal manifestations similar to those seen in AGS patients [39]. Additionally, the spatial and temporal expression pattern of *Notch2* in tissues involved in AGS made it an excellent candidate to be the Jagged1 interacting receptor [38, 39].

These data led to the screening of a cohort of *JAG1* mutation-negative AGS patients for alterations in *NOTCH2*. To date, only two families

Table 7.2
Selected notch pathway mutant mouse models

Genotype	*Phenotype*
$Jag1^{+/-}$	Mild anterior chamber defects and inner ear defects; no liver disease [37]
$Jag1^{-/-}$	Lethal at E10.5 due to vascular defects [37]
Liver-specific conditional/null $Jag1^{F/-}$ *AlfpCre*	Increased bile duct numbers and irregular bile duct structures around portal tracts in adult mice [58]
$Jag1^{+/-}$, $Notch2^{+/-}$	Cholestasis, bile duct paucity, pulmonary artery hypoplasia, renal anomalies, eye anomalies [39]
$Notch2^{-/-}$ (null)	Lethal at E11.5 due to increased cell death [55]
$Notch2^{del1/del1}$ (hypomorphic)	Lethal at birth due to glomerular hypoplasia; also exhibit bile duct paucity and myocardial hypoplasia [38]
Liver-specific conditional $Notch2^{F/F}$ *AlbCre*	Failure of ductal plate remodeling, irregular ductal structures, progressive portal inflammation, and fibrosis [59]
$Hes^{-/-}$	Hypoplastic extrahepatic biliary tree, conversion of extrahepatic bile duct to pancreatic tissue [56], failure of ductal plate remodeling [53]

(five individuals) have been reported with a *NOTCH2* mutation [12] and one additional family (five individuals) has been identified (unpublished data, personal communication, Spinner 2008). The initial report suggested a prominent renal phenotype in *NOTCH2* mutation carriers but this has not yet been validated as insufficient affected individuals have been identified.

The NOTCH2 protein, like the other Notch receptors, is a single-pass transmembrane protein. The *NOTCH2* gene is made up of 34 exons, occupying 158,099 base pairs of genomic DNA, which codes for an

11,433-base-pair message. Screening of this gene for mutations in AGS patients has been accomplished by sequencing of the genomic DNA.

ROLE OF *JAGGED1/NOTCH* SEQUENCE IN BILE DUCT DEVELOPMENT

The identification of *JAG1* as the disease gene in AGS and the classic histologic finding of bile duct paucity have led to interest in the role of the Notch signaling pathway in intrahepatic bile duct development. Mutant mouse models have been particularly important in studying this area (Table 7.2). The doubly heterozygous *Jag1* null/*Notch2* hypomorphic mouse has bile duct paucity and a few biliary cells adjacent to the portal vein. Similar findings are seen in mice homozygous for the *Notch2* hypomorphic allele. This supports a role for *JAG1* and *NOTCH2* in bile duct development, but the mechanism of this role is only just beginning to be revealed.

In normal embryonic bile duct development, hepatoblasts (hepatic progenitor cells) are bipotential, i.e., those in the parenchyma become hepatocytes and those adjacent to portal veins differentiate into biliary cells [57]. Prior to birth, these form a single layer around portal vein branches and become the ductal plate. The ductal plate becomes bilayered and certain areas give rise to tubular structures, which form mature ducts. The remainder of the ductal plate disappears in the first few weeks postnatally. The exact role of Notch signaling in this process has been postulated to be involved in cell fate specification of biliary cells or the development of ducts from the ductal plate. In the *Jag1/Notch2* doubly heterozygous mouse and the liver-specific *Notch2* conditional knockout, the ductal plate forms normally but the ductal plate remnants are present postnatally (unlike in controls) [58]. In separate work, liver-specific inactivation of *Notch2* (but not *Notch1*) in mice resulted in the presence of biliary cells but irregular ductal plate structures and deranged postnatal development of bile ducts with associated portal inflammation and fibrosis [59]. Both groups concluded that *Notch2* is required for bile duct formation and morphogenesis but is not necessary for biliary cell specification from bipotential hepatoblasts [58, 59]. It appears that the role of Notch signaling is in remodeling of the ductal plate into mature bile ducts late in gestation and in the early postnatal period. In further support of this notion, earlier data demonstrate that *Hes1*-deficient mice developed normal ductal plates but these did not give rise to normal tubular structures [53]. Summarizing this data would suggest a model of bile duct development in which *JAG1* activates *NOTCH2* and downstream activation of *HES1* in the ductal plate induces duct formation.

Zong and colleagues [60] have extensively studied temporal and spatial roles of Notch signaling in mice models. They demonstrated that Notch signaling plays a role in the differentiation of the second biliary layer where it regulates tubulogenesis. Furthermore it appears that Notch signaling maintains biliary epithelial cells postnatally and can drive mature hepatocytes toward a biliary fate. These data will help better explain the role of the Notch signaling pathway in normal development and also in the pathogenesis of bile duct paucity in AGS.

The role of *Jag1* in liver and bile duct development has also been investigated in a mouse model. Of note, mice carrying a *Jag1* mutation targeted to hepatoblasts had no significant phenotype, and the liver and bile ducts were normal up to 1 year of age [61]. These findings indicate that although *Jag1* expression has been identified in the ductal plate in some studies, hepatoblast *Jag1* expression is not crucial for normal bile duct development. Mice doubly heterozygous for a *Jag1* null allele and a liver-specific *Jag1* conditional allele are remarkable for normal intrahepatic bile duct development in the embryonic period; however, in early adulthood, a subset of these animals develop a striking phenotype of bile duct proliferation [61]. The hepatic phenotype in these animals is

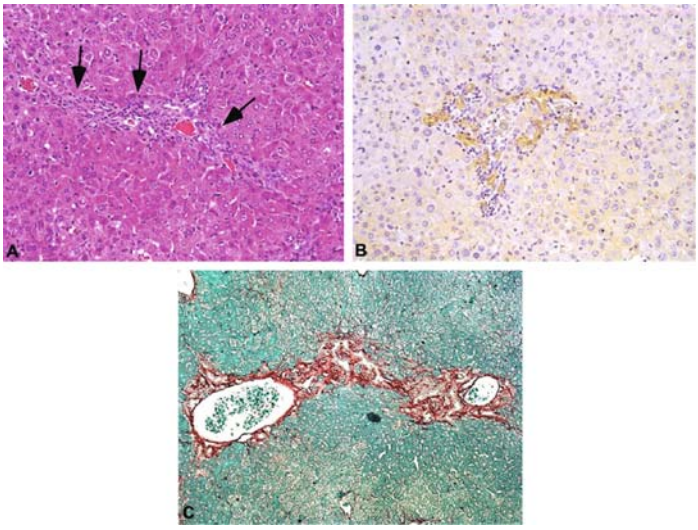

Fig. 7.6. Bile duct phenotype in *Jag1* conditional/null adult mice. Mice heterozygous for a null mutation in *Jag1* and a liver-specific conditional *Jag1* mutation show increased numbers of irregular bile ducts around portal tracts at 4 weeks of age (**a**, hematoxylin and eosin stain, *arrows*; **b**, cytokeratin 19 stain). Sirius *red stain* shows increased fibrosis and portal expansion (**c**).

characterized by the early onset of fibrosis and disorganized bile ducts surrounding portal tracts (Fig. 7.6). These studies point to an important role of the Notch pathway in postnatal bile duct growth and remodeling.

ROLE OF *JAGGED1/NOTCH* SEQUENCE IN CARDIAC DEVELOPMENT

A full discussion of cardiac development is beyond the scope of this review; however, as would be expected on the basis of significant cardiac involvement in AGS, the Notch signaling pathway has a key role in this process [62]. The heart is the first organ to develop during embryogenesis and the early myocardium is derived from the primary heart field, a region of mesoderm. As the heart tube develops, some cells within this field develop into cardiomyocytes and several studies suggest that Notch signaling inhibits this cardiac cell fate specification [63]. Notch signaling also appears to influence atrioventricular canal and valvular development as well as ventricular trabeculation [62]. Specifically Notch activation in endothelial cells is required for epithelial to mesenchymal transition, which is fundamental to the formation of endocardial cushions and valves.

The importance of Notch signaling in cardiac development has been substantiated by the finding that *NOTCH1* mutations are associated with hereditary structural abnormalities of the aortic valve, such as bicuspid aortic valve [40]. In AGS, the typical cardiac lesions are right sided, involving the outflow tract. Since the *Jag1/Notch2* mouse has a similar phenotype, this ligand–receptor pair is implicated in cardiac outflow tract development. The importance of the Notch signaling pathway in this process is further underscored by the analysis of presenilin knockout mice which also display pulmonary artery stenosis, ventricular septal defects, and double-outlet right ventricle [64]. It appears that Notch signaling in the cardiac neural crest is pivotal in inducing cell fate decisions, and Notch inhibition in this area leads to an AGS-like right-sided cardiac lesions [65]. Therefore, a possible mechanism for cardiac disease in AGS would be that *JAG1* haploinsufficiency leads to reduced Notch activity in embryogenesis, insufficient smooth muscle development and stenosis of blood vessels such as the pulmonary artery, and others.

ROLE OF *JAGGED1/NOTCH* SEQUENCE IN VASCULAR DEVELOPMENT

Vascular involvement in AGS is emerging as an important clinical feature, often manifested by devastating consequences. Beyond the

classic peripheral pulmonary artery stenosis, intracranial bleeding is a known complication with a prevalence of 15% [4, 5]. Some of these events have been associated with the presence of documented structural vessel abnormalities. Furthermore, review of a large cohort of individuals with AGS revealed that 9% had a non-cardiac vascular anomaly or significant vascular event [66]. In fact, vascular abnormalities accounted for 34% of the mortality in this AGS cohort. Thus there is clinical evidence of widespread vasculopathy in AGS. Review of the recent literature reveals a body of work, implicating the Notch signaling pathway both in embryonic blood vessel development and tumor angiogenesis.

It is clear that Notch signaling is required for arterial cell fate differentiation [67, 68] and that two Notch ligands Jagged1 and Delta-like 4 are expressed prominently in the vasculature. It has been well established in mice models that Delta-like 4 is required for vascular development and is the critical ligand signaling between endothelial cells [69–71]. Recent data from zebrafish and mouse retina suggest that inhibition of Notch signaling leads to increased branching and sprouting of blood vessels and this is mediated via Delta-like 4/Notch signaling [72, 73]. The mechanism of this effect is believed to be via influence on the formation of "tip cells" which are specialized endothelial cells at the leading edge of sprouting vessels. Inhibition of Notch signaling leads to increased numbers of tip cells and vessel branching, though the new vessels function poorly. Vascular endothelial growth factor (VEGF) promotes Notch signaling in this model and thereby suppresses the formation of tip cells and inhibits angiogenesis. Thus both Notch signaling and VEGF are integral to normal blood vessel development and it is conceivable that disordered Notch signaling, as in AGS, could result in vascular anomalies.

Jagged1 is well expressed in developing arterial structures [35]. Mice homozygous for a *Jag1* null mutation die early in embryogenesis of yolk sac and vasculature defects [37]. It has recently been demonstrated in mice that endothelial-specific deletion of *Jag1* results in embryonic lethality and a vascular phenotype similar to that described in the *Jag1* knockout [74]. Furthermore, the expression of vascular smooth muscle markers is diminished in the endothelial-specific *Jag1* knockouts, suggesting that endothelial *JAG1* modulates the differentiation of adjacent vascular smooth muscle and thus is required for embryonic blood vessel development. In an AGS model of haploinsufficiency where there is diminished *JAG1* expression, it is suggested that this results in abnormal vascular smooth muscle development, which may be implicated in the development of vascular anomalies. This could

account for the pulmonary artery anomalies and other extra-cardiac vessel abnormalities.

GENETIC TESTING IN ALAGILLE SYNDROME

Molecular analysis of the *JAG1* and *NOTCH2* genes is available to screen for mutations associated with AGS. An evaluation of deletions including the *JAG1* gene by fluorescence in situ hybridization (FISH) will identify these deletions in less than 7% of patients. Molecular testing is now available in several centers for *JAG1*, and hopefully availability of *NOTCH2* sequencing will follow. The most straightforward approach is direct sequencing of the coding region of these genes, as recent data shows that this approach identifies mutations in 94% of patients [11]. Once a *JAG1* mutation is identified in a proband, it is relatively simple to test parents and other relatives for the identified mutation. Mutations are inherited from an affected parent in 30–50% of patients, while the mutations appear de novo in 50–70% [6, 19, 25]. If a parental mutation is identified, there is a 50% risk for each future offspring to inherit the *JAG1* mutation. However, it should be emphasized that expressivity of the disorder is highly variable, and it is not currently possible to predict disease severity. If no parental mutation is identified, then the recurrence risk is limited to the chance of germline mosaicism, which for multiple different disorders is estimated at 1–3%. There have also been cases of parental somatic mosaicism observed in apparently unaffected individuals [19, 75]. Prenatal testing can also be carried out in a family once the proband's mutation is identified. Testing can help to reassure parents of children with de novo mutations who may be concerned about germline mosaicism. Testing has also aided in the diagnosis of AGS for patients with minor or atypical manifestations, and has expanded the spectrum of the AGS phenotype.

SUMMARY

AGS is a fascinating multi-system disorder caused by mutations in *JAG1* and in a minority of cases, *NOTCH2,* both integral members of the Notch signaling pathway. The involvement of the different organ systems has led to significant insights into the role of this pathway in normal human development and disease states. The study of Notch signaling in tumor angiogenesis has already led to the development of potential therapeutic agents, which target Notch members. This opens the door for manipulation of the *JAG1/NOTCH* sequence, which one day may have a therapeutic role in AGS, perhaps by maintaining postnatal bile duct development and alleviating cholestasis.

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8 Biliary Atresia and the Ductal Plate

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CONTENTS

Summary

Biliary atresia (BA) is a unique neonatal disease, presenting with complete obstruction of the extrahepatic biliary tree within the first 3 months of life. Current therapy requires a hepatic portoenterostomy within the first months of life; however, progressive intrahepatic bile duct injury, sclerosis, and obstruction lead to cirrhosis and the need for liver transplantation in the majority of patients. Medical treatments are inadequate largely because the pathogenesis of BA is poorly understood. Two major forms of BA have been described. The *embryonic* form is associated with other congenital malformations

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and presumably represents failure of normal bile duct formation. Mutations or epigenetic modifications in genes involving laterality and bile duct morphogenesis or ductal plate regression are proposed to be involved. The *perinatal* form is not associated with other malformations and is proposed to be caused by an immune or an autoimmune response to a perinatal insult (e.g., cholangiotropic viral infection). There is conflicting evidence for the role of several viruses, although the mouse rhesus rotavirus model of BA makes human rotavirus and reovirus intriguing candidates. Future investigations in coming years are anticipated to unlock the mysteries surrounding the etiology of BA with the promise of new therapeutic strategies and potential prevention.

Key Words: Reovirus, Rotavirus, Cholangiocyte, Autoimmunity

Abbreviations

APC	antigen-presenting cell
BA	biliary atresia
CD	clusters of differentiation
CMV	cytomegalovirus
HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
INV	inversin
LBW	low birth weight
MHC	major histocompatibility complex
OR	odds ratio
RNA	ribonucleic acid
RR	risk ratio
RT-PCR	reverse transcriptase-polymerase chain reaction
SGA	small for gestational age
TNF	tumor necrosis factor

BILIARY ATRESIA – CLINICAL PERSPECTIVE

Biliary atresia (BA) is a progressive, inflammatory obstructive cholangiopathy affecting both the extrahepatic and the intrahepatic bile ducts with clinical onset restricted to the first three months of life. BA is the most common cause of neonatal cholestasis, accounting for 30–40% of all cases, occurring in 1 in 8,000 to 1 in 18,000 live births. BA typically presents with jaundice, acholic stools, dark urine, and hepatomegaly in an otherwise apparently healthy infant [1]. Conjugated hyperbilirubinemia, elevated aminotransferases, alkaline phosphatase, and γ-glutamyl

transpeptidase (GGT) are characteristics. Without surgical correction or liver transplantation, BA progresses to biliary cirrhosis and is uniformly fatal within 2–3 years of life. If diagnosed in the first 2–3 months of life, hepatic portoenterostomy (HPE; the Kasai procedure) will restore bile flow from the liver into the intestinal tract in 30–80% of patients [1–3]. Recent analyses of large cohorts of patients suggest that the best HPE outcomes are achieved if surgery is performed before 45 days of age at a center that performs at least five HPEs per year [4]. After 90 days of life, surgical outcomes fall off dramatically such that after 120 days, most patients are spared HPE surgery and proceed directly to liver transplant. However, despite successful HPE surgery, continued inflammation and progressive fibrosis of intrahepatic bile ducts develop frequently, culminating in biliary cirrhosis and end-stage liver disease requiring liver transplantation in up to 70% of patients by age 20 years. Because of late diagnosis or unsuccessful HPE surgery, approximately 40–50% of BA patients undergo liver transplantation by age 2 years at many centers [5–7]. BA remains the most common indication for pediatric liver transplantation and accounts for 40–50% of such transplants. Based on these humbling survival statistics, there exists a vast opportunity to improve the transplant-free survival of children with BA. Achieving this goal will require a more thorough understanding of the etiology and pathogenesis of BA and the development and implementation of effective measures to establish the diagnosis at the youngest possible age. Because BA is such a rare disease, making substantial progress in these arenas requires multi-centered collaboration in clinical and translational research. Efforts are currently underway in the United States, Europe, Japan, Taiwan, and other countries to better understand BA, establish tissue and DNA repositories and patient registries, and conduct controlled clinical trials of screening methodologies and promising treatments.

PATHOGENESIS OF BILIARY ATRESIA

BA is now regarded as a clinical phenotype that results from several putative pathogenic processes that can be segregated into at least two forms [1, 8]. Approximately 10–20% of BA cases have been referred to as the *embryonic* or the fetal form in which defective morphogenesis of the extrahepatic bile duct is believed to be responsible. Presumed germline or somatic mutations in genes or epigenetic factors that regulate bile duct development or remodeling lead to atresia at an early stage of bile duct embryogenesis, with complete bile duct obstruction being present at birth. This form of BA is frequently associated with other congenital malformations that have their origin in the first trimester,

many involving right–left axis differentiation (laterality genes) [1]. Other patients have only a single major congenital malformation unassociated with laterality defects. Defective remodeling of the ductal plate has also been proposed in this form. These infants frequently present at birth with early onset of jaundice, conjugated hyperbilirubinemia, and acholic stools. Outcome from the HPE is poorer compared to other

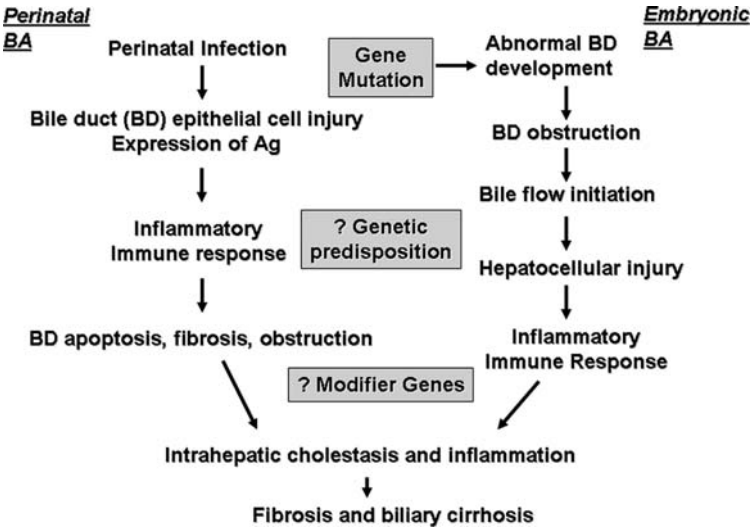

Fig. 8.1. Proposed pathways for pathogenesis of two forms of biliary atresia (BA). *Perinatal BA* may develop when a perinatal insult, such as a cholangiotropic viral infection, triggers bile duct (BD) epithelial cell injury and exposure of self- or neo-antigens which elicit a subsequent immune response. The resulting inflammation induces apoptosis and necrosis of extrahepatic BD epithelium, resulting in fibro-obliteration of the lumen and obstruction of the BD. Intrahepatic bile ducts may also be targets in the ongoing TH1 immune (?autoimmune) attack and the cholestatic injury, resulting in progressive portal fibrosis culminating in biliary cirrhosis. *Embryonic BA* may be the result of mutations in genes controlling normal bile duct formation or differentiation, which secondarily induces an inflammatory/immune response within the common bile duct and liver following the initiation of bile flow at approximately 11–13 weeks of gestation. Secondary hepatocyte and intrahepatic bile duct injuries ensue either as a result of cholestatic injury or as targets for the immune (?autoimmune) response that develops. The end result is intrahepatic cholestasis and portal tract fibrosis, culminating in biliary cirrhosis. Other major factors may be the role played by genetic predisposition to autoimmunity and modifier genes that determine the extent and type of cellular and immune response and the generation of fibrosis.

forms of BA. This form of BA is less common among Asian populations. Another 80% of BA cases have been labeled the *perinatal* or the acquired form, presuming that the pathologic trigger is acquired toward the end of pregnancy or early in life. It is believed that a patent biliary system present at birth undergoes an initial insult (perhaps viral infection) that injures bile duct epithelia, triggering a progressive inflammatory, autoimmune fibro-obliterative process. Proposed triggers include infectious, toxic, or vascular factors that impose biliary injury in a genetically susceptible individual. The two different pathologic pathways leading to these two major phenotypic forms of BA are illustrated in Fig. 8.1. In this review, recent advances that support these proposed pathogenic processes will be examined and updated.

DEFECTIVE MORPHOGENESIS AND BILIARY ATRESIA

Embryogenesis and Visceral Isomerism

The embryonic form of BA has been assumed to be caused by abnormal development of the biliary tree. Supporting this is the observed strong association of visceral organ asymmetry with BA (the polysplenia or BA splenic malformation syndrome; BASM), implying that genes that control normal situs development also regulate normal extrahepatic bile duct development. The BASM clinical phenotype is characterized by distinctive anatomic features that may be present in varying combinations in the presence of polysplenia, a double spleen, or asplenia. These malformations include a midline liver, intestinal malrotation, preduodenal portal vein, interrupted IVC with azygous continuation, situs inversus, and congenital heart disease. Reports of bile duct abnormalities in animal models of situs further support this pathogenetic link. The autosomal recessive deletion of the mouse inversion (*inv*) gene has been associated with both laterality defects in abdominal organ placement and anomalous development of the hepatobiliary system [9, 10]. This led to mapping of the human *INV* gene, which permitted evaluation of 64 patients with heterotaxia [11]. Unfortunately, no mutations were found in seven patients with BA and laterality defects, militating against the involvement of the *INV* gene in embryonic cases of BA. Interestingly, recessive mutations in *INV* were recently described in children with nephronophthisis type 2 [12], a disease in which perturbed biliary development (including ductal plate abnormalities) has been described [13]. Moreover, the inversin protein was observed to interact with nephrocystin within the primary cilia of renal tubular cells [12]. Conversely, gene products localized to the mechanosensory primary cilium of cholangiocytes could potentially produce aberrant

biliary development with predominance of ductal plate malformation. In this regard, there is a subset of patients with BA with portal tract histology resembling that of a ductal plate pattern and who are believed to have a worse prognosis [14]. It remains to be seen if mutations or polymorphisms of genes that code for proteins regulating critical functions of the primary cilia will play a role in the pathogenesis of BA.

More than 30 other mammalian proteins are involved in the establishment of left–right patterning during embryogenesis. Several have been examined in BA patients. Bamford et al. analyzed the *CFC1* gene (which encodes the human CRYPTIC protein) in genomic DNA from 144 cases of familial and sporadic cases of laterality defects [15]. Heterozygous mutations were identified in nine patients, including one with BA and the polysplenia syndrome. Jacquemin et al. subsequently found heterozygous gene mutations in two brothers with laterality defects, one of whom had BA, which were inherited from their unaffected mother [16]. More recently, this group of investigators genotyped the six *CFC1* exons and intron–exon boundaries in 10 additional patients with BASM and found a c.443G>A heterozygous mutation in five patients, leading to an Ala145Thr amino acid substitution [17]. This mutation was also detected in 18 of 144 chromosomes from healthy individuals, thus it appears to be twice as common in these BASM patients. Although heterozygous *CFC1* mutations are believed to predispose to BA, it also appears to require a second genetic or environmental factor to result in the BA phenotype. The CRYPTIC protein acts as a cofactor in the nodal pathway that determines left–right axis development potentially through its function in the transforming growth factor beta signaling pathway [16]. More work on this interesting gene and signaling pathway in BA are clearly indicated.

Another laterality gene that has received attention in BA is *ZIC3*, which encodes a zinc finger transcription factor that translocates to the nucleus and influences left–right axis determination. In 2004, Ware et al. reported eight *ZIC3* mutations in 194 heterotaxia patient samples and calculated that mutations in this gene were responsible for approximately 1% of patients with sporadic heterotaxy [17]. Among the patients with complex congenital heart disease and *ZIC3* mutations, two had BA, one had abnormal liver lobulation, and one had cholelithiasis and cholecystitis. Based on this study, *ZIC3* appears to be a promising candidate gene for BASM in isolated cases of BA with heterotaxia. Evaluation of *ZIC3* in a larger population of patients with BA is needed to determine its overall role. Other human genes that determine laterality *(LEFTYA* and *ACVR2B*) and cell adhesion (*CRELD1* and *NKX2.5*) have been associated with a small percentage of human situs defects [77]. Moreover, Zhang et al. reported no differences in mRNA

expression of a panel of laterality genes in liver from embryonic vs. perinatal cases of BA [18]. Genotyping of laterality genes and regulatory transcription factors, evaluation of DNA copy number variations, and assessment of epigenetic factors will be essential to determine the role of inherited or somatic mutations/deletions/copy number variations in the pathogenesis of embryonic cases of BA.

BA and Other Major Congenital Malformations

Although the association of BA is strongest with BASM, an equal number of BA cases have one or more other major congenital malformations but with no evidence of BASM. Two studies have now demonstrated that approximately 10% of BA cases fall into this latter group [19]. Included in this group are cardiac, gastrointestinal, renal, and genitourinary malformations. In the BA Research Consortium (BARC) study, BA cases with non-BASM malformations comprised 11% of 149 BA cases.

Intrahepatic Bile Duct Development and the Ductal Plate Malformation

Normal embryologic development of intrahepatic bile ducts is controlled by interactions between hepatic mesenchyme and portal venous radicals, inducing the formation of the ductal plate. The ductal plate, derived from a subset of hepatoblasts that differentiate into a single-layered ring of biliary precursor cells, surrounds terminal portal venous branches within the liver and their encompassing mesenchyme (reviewed in [20, 21]) and is present by 5 weeks of gestation. During the ensuing 6–7 weeks, this single layer of cells becomes partly bi-layered and is further remodeled into focal dilated areas between the two cell layers, soon giving rise to the portal tract bile ducts. Abnormal or failed remodeling of the ductal plate produces the ductal plate malformation lesion that is characteristic of congenital hepatic fibrosis/autosomal recessive polycystic kidney disease and other bile duct dysplasias. Of interest, a subgroup of infants with BA have been described with some evidence of the ductal plate malformation on liver biopsy, but not the full blown ductal plate malformation lesion of congenital hepatic fibrosis [14]. This lesion is not easily recognizable by all pathologists, raising the question of whether the immature liver in BA remodels in a ductal plate pattern in a subset of patients. At least one study suggests that those BA infants with the ductal plate pattern have a worse prognosis, suggesting that their phenotype is different. Further studies will be needed to identify genetic or environmental factors that

might predispose to this lesion and determine if it carries prognostic significance.

A growing family of genes has been shown to regulate development, differentiation, and regression of bile duct elements during fetal life. Polymorphisms in *HNF6, HNF1-β, JAGGED1, PKDH1*, or other genes that regulate remodeling of the ductal plate could act as susceptibility factors or modifier genes necessary, but not sufficient, for the development of BA [22–24]. In this regard, Kohsaka et al. identified *JAGGED1* (the gene causing Alagille syndrome) missense mutations in 9 of 102 Japanese patients with BA, all of whom had no phenotypic features of Alagille syndrome [25]. Prognosis was worse in the group with mutations, suggesting that the presence of abnormal *JAGGED1* signaling could be a modifying factor in BA. Unfortunately, other factors known to influence prognosis in BA were not described in this report, such as age at the time of portoenterostomy. Thus it is not clear if these patients had an unusual form of BA caused by mutations in *JAGGED1*, if the phenotype reported was an unusual presentation of Alagille syndrome, or if the gene functioned as a modifying factor in BA. These provocative observations remain to be confirmed.

The pathway by which a defect in ductal plate remodeling could lead to atresia of the *extrahepatic* biliary tree is not intuitively obvious. The work of Tan and colleagues may provide an explanation for this paradox. Tan et al. have reported the presence of extravasated bile in bile duct remnants of BA patients and have proposed that this bile may have originated from breaches in bile duct mucosa at the porta hepatis [26]. This group proposes that there exists a stage of human biliary development between 11 and 13 weeks of gestation during which failure of complete remodeling of the ductal plate structures could lead to abnormal development of the mesenchymal cuff that surrounds developing hilar bile ducts. Alternatively, failure to establish normal connections of the hepatic ducts (which are derived from tubulization of the ductal plate structures) to the extrahepatic bile ducts could produce this breach in ductal integrity. Either of these alterations could potentially result in the rupture of hilar bile ducts in response to initiation of bile flow at 12–13 weeks of gestation. Extravasated bile, with its detergent properties, into adjacent periductal tissues would then lead to protracted inflammation and sclerosis in the submucosa, causing secondary obliteration and obstruction of the more distal extrahepatic bile duct. This hypothetical mechanism was first described by Rolleston and Hayne in 1901, who coined the term “descending cholangitis” to describe a similar process [27]. Although provocative, this postulated mechanism to explain atresia of the extrahepatic bile ducts has not been well accepted by many authorities.

IMMUNE AND AUTOIMMUNE MECHANISMS CAUSING BILE DUCT INJURY AND OBSTRUCTION

At the time of diagnosis of BA, a combination of sclerosis and lymphocytic inflammation characterize the extrahepatic bile duct remnant [28], while the portal tract infiltrate is composed of lymphocytes, macrophages, and eosinophils [29]. The potential roles of T-cell-mediated inflammation, innate immunity and Kupffer cell activation, and autoimmune processes have received the most attention and will be reviewed.

T-Cell-Mediated Inflammation

In general, there is a marked inflammatory infiltrate of T lymphocytes that surround and invade intrahepatic bile ducts, and may even lead to direct obstruction of the bile duct lumen [30]. A mixture of $CD4^+$ T cells [39–41] and $CD8^+$ T cells [31, 32] have been described in the portal infiltrates, which exhibit expression of lymphocyte activation (LFA-1, IL-2 receptor) and proliferation (transferrin receptor) markers [33, 34]. Bile duct injury can result from cytokines generated by activated effector T cells or by other immune pathways stimulated by T cells. It is now clear that a TH1 immune response is present in human liver and the rhesus rotavirus (RRV)-induced mouse model of BA. A TH1 cytokine repertoire composed of IL-12, IFN-γ, IL-2, and TNF-α has been identified in portal inflammatory cells of BA livers but not other neonatal cholestatic diseases by Mack et al. [32]. Generating similar results, Bezerra et al. used mRNA transcriptome microarrays to analyze liver biopsies from BA compared to those from intrahepatic cholestasis infants [35]. They identified upregulation of TH1 pro-inflammatory genes (including IFN-γ and osteopontin) and decreased expression of *TH2* genes in BA infants. In addition, they demonstrated in the RRV mouse model of BA that IFN-γ-knockout mice had increased survival and resolution of bile duct obstructive lesions compared to wild-type controls [36]. Whether polymorphisms in IFN-γ or other key cytokines are involved in determining human susceptibility to BA is still unknown. The potential for targeted therapies directed toward interrupting these inflammatory pathways requires further investigation.

Activation of T cells requires presentation of antigenic peptides by antigen-presenting cells (APCs). The role of professional APCs (macrophages, dendritic cells, and B cells) has been implicated by the presence of macrophages in the portal tracts of both human and murine BA. Endothelial cells and cholangiocytes have also been implicated in BA by Kobayashi et al., who reported increased expression of B7-1 and

B7-2 on intrahepatic bile duct epithelium and Kupffer cells in livers with more advanced lesions at presentation [37]. Amplified expression of ICAM on bile duct epithelium has also been well described in BA liver [33, 38, 39]. The role of dendritic cells as APCs in BA has not been reported.

Innate Immunity in BA

The intensity of the activated macrophage portal tract infiltrate in BA is associated with a worse prognosis [32, 40, 41]. Kobayashi et al. reported markedly increased numbers of portal tract macrophages at BA diagnosis in patients with absent bile flow following portoenterostomy compared to those with good bile flow [40]. Davenport et al. similarly reported that the intensity of macrophage activation correlated with poor outcome following portoenterostomy [33]. Thus, it can be proposed that macrophages (Kupffer cells) may be eliciting cytokines and chemokines that produce more serious injury to bile ducts that impair their ability to permit bile flow following portoenterostomy. In support of this hypothesis is an accumulating body of evidence.

Activated macrophages produce IL-12 and IL-18 which function together to promote Th1 cellular differentiation. Increased mRNA expression of both IL-12 and IL-18 has been reported in livers and serum, respectively, of BA patients at diagnosis [32, 42]. Macrophages secrete TNF-α and other cytotoxic cytokines which are markedly upregulated in BA [32]. TNF-α binding to cell death receptors may be partly responsible for the bile duct epithelial apoptosis or necrosis observed by Funaki et al., in almost 50% of intrahepatic cholangiocytes in BA liver compared to only 2.5–3.6% in control groups [43]. Another important cholangiocyte death pathway is triggered by the interaction of Fas ligand (FasL) with Fas-bearing cells. FasL is expressed predominantly on activated T cells and monocytes/macrophages. Liu et al. reported strong expression of Fas in 25% of BA samples and none of controls [44]. Moreover, aberrant FasL expression was found in cholangiocytes in approximately 30% of BA specimens as well as in the majority of portal tract monocytes, the former being associated with poor bile flow post-portoenterostomy.

Recent studies by Jafri et al. showed that cholangiocytes from the murine RRV BA model secreted the chemokines macrophage inflammatory protein 2, monocyte chemotactic protein 1, and others [45]. Moreover, an RRV-infected cholangiocyte cell line also produced these chemokines, suggesting that chemokine expression by infected bile duct epithelia may play a role in triggering the immune response that causes bile duct obstruction in BA. These data are consistent with

studies by Barnes et al., in which mouse cholangiocytes were demonstrated to express transcripts for many pro-inflammatory cytokines and chemokines [46]. This possible key role of cholangiocytes in mediating innate immune responses needs to be further explored in the pathogenesis of BA.

In summary, a hypothesis (Fig. 8.2) is put forth by which an initiating factor (e.g., viral, toxin, vascular) injures cholangiocytes in a unique window of time early in life leading to both cholangiocyte apoptosis and HLA-DR-restricted Kupffer cell (or bile duct epithelium) presentation of antigens (derived from virus or altered self-antigens on bile duct epithelia) to $CD4^+$ T cells. Effector Th1 cells and cytotoxic $CD8^+$ cells are generated that re-migrate back to the intrahepatic and extrahepatic bile ducts and locally release IL-2, IFN-γ and TNF-α. Chemokines and adhesion molecules released by cholangiocytes and vascular endothelial cells recruit local macrophages and neutrophils that then release TNF-α, nitric oxide, and other reactive oxygen species that produce further cholangiocyte apoptosis and necrosis, obstruction, and finally fibrotic luminal obliteration after the recruitment and activation of hepatic stellate cells.

Evidence for Autoimmunity in Biliary Atresia

Although the extrahepatic bile duct remnant is removed during surgical treatment of BA, intrahepatic bile duct injury continues in the majority of patients even when bile flow is established by Kasai portoenterostomy. This persisting and progressive cholangiopathy suggests that there is a failure to downregulate the inflammatory response described above [47, 48]. It has been proposed that a secondary autoimmune response develops following the initial triggering factor in the following manner. Once cholangiocyte cell death occurs, exposure of altered or previously sequestered self cholangiocyte antigens is recognized as "foreign" and elicits a persistent autoimmune-mediated bile duct injury. Molecular mimicry, in which virus antigens cross-react with self cholangiocyte antigens, is another pathway that could initiate such an immune response against both viral and self-antigens [48, 49].

Several lines of evidence are consistent with autoimmunity in BA. In a preliminary report, Vasiliauskas et al. reported serum IgG and IgM antineutrophil cytoplasmic antibodies (ANCAs) in 10 of 11 BA patients, with higher IgM-ANCA titers in BA compared to other liver diseases [50]. Burch et al. determined if maternal transfer of autoantibodies could be involved in BA pathogenesis by measuring lupus autoantibodies in mothers of children with BA and idiopathic neonatal

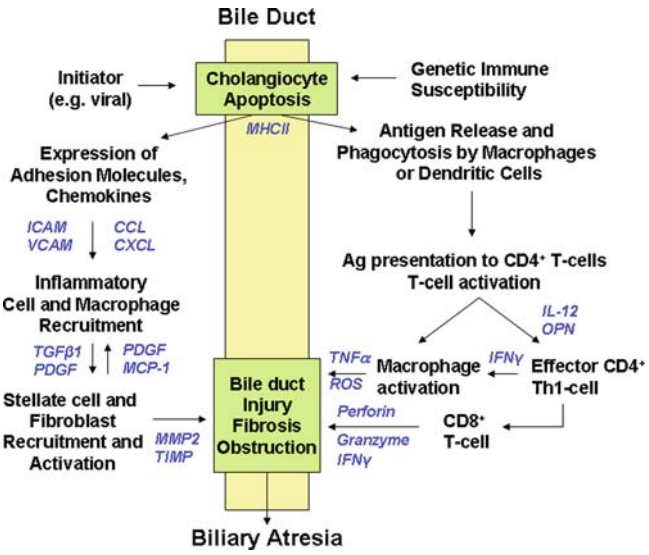

Fig. 8.2. More detailed proposed pathogenetic model of perinatal (acquired) biliary atresia, incorporating elements from human and murine model investigations. A perinatal initiating event (e.g., viral or other insult) triggers cholangiocyte apoptosis and aberrant MHC class II expression in extrahepatic and intrahepatic bile ducts in an immunologically susceptible host. Viral and/or native or altered bile duct antigens are phagocytosed by macrophages or dendritic cells and presented to naïve T cells in local lymph nodes in which virus/bile duct-specific $CD4^+$ T cells are activated and proliferate, stimulated by IL-2 (*right side of figure*). These activated $CD4^+$ T cells (which may be autoreactive) home back to the original site of antigen exposure and elicit T-cell effector functions, including IFN-γ-induced macrophage stimulation and activation of cytotoxic $CD8^+$ T cells. Release of TNF-α, nitric oxide (NO), and reactive oxygen species (ROS) by macrophages and release of granzyme, perforin, and interferon-γ (IFN-γ) by $CD8^+$ T cells produce further cholangiocyte injury through apoptotic or necrotic pathways. Simultaneously, cholangiocytes and vascular endothelial cells upregulate the expression of adhesion molecules and secrete chemokines to recruit neutrophils and macrophages to the site of bile duct injury (*left side of figure*). Through release of soluble mediators, these cells then recruit and activate hepatic stellate cells (myofibroblasts) and fibroblasts which initiate extracellular matrix deposition and fibrosis of injured bile ducts. The resulting inflammation, cholangiocyte injury, and fibrosis lead to complete bile duct obstruction and the phenotype of biliary atresia. Soluble inflammatory mediators in these pathways are shown in *blue*.

hepatitis [51]. Low-titer anti-Rho antibodies were more prevalent in mothers of infants with BA and idiopathic neonatal hepatitis than in controls. This suggested that there may be maternal transfer or a familial predisposition to autoimmunity in BA. Preliminary data

(unpublished) from the BA Research Consortium suggests that a family history of autoimmunity is no different in BA than in other neonatal cholestatic liver diseases. Another possible clue to a predisposition to autoimmunity in BA is the observation that children with BA have a 2.5% risk of developing de novo autoimmune hepatitis following liver transplantation [52, 53].

An inherited predisposition to autoimmunity is closely linked to the presence of specific HLA gene alleles, especially *HLA-DR*, *DQ*, or *DP* (MHC class II equivalent), as in other autoimmune liver diseases [54]. For example, there is an increased frequency of HLA B8 and DR3 in patients with primary sclerosing cholangitis [55, 56] and HLA A1-B8-DR3 and DR4 in autoimmune hepatitis [57]. Several studies have reported conflicting results in BA. Rosenthal et al. found an increased frequency of HLA-Cw4/7 in BA, while Silveira et al. reported a higher prevalence of HLA-B12 in patients from the United Kingdom [58, 59]. A-Kader et al. from Egypt observed an increased prevalence of both HLA B8 and DR3 [60]. These studies were of limited size and performed by HLA serological techniques. More recently, using the more robust HLA genotyping methodology, Donaldson et al. reported no specific HLA associations in 101 BA patients from the United Kingdom [61]. Indeed, a large study of genotyping for both HLA class II and class I is needed to resolve this controversy, including a sample size that is calculated to generate statistically meaningful results.

ROLE OF INFECTIOUS AGENTS IN BILIARY ATRESIA

Clues from the Epidemiology of BA

Epidemiologic studies support a possible infectious etiology of BA. Seasonal clustering of cases, which suggests environmental exposure to an infectious agent, has been reported in some series but not in others. One of the best conducted studies was a population-based study in metropolitan Atlanta from 1968 to 1993. The incidence of BA varied greatly by year and by season. Seasonal clustering was observed with rates threefold higher in December through March (OR = 2.63 for winter compared to spring) [62]. Strickland et al. reported BA case clustering in northeast Texas and temporal association with autumn compared to the remainder of the year [63]. A recent New York state study reported seasonal patterns of BA cases which varied by the region of the state [64]. Spring births were at highest risk for BA in New York City and September to November births were at highest risk in other regions of the state. Thus, it may not be possible to draw conclusions about case clustering by investigating seasonal incidence of BA across

different locales or in entire countries. This may explain why epidemiologic studies from Europe have failed to identify clustering of cases [6, 65].

A second potential link of BA to an infectious agent can be inferred from studies examining the association of birth weight and gestational age with BA. Low birth weight and premature delivery are known to be associated with intrauterine viral infections. The case-controlled study from New York state demonstrated an increased risk of BA in low birth weight (LBW) preterm and LBW term infants compared to normal weight infants (OR = 2.92 and 2.36, respectively) [64]. A similar association was observed in the Atlanta study, with term LBW infants having an increased risk compared to term normal birth weight infants (OR = 3.52) [62]. Both small for gestational age (SGA) and preterm status were associated with BA in a cohort study from Sweden [65]. SGA infants had a risk ratio (RR) of 5.75 compared to AGA infants. Infants born between 2,232 weeks (RR 6.75) and 33–36 gestation (RR 2.48) were at increased risk for BA compared to term infants. Thus, the increased risk for BA in premature and LBW infants is supportive of a possible intrauterine viral infection.

Viruses and BA

Although definitive evidence of the involvement of a viral infection in the pathogenesis of BA is lacking, three viruses have received the most attention. *Reovirus* was first evaluated in BA because weanling mice infected with certain reovirus strains developed extrahepatic bile duct cysts and histology of intrahepatic bile ducts that resemble BA [66]. Seminal studies reported serologic evidence of increased rates of reovirus infection in BA and neonatal hepatitis infants compared to controls [67, 68]. Two subsequent studies did not confirm these findings [69, 70]. Another important publication was the report of reovirus antigens in the bile duct remnants of a rhesus monkey who developed a disease indistinguishable from BA [71] and from human infants with BA [72]. Once again, others could not replicate finding reovirus antigen in BA infant liver and bile duct [69]. More recently, three research groups have independently used molecular techniques to examine hepatobiliary tissues from infants with BA for reovirus RNA. Using nested reverse transcription (RT)-PCR, Steele et al. did not detect reovirus RNA in archived, formalin-fixed preserved hepatic tissues of 14 BA patients [73]. Saito et al. from Japan also failed to identify reovirus RNA in frozen liver and bile duct tissue from BA patients; however, the average age at the time of collection of the tissue was 8.7 months [74].

In contrast, Tyler et al. detected reovirus RNA by RT-PCR in snap-frozen liver, gall bladder, or bile duct remnants from 55% of cases of BA (average age 2.2 months) and in only 8–15% of controls [75]. There were major methodologic differences among these studies that may explain these discrepant results, including the age of the patients when liver and bile duct were obtained, methods of preparation of the tissue, and the use of PCR primers for different reovirus genes. These factors may be quite important because it is likely that a perinatal viral infection of the liver and the biliary tree is cleared within a relatively short period of time, limiting the time during which detection of virus is possible. This is precisely the situation in the RRV mouse model of BA described below.

Group C rotavirus has been the focus of much study in recent years, stimulated by the newborn mouse model of RRV infection. Riepenhoff-Talty et al. were the first to demonstrate Group C rotavirus in human BA tissues by RT-PCR, with 10 of 18 BA patients and 0 of 12 liver disease control patients positive for rotavirus RNA [76]. Bobo et al. were unable to replicate these findings by failing to detect RNA of rotavirus Groups A, B, or C in livers from 10 BA patients and 14 liver disease controls [77]. Importantly, almost half the patients in this study were over 12 months of age at the time the tissues were obtained, limiting the chances that virus would still be present. The most exciting association of rotavirus with BA is based on the mouse model. Rhesus rotavirus (RRV) infection in the first 12–24 h of life in certain mouse strains produces extrahepatic bile duct obstruction by 2–3 weeks of life despite viral clearance by 1 week of life [78, 79]. As already discussed, the hepatic T-cell populations and cytokine profiles of BA mice recapitulate that found in human BA, supporting a viral pathogenesis (perhaps due to rotavirus) in infants with BA.

An interesting study employed an indirect approach to demonstrate possible involvement of *Reoviridae* infections in human BA. Harada et al. used cultured human biliary epithelial cells and liver tissues from BA patients to demonstrate that double-stranded (ds) viruses could trigger Toll-like-3 receptors (TLR3) in biliary epithelia and that BA livers had evidence of activation of this pathway with NF-KB and TRAIL enhancement [80]. These data suggested that dsRNA viruses (such as *Reoviridae*) could directly induce expression of TRAIL and apoptosis of bile duct cells as a result of the biliary innate immune response. In contrast, Huang et al. reported that TLR7 was overexpressed and TLR3 and TLR4 were lower in liver from infants with early stages of BA liver and that type I interferon signaling was enhanced with upregulation of MxA and IL-8 [81]. Thus, the role of TLR activation in the pathogenesis of BA requires further study.

The third candidate virus receiving considerable study is *cytomegalovirus (CMV*). In one study, 5 of 23 patients with BA were positive for CMV based on liver histology, CMV-IgM titers, or viral culture [82]. A study from Brazil likewise reported positive CMV-IgM titers in 28.5% of patients with BA or choledochal cysts [83]. A Swedish study demonstrated a higher prevalence of CMV antibodies in mothers of BA patients and CMV DNA was present in livers from 50% of those infants [84]. In contrast, a Canadian study did not identify CMV in bile duct remnants from 12 children with BA and other studies failed to detect an association with BA [85]. Since CMV is a common infection in the first several months of life, defining an association with BA will require a very carefully designed study in a large number of infants. Other viruses have also been studied in BA, including human papillomavirus and human herpes virus-6 with similar conflicting results [86, 87].

In summary, continued search for a viral basis of BA is of interest, given the remarkable similarities of the RRV mouse model and the human disease. However, difficulties in establishing this relationship may arise by using current available technologies to detect fingerprints of prior viral infection. Newer methods for identification of viral nucleic acids and proteins are currently being evaluated in human BA tissues.

The possible role of bacterial infection has also received attention in the pathogenesis of BA. Tracy et al. reported the presence of CD14 on sinusoidal and portal tract macrophages in BA liver biopsies obtained at portoenterostomy but not in control specimens [88]. CD14 is a monocyte cell surface receptor activated by bacterial LPS. In addition, Ahmed et al. compared the in situ expression of CD14 between early and late stages of BA [89]. Almost 70% of BA specimens at the time of portoenterostomy had extensive CD14 expression, especially on sinusoidal Kupffer cells, whereas it was markedly decreased in BA livers at the time of transplantation. The investigators postulated that exposure to gut-derived LPS in BA stimulated CD14 expression on activated macrophages which released pro-inflammatory cytokines and contributed to bile duct damage. In as much as cholestasis alters bacterial flora in the intestinal tract and portal tract macrophages are characteristic of BA, this provocative mechanism merits further study.

CONCLUSIONS

At least two forms of BA are recognized, each culminating in common pathways that initiate an aberrant immune/autoimmune response

that perpetuates intrahepatic bile duct injury that eventually results in biliary cirrhosis in the majority of BA patients. The embryonic form of BA appears to result from as yet undefined mutations in, or epigenetic modification of, genes that regulate bile duct morphogenesis. Perinatal BA may develop following, or epigenetic modification of a pre- or postnatal insult that initiates cholangiocyte injury and a subsequent immune response that generates sclerosis and obstruction of both extrahepatic and intrahepatic bile ducts. Predisposing genetic factors may play a role in determining the extent and type of cellular and immune response and the generation of fibrosis. It is hoped that determining these specific causes of BA will lead to not only a better understanding of its pathogenesis but also the possibility of earlier diagnosis and developing new more effective therapeutic and possibly preventative strategies.

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9 Nephronophthisis and Renal–Hepatic–Pancreatic Dysplasia of Ivemark

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CONTENTS

Summary

Nephronophthisis is a disease of renal tubular dysfunction that progresses to end-stage renal disease; it can occur in combination with abnormalities of other organs including the liver. Growing knowledge of the genes associated with nephronophthisis indicates that the disease is associated with dysfunction of primary cilia and related structures.

Renal–hepatic–pancreatic dysplasia of Ivemark is a rare, typically fatal congenital anomaly sequence, characterized by renal dysplasia, hepatic dysgenesis, and pancreatic fibrosis. Newer data suggest

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that it, too, is related to abnormalities of cilia and may share gene abnormalities with syndromes associated with nephronophthisis.

Knowledge of the various genes involved in these and other cystic/dysplastic syndromes continues to grow. Diverse mutations in genes associated with the primary cilium can lead to a variety of phenotypes; epistasis and oligogenic inheritance may explain some of the variety in clinical findings.

Key Words: Nephronophthisis, Primary cilia, Basal body, Centrosome, Renal dysplasia, Hepatic fibrosis, Cystic disease, Chronic kidney disease, End-stage renal disease, Epistasis, Oligogenic inheritance

INTRODUCTION

This chapter focuses on two different forms of renal and hepatic dysplasia. Nephronophthisis is a disease of renal tubular dysfunction that progresses to end-stage renal disease; it can occur in combination with abnormalities of other organs including the liver. Renal–hepatic–pancreatic dysplasia of Ivemark is a rare, typically fatal congenital anomaly sequence, characterized by pancreatic fibrosis, renal dysplasia, and hepatic dysgenesis. Growing knowledge of the genes associated with nephronophthisis indicates that the disease is associated with dysfunction of primary cilia, basal bodies, and centrosomes; data suggest that similar abnormalities may lead to renal–hepatic–pancreatic dysplasia.

NEPHRONOPHTHISIS

Nephronophthisis was first described in 1945 by Smith and Graham [1], and a family cohort was described by Fanconi in 1951 [2] in which he titled the disorder familial juvenile nephronophthisis as an autosomal recessive disease. Hundreds of cases have been described since the first publications and in the last decade there has been extensive progress on the genetic etiologies. Much of the early literature on nephronophthisis is mixed with that on medullary cystic kidney disease, an autosomal dominant cause of adult-onset, end-stage renal disease, but since the late 1980s they are recognized as different disease processes.

Nephronophthisis, though an infrequently occurring renal disorder, is reported to be the most common cause of end-stage renal disease in children and young adults for which there are defined genetic mutations [3]. The classic presentation of nephronophthisis is a late school-aged child with polyuria (usually presenting as secondary nocturnal enuresis) and polydipsia who is found to have urinary concentrating deficits

and later anemia with growth failure. These represent a defect of renal tubular function over any loss of glomerular function.

Clinical Subtypes

Nephronophthisis has three clinical subtypes – infantile, juvenile, and adolescent – differentiated by the age of onset of end-stage renal disease (see Table 9.1) [4]. All three clinical subtypes share clinical features of polyuria without severe electrolyte abnormalities, progression to end-stage renal failure, and anemia out of proportion to the degree of renal injury. In many cases, nephronophthisis presents as part of a complex of variably dysfunctional organs within the context of primary cilia-related diseases. This chapter will focus on isolated nephronophthisis or that associated with retinal degeneration (Senior–Loken syndrome) as other defined syndromes are presented elsewhere.

Juvenile nephronophthisis is the most common and the most studied disease. Infantile nephronophthisis is a more rare phenotype with the onset of end-stage renal disease before 3 years of age. Adolescent nephronophthisis, as described in a Venezuelan cohort, typically presents with end-stage renal disease in the late teens and early twenties [5, 6].

Juvenile Nephronophthisis

The incidence of juvenile nephronophthisis is reported in the United States at 9 per 8.3 million live births [7]. Symptoms of polyuria and polydipsia in juvenile nephronophthisis classically start around 6 years of age. Nighttime drinking is common. Patients may report nocturnal enuresis, which may present as primary but more commonly will manifest as secondary nocturnal enuresis. Median age of end-stage renal disease is 13 years of age with a range of 4–20 years [8].

Patients with juvenile nephronophthisis may have multi-organ involvement (see Table 9.2). In these syndromes, nephronophthisis describes the renal phenotype seen in conjunction with extra-renal manifestations. Typically, syndromes with more severe extra-renal findings will present renal manifestations earlier and with more features of renal dysplasia.

Senior–Loken Syndrome

Contreras, Loken, and Senior independently described an association with nephronophthisis and retinal degeneration in 1960 and 1961 [9, 10]. The ocular manifestations take two forms. The early more severe ocular abnormality is a form of Leber congenital amaurosis, a disorder presenting in infancy with profound visual loss, nystagmus,

Table 9.1
Clinical phenotypes of nephronophthisis

Type	*Inheritance*	*Onset*	*End-stage renal disease*	*Clinical features*
Infantile	Autosomal recessive	Infancy	< 3 years of age[a]	Polydipsia, polyuria, poor growth, renal enlargement with some cysts, arterial hypertension, situs inversus, ventricular septal defects, and pancreatic dysplasia
Juvenile	Autosomal recessive	Childhood	Childhood to early adolescence	Polydipsia, polyuria (primary or secondary nocturnal enuresis), poor growth, mild electrolyte abnormalities, anemia
Adolescent	Autosomal recessive	Late childhood to early adolescence	Mid adolescence to early adulthood	Polydipsia, polyuria, normal urine sediment, anemia

[a] By definition.

Table 9.2
Multi-organ syndromes associated with nephronophthisis

Syndrome	*Extra-renal manifestations*[a]
Joubert syndrome	Cerebellar vermis hypoplasia; ataxia; developmental delay (213300)
Senior–Loken syndrome	Tapetoretinal degeneration (266900)
Meckel syndrome	Encephalocele; polydactyly; oral clefts; liver fibrosis [249000 (MKS Type 1), 603194 (MKS Type 2), 607361 (MKS Type 3), 611134 (MKS Type 4), 611561 (MKS Type 5), 612284 (MKS Type 6)]
RHYNS syndrome	Retinitis pigmentosa; hypopituitarism; mild skeletal dysplasia (602152)
Mainzer–Saldino syndrome (conorenal syndrome)	Skeletal dysplasia; retinitis pigmentosa (266920)
Jeune syndrome (asphyxiating thoracic dystrophy)	Skeletal dysplasia; narrow thorax; micromelia (208500)
Sensenbrenner syndrome (cranioectodermal dysplasia)	Dolichocephaly; rhizomelic dwarfism; dental and nail dysplasia; congenital hepatic fibrosis (218330)
Bardet–Biedl syndrome	Retinal dystrophy; polydactyly; developmental delay; obesity (209900)
COACH syndrome	Cerebellar vermis hypo/aplasia; oligophrenia, congenital ataxia; ocular coloboma; hepatic fibrosis (216360)

[a] Number is reference number for disorder in Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov.offcampus.lib.washington.edu/sites/entrez?db=omim).

poor pupillary reflexes, and either a normal retina or varying degrees of atrophy and pigment changes within the first 2 years of life. The milder retinal lesion, tapetoretinal degeneration, presents as peripheral vision loss and night blindness. Examination in tapetoretinal degeneration demonstrates atrophy and retinal pigment changes usually by the age of 10 years.

Infantile Nephronophthisis

The infantile form of nephronophthisis is associated with early polyuria, anemia, and azotemia with the onset of end-stage renal disease by 3 years of age. It can also be associated with situs inversus

and ventricular septal defect. A hallmark of infantile nephronophthisis is hypertension presenting well before development of end-stage renal disease, a characteristic which is not present in juvenile or adolescent nephronophthisis. Unlike juvenile nephronophthisis, a history of polyuria may not be present at diagnosis. The fetus may demonstrate oligohydramnios and newborns may have a Potter-like sequence of abnormalities, including flat facies and pulmonary hypoplasia. Gagnadoux first described infantile nephronophthisis in 1989 [11].

A difficulty in diagnosing infantile nephronophthisis is the clinical overlap that may exist with autosomal recessive polycystic kidney disease (ARPKD). Both are associated with neonatal and prenatal presentation. Both may have significant hypertension early on. Infantile nephronophthisis is associated with renal enlargement in contrast to the other clinical forms of nephronophthisis, but the degree of renal enlargement at birth is much greater in ARPKD than in infantile nephronophthisis. ARPKD is much more common and evaluation for this diagnosis should take precedence, but if a case lacks the genotype for ARPKD, then infantile nephronophthisis should be considered.

Adolescent Nephronophthisis

Adolescent nephronophthisis was first described in a kindred in Venezuela in 1999 at the same time linkage analysis was completed for this disease [5]. Unlike juvenile nephronophthisis, this form has a median age of onset at 19 years. The presenting complaint was polyuria, polydipsia, secondary enuresis, and anemia in this initially described cohort of 340 family members with 24 affected individuals.

Pathologic findings include small kidneys with cyst formation at the corticomedullary junction and renal tubular ectasia. With progression, kidneys show interstitial and cortical fibrosis [6]. Some patients with adolescent nephronophthisis have demonstrated retinal findings.

DIAGNOSIS

As with all forms of renal disease, clinical suspicion for nephronophthisis is important for establishing a diagnosis. Nephronophthisis should be considered in any child presenting with secondary enuresis, anemia, or growth failure. These symptoms may predate the onset of chronic renal insufficiency and renal failure by several years. Other symptoms include awakening at night to drink and susceptibility to dehydrating illnesses; later, there can be fatigue secondary to anemia. Except in the

infantile form, hypertension is not typically a problem for children with nephronophthisis until late in their course as they develop volume sensitivity from end-stage renal disease. A history of urinary tract infections is usually not present, and they typically lack edema and are volume contracted due to polyuria.

The differential diagnosis includes other interstitial renal diseases such as oligomeganephronia, a condition with few overly large glomeruli on renal biopsy; autosomal dominant or autosomal recessive polycystic kidney disease, both of which have characteristic findings and ages of onset; medullary cystic kidney disease, which typically presents later in life; and renal dysplasia secondary to congenital obstructive uropathies such as partial posterior urethral valves, ureteropelvic junction obstruction, or severe vesicoureteral reflux.

Evaluation includes blood testing to check for anemia and biochemical imbalance associated with renal dysfunction. Urine assessment will likely show concentrating defect; heavy proteinuria should be absent. Elevated serum creatinine and azotemia are very late signs in the progression to end-stage renal disease.

Ultrasound of the kidneys provides information key to establishing the diagnosis of nephronophthisis. Renal size is normal to mildly decreased early in the course but the kidneys are often small at end-stage disease. Increased echogenicity with loss of corticomedullary differentiation is common in all forms of nephronophthisis. In late presenting cases there may be small cysts typically along the corticomedullary junction [12, 13]. Infantile nephronophthisis may present with enlarged kidneys at birth though not to the same degree as is seen in autosomal recessive polycystic kidney disease [11].

Renal histology of patients with nephronophthisis is usually characterized by disintegration of the renal tubular basement membrane, renal tubular cysts, tubulointerstitial inflammation, and fibrosis [14–16]. Renal biopsy of children presenting late in the course of nephronophthisis usually does not provide any clinical information due to the degree of scarring and fibrosis present in the kidney, a common appearance in many forms of end-stage renal disease.

Patients who have nephronophthisis should undergo retinal examination by an ophthalmologist to look for signs of tapetoretinal degeneration or Leber congenital amaurosis. The clinician must recognize that tapetoretinal degeneration may not be present in young children; follow-up screening is needed. If any neurological or developmental problems exist, magnetic resonance imaging of the brain is also indicated to look for evidence of cerebellar hypoplasia or other structural abnormality.

CLINICAL CARE

Chronic Kidney Disease Care

All children with nephronophthisis are at risk for the usual complications of chronic kidney disease, including anemia, metabolic bone disease, poor growth, acidosis, and electrolyte abnormalities. Since they have poor renal concentrating ability, patients with nephronophthisis have obligate daily water requirements that are in excess of typical childhood fluid needs. As the disease progresses and renal function declines, the urine output may decrease. This is important when planning procedures and dietary modifications as excess caloric support can drive polyuria. Some patients demonstrate mild electrolyte abnormalities but profound natriuresis is uncommon.

Growth failure can result from a combination of acidosis and progressive loss of renal function. Correction of acidosis is one goal of supportive therapy. Growth hormone should be considered if, after normalizing acidosis, anemia, bone health, and volume status, the patient continues to have poor growth.

Anemia in patients with nephronophthisis may be out of proportion to the degree of renal failure [17]. Patients often need erythropoiesis-stimulating agents to maintain hemoglobin at an acceptable level. Use of erythropoiesis-stimulating agents must be balanced by evidence in the adult population that high-dose use is associated with increased mortality especially in the end-stage renal disease population [18, 19].

Patients with chronic kidney disease merit careful observation. Coordination with the pediatric nephrologist is required for appropriate ongoing care.

Factors Affecting Progression

No study of patients with juvenile nephronophthisis has had enough patients to look at risk factors for progression to end-stage renal disease. For all children with chronic renal disease, factors such as urinary tract infections/pyelonephritis, hypertension, chronic use of nonsteroidal anti-inflammatory medications, persistent dehydration, and poor nutritional status can accelerate the progression to end-stage renal disease.

End-Stage Renal Disease Care

Patients with advanced chronic kidney disease are candidates for renal transplantation. Though preemptive renal transplantation for patients with identified donors is becoming more common, many patients still require a period of time on dialysis prior to transplant.

PATHOLOGY

Most of the pathological descriptions of kidneys affected by juvenile nephronophthisis are from the early descriptions of the phenotype, so exact genotype/pathological phenotype associations are not available. Ivemark and colleagues, in 1960, using two autopsy specimens, found renal hyalinization and microcystic tubular dilatation [20]. The renal tubular basement membrane was thickened and the course of the proximal tubule and the loop of Henle was elongated and tortuous. Tubules often ended in a microscopic cyst. No changes in the glomeruli other than atrophy were noted.

In infantile nephronophthisis, Gagnadoux et al. noted tubulointerstitial injury as the predominant feature with variable amounts of cystic dilatation of the renal tubules in the cortical and juxtaglomerular regions [11]. Cysts were lined with undifferentiated cuboidal epithelium and the characteristic thickened tubular basement membrane of nephronophthisis was present.

GENETICS AND NEPHRONOPHTHISIS

Discovery

Understanding of cystic renal diseases has benefited greatly over the last decade from genetic research into their etiologies. In 1997, the gene for the most common form of juvenile nephronophthisis was cloned [21, 22]; since then there has been an explosion of information regarding the genes involved in nephronophthisis.

A Shift in Paradigm

The literature on nephronophthisis is in flux between the older clinical classifications and newer gene-based clustering of phenotypes. This transition makes comparison between old and new literature more difficult. Not only do the clinical types of nephronophthisis have overlapping genetic components, but some of the genes may be present in two to three different clinical phenotypes. A mutation in one gene (*NPHP2*) has been linked to the infantile form of nephronophthisis; the other clinical forms of nephronophthisis are associated with mutation in a variety of other genes (*NPHP1, 3, 4, 5, 6, 7* and other associated genes). Further gene defects may be discovered in the future. A brief overview of the current understanding of the genetics in nephronophthisis is presented below.

With the cloning of the various genes associated with nephronophthisis (see Table 9.3), it has become more clear that the products of all

Table 9.3
Genes associated with nephronophthisis

Gene (OMIM ID)	*Protein*	*Mutation type*	*Phenotype*
NPHP1 (607100)	Nephrocystin-1	Any	Juvenile NPHP, JS Type 4 (CNS), SLS Type 1 (RP)
NPHP2/INVS (243305)	Inversin	Any	Infantile NPHP, hepatic fibrosis, hypertension
NPHP3 (608002)	Nephrocystin-3	Functional nsSNPs	Adolescent NPHP, RP
		Non-functional nsSNPs, deletions, truncations	MKS, RHPD, renal and genitourinary anomalies, patterning defects
NPHP4 (607215)	Nephrocystin-4 or nephroretinin	Unconserved nsSNPs	Juvenile NPHP, SLS Type 4
		Conserved nsSNPs, frameshift, deletions	SLS Type 4, Cogan syndrome, Usher syndrome, bronchitis

NPHP5/IQCB1 (609237)	IQ calmodulin-binding motif-containing protein B1	Only truncating mutations observed to date	SLS
NPHP6/CEP290 (610142)	Centrosomal protein 290 kDa	Any	Bardet–Biedl syndrome 14, JS Type 5, Leber congenital amaurosis, MKS Type 4, SLS Type 6
NPHP7/GLIS2 (608539)	GLIS family zinc finger 2	Single mutation known nsSNP with abnormal splicing	Juvenile NPHP
NPHP8/RPGRIP1L (610937)	RPGRIP1-like	nsSNP with substitutions or premature terminations	JS Type 7, MKS Type 5
NPHP9/NEK8 (609799)	NIMA-related kinase 8	nsSNP in conserved region	NPHP, RP

NPHP, nephronophthisis; JS, Joubert syndrome; SLS, Senior–Loken syndrome; MKS, Meckel syndrome; RHPD, renal–hepatic–pancreatic dysplasia; nsSNP, non-synonymous single-nucleotide polymorphism; CNS, hypoplasia of cerebellar vermis/molar tooth signs; RP, retinitis pigmentosa.

Data from Entrez and OMIM.

genes that are mutated in cystic kidney diseases of humans or animal models (mice or zebrafish) show expression of their encoded proteins in primary cilia, basal bodies, adherens junctions, or centrosomes. Thus, abnormalities of primary cilia and centrosomes must underlie these disease processes.

NPHP1

Mutation of the *NPHP1* gene is the most common gene defect responsible for juvenile nephronophthisis. The gene is located on chromosome 2q12–q13 and was identified in 1993 by positional cloning [23]. It accounts for 27–60% of isolated nephronophthisis cases and is one of the most frequent genetic causes of end-stage renal disease in children. In 80% or more of patients, the gene defect is related to a 290-kb deletion [24].

Nephrocystin-1, the protein product of *NPHP1*, interacts with numerous proteins including the products of other *NPHP* genes. In particular, nephrocystin-1 interacts with the basal body of the cilia and is present along the length of the cilia, intracellular aspects of the basal membrane's focal adhesion complex, and the lateral membrane's adherens junction in retinal cells. At the cell membrane it co-localizes with focal adhesion kinase 2, tensin, and p130 Cas, proteins involved with cell–cell and cell–basement membrane signaling [25].

NPHP2/INV

The locus for infantile nephronophthisis was originally mapped by linkage analysis in a large Bedouin kindred with significant consanguinity in which there were seven children affected by infantile nephronophthisis [26]. The gene, called *NPHP2*, appeared at the same region as the *INVS* gene, which encodes the protein inversin, critical in left–right patterning during embryonic development. Otto et al. showed mutation of *INVS* to be the cause of infantile nephronophthisis both with and without situs inversus [27]. They further demonstrated that inversin interacts with nephrocystin-1 and that both proteins localize to primary cilia of renal tubular cells.

NPHP3

NPHP3, located on chromosome 3q22, was originally identified by linkage analysis from the previously mentioned large Venezuelan kindred [5]. In this group, affected members were homozygous for a 3-base-pair deletion. Continuing investigation demonstrates that defects in *NPHP3* can cause nephronophthisis in multiple age groups, not just

in adolescents as suggested by the original kindred [6, 28]. *NPHP3* has been implicated in numerous defects of embryonic patterning including situs inversus, polydactyly, malformations of the central nervous system, congenital heart abnormalities, and multicystic kidneys [29]. The variation in phenotype related to the type of defect in the transcribed protein is consistent with the growing recognition of a link between the severity of genetic interruption of protein function and the degree of malformation in target organs. As an example, *NPHP3* has been linked to renal–hepatic–pancreatic dysplasia, a more profound phenotype described later in this chapter.

NPHP4

NPHP4 is located at 1p36 and was localized via linkage analysis using families with nephronophthisis and retinal findings of Senior–Loken syndrome [30]. Nephrocystin-4/nephroretinin, the gene product, has no sequence similarity with nephrocystin-1 and has highly conserved regions in prokaryotes [31]. It interacts with nephrocystin-1 and retinitis pigmentosa GTPase regulator-interacting protein (RPGRIP1) [32]. Within quiescent cells, it localizes to primary cilia, basal bodies, and the actin cytoskeleton. In dividing cells, it localizes to centrosomes.

NPHP5/IQCB1

The IQ motif-containing protein B1 gene *(IQCB1)* was originally isolated from cDNA library screening of an immature myeloid cell line [33]. It was later localized by linkage analysis in a Turkish family with Senior–Loken syndrome phenotype and dubbed *NPHP5* [34]. Typical mutations are frameshift deletions and non-synonymous nucleotide transitions, resulting in premature stop codons. The gene product, nephrocystin-5, is expressed in connecting cilia of photoreceptors and in the primary cilia of renal epithelial cells.

NPHP6/CEP-290 (Centrosomal Protein 290 kDa)

The *NPHP6* gene, encoding centrosomal protein 290 kDa, is associated with Meckel syndrome type 4, Joubert syndrome, Senior–Loken Syndrome, and isolated Leber congenital amaurosis. The gene product activates ATF4, a transcription factor that participates in cell cycle control [35]. Screening of 12 families with Joubert syndrome revealed 13 different mutations [36].

NPHP7/GLIS2 (Glis Family Zinc Finger Protein 2)

A Canadian Oji-Cree kindred with isolated juvenile nephronophthisis in three children was found to have a mutation in the *NPHP7* gene, also known as *GLIS2* [37]. The gene, located at 16p13.3, was cloned by searching for sequences similar to the murine homologue *Glis2* [38]. The 525-amino acid gene product is most closely related to members of the *GLI* and *ZIC* subfamilies of Kruppel-like finger proteins, which function as activators and/or repressors of gene transcription. In the three affected members of the kindred described above, the mutation is a homozygous G-to-T transversion that interferes with normal RNA splicing patterns leading to a non-functional protein [37].

Other Genes Associated with Nephronophthisis

RPGRIP1L encodes a cytosolic protein that associates with nephrocystin-4 and nephrocystin-6 around basal bodies, centrosomes, and primary cilia of renal tubular cells [39]. Defects in this gene, which has also been referred to as *NPHP8*, have been associated with Joubert syndrome and Meckel syndrome [40, 41]. *NEK8*, a gene whose product co-localizes with primary cilia in renal epithelial cells, was found to be a rare cause of nephronophthisis in a screening cohort of 588 families with the disease [42]. This gene has also been called *NPHP9*.

Evidence for Epistasis and Oligogenic Inheritance

With the cloning of multiple genes related to nephronophthisis, evidence for oligogenic inheritance, in which multiple gene defects lead to disease, is beginning to surface. Multiple gene defects have been noted in some patients with Joubert syndrome [43] and a study of 94 families with nephronophthisis demonstrated six kindreds in which multiple mutations were detected in the *NPHP1*, *NPHP3*, and *NPHP4* genes [44]. These studies suggest that gene/gene interactions affect severity of the disease process (epistatic gene interaction).

Genetic tests for most of the genes causing nephronophthisis are not approved for commercial use in the United States and are used only in the research setting. When clinical consideration of genetic testing occurs, reference to genetic testing databases [e.g., GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1993–2008. Available at http://www.genetests.org] would be an appropriate method for up to date information on genetic testing.

NEPHRONOPHTHISIS AND THE LIVER

Hepatic Manifestations in Nephronophthisis Syndromes

Hepatic involvement can be seen in nephronophthisis and in several of its associated multi-organ syndromes (see Table 9.4). Hepatic fibrosis is rare in classic juvenile nephronophthisis. One case series from Israel demonstrates a family with findings of mild hepatic fibrosis in association with nephronophthisis [45]. In this family, end-stage renal disease developed well before any signs of mild hepatic impairment.

Table 9.4
Syndromes associated with nephronophthisis and congenital hepatic fibrosis

Joubert syndrome
Meckel syndrome
Jeune syndrome
Sensenbrenner syndrome
Bardet–Biedl syndrome

In the case series by Gagnadoux describing seven children with infantile nephronophthisis, cholestasis was the clinical presentation of liver disease in only one patient [11]. Liver biopsy was performed on this one patient on two occasions. These biopsies showed portal fibrosis with inflammatory cells. The second biopsy demonstrated bile duct proliferation, signs of cholestasis, and multinucleated hepatocytes. One other patient had mild hepatomegaly without other abnormality. During the linkage analysis study that lead to the isolation of *NPHP2*, Haider and colleagues found no evidence of liver disease in the large Bedouin family with infantile nephronophthisis [26]. This finding contrasts with the earlier description by Gagnadoux [11].

Hepatic Fibrosis and Genetics in Nephronophthisis Syndromes

Mutation of the *NPHP3* gene is described in a family kindred from Yugoslavia with hepatic fibrosis and clinical juvenile nephronophthisis [6]. Affected individuals were heterozygotes for a 1079 G-to-C transversion which causes an S360T mutation in the evolutionarily conserved 360th amino acid residue on one allele and a 1381 G-to-T transversion creating a premature stop codon on the other allele.

Recently, several patients with renal–hepatic–pancreatic dysplasia were shown to have truncating mutations in the *NPHP3* gene [29]. These patients came from different kindreds and additional phenotypic

elements included situs inversus, postaxial polydactyly, preauricular fistulas, and Dandy–Walker central nervous system malformations. These were in addition to the Meckel-like syndrome findings of multicystic dysplastic kidneys, ductal plate abnormalities of the liver, and cystic pancreatic lesions. Not all of these cases were fatal at birth. Two children later received combined liver kidney transplants.

Renal–Hepatic–Pancreatic Dysplasia (RHPD)

Renal–hepatic–pancreatic dysplasia was first described in the literature by Ivemark in 1959 [46]. It is a rare sporadic and autosomal recessive disorder characterized by renal dysplasia, hepatic dysgenesis, and pancreatic fibrosis that is uniformly fatal [47]. Most infants die in the neonatal period but some are lost during pregnancy. Descriptions are mostly case reports and until recently no genetic cause is known [48–50].

Infants are typically still born with low birth weight and shortened crown-to-heel length. Ultrasound was reported to detect prenatal bile duct dilatation at 22 weeks of gestational age in a fetus where pregnancy was terminated and RHPD diagnosed [51]. Bile duct dilatation was confirmed by postmortem cholangiography.

At autopsy, fetuses and neonates are typically small and short. Postaxial polydactyly is common. Kidneys are uniformly mildly enlarged with small cysts (0.2–1.4 cm diameter) and smooth surface. Normal fetal renal lobulations are present. The liver is typically moderately enlarged and firm. Pancreases are enlarged for age, firm, and only variably contain cysts. Some cases demonstrate Dandy–Walker malformations of the central nervous system and partial or complete situs inversus [52]. Case reports also included cardiac transpositions, polysplenia/asplenia [48, 50], hypertrophic cardiomyopathy, and exocrine pancreas insufficiency [53].

Microscopically, the kidneys are highly dysplastic with immature mesenchyme. Renal dysplasia has been described at 16 weeks of gestation [54]. The livers contain features of dysplasia with primitive ducts in the portal tracts and varying degrees of fibrosis [55]. The pancreases contain areas of immature mesenchyme and typically reduced numbers of islets. When present, pancreatic cysts are lined with cuboidal epithelium [50].

Work by Bergmann et al. demonstrates the broad clinical spectrum of mutations associated with *NPHP3* [29]. This spectrum includes RHPD when deletions or transitions/transversions lead to truncating or splice variant mutations. This is in contrast to the hypomorphic alleles like

non-synonymous transversions and transitions seen in patients with juvenile nephronophthisis and *NPHP3* mutations.

SUMMARY

Our knowledge of the genetic basis of nephronophthisis and renal–hepatic–pancreatic dysplasia continues to grow. Diverse mutations in genes associated with the primary cilium can lead to a variety of phenotypes. Care of patients with nephronophthisis remains supportive at this time and genetic counseling is the only medical means of helping families with children who had renal–hepatic–pancreatic dysplasia.

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10 Meckel and Joubert Syndromes

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CONTENTS

Summary

Both Meckel (Gruber) syndrome (MKS; MIM 249000) and Joubert syndrome and related disorders (JSRD; MIM 213300) are rare, autosomal recessive genetic disorders that share congenital malformations of the posterior fossa or the hindbrain and are associated with defects in the structure and/or the function of the primary cilium. The cardinal features of Meckel syndrome include the triad of brain anomalies (usually an occipital encephalocele), cystic renal dysplasia, and hepatic ductal plate malformation, in conjunction with a variety of other more variable malformations, such as polydactyly. MKS is typically lethal in the perinatal period. The genetic defects in MKS involve at least five genes that localize to the primary cilium and/or the basal body. Joubert syndrome and related disorders are characterized by a complex hindbrain malformation identified on axial magnetic resonance imaging (MRI) known as the molar tooth sign (MTS), as well as intellectual disability, hypotonia, and often, abnormal respiratory pattern and/or abnormal eye movements. In

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addition, a broad spectrum of other congenital anomalies, including retinal dystrophy, ocular coloboma, oral frenulae and tongue tumors, polydactyly, cystic renal disease (including cystic dysplasia or juvenile nephronophthisis), and congenital hepatic fibrosis, have been described. In contrast to MKS, most children with this condition survive infancy to reach adulthood. At least seven genes cause JSRD, with the *MKS3* gene more likely to be associated with hepatic fibrosis and the JSRD subtype known as COACH syndrome. In fact, the JSRD genes associated with liver (and other) phenotypes overlap with the MKS genes, illustrating the close association of these two disorders and their shared etiology in coding for proteins important in the function of the primary cilium.

Key Words: Meckel syndrome, Joubert syndrome, Molar tooth sign, Nephronophthisis, Ductal plate malformation, Ciliary disorder, Ciliopathy, Congenital hepatic fibrosis, Encephalocele, Cerebellar vermis hypoplasia

MECKEL SYNDROME

Overview

Historical. Meckel syndrome or MKS was first described in 1822 in two siblings who perished at birth with microcephaly, occipital encephalocele, cleft palate, large cystic kidneys, and polydactyly with cryptorchidism in the male sibling [1]. Thereafter, Gruber collected other cases from the literature and added three more case reports of his own, concluding that it had a genetic basis [2]; he renamed the disorder dysencephalia splanchnocystica, and it later came to be known as Meckel syndrome.

In their landmark 1969 historical review, Opitz and Howe revived this entity for the English-speaking world. They delineated the clinicopathological basis of MKS, established the autosomal recessive mode of inheritance, and solidified the name, Meckel syndrome, based on the significance of the original description by Meckel [3]. Succeeding reports confirmed the non-random nature of the association of the clinical features of occipital encephalocele, cystic renal dysplasia, and polydactyly [4, 5]. Autosomal recessive inheritance in the Finnish population using these ascertainment criteria was described in the definitive work by Salonen and others [6, 7]. However, review of case material identified variable hepatomegaly, cystic changes in the liver, absence of the gall bladder, and the constant findings of portal fibrosis and proliferation of the bile ducts (when histopathological examination could be completed), indicating that congenital hepatic fibrosis and hepatic

ductal plate malformation are major and invariant features of MKS [6, 7].

Since these pioneering studies, it has become apparent that the MKS phenotype is broader than originally described and that congenital liver disease is always present in MKS. Further, the recognition of cerebellar vermis hypoplasia/aplasia in MKS cases [8] and overlapping clinical features of these conditions suggested genetic allelism between MKS and Joubert syndrome and related disorders (JSRD; see below), subsequently confirmed by the identification of shared genetic causes.

Prevalence. MKS has been reported in many regions worldwide, including Europe, Asia, the Americas, the Middle East, and Africa, but prevalence estimates vary greatly between populations and ethnicities. These estimates range from 1 in 3,408 births in Belgium [9], 1 in 9,000 in Finland [7], 1 in 13,250 in the United States [10], to 1 in 140,000 births in the United Kingdom [11].

Clinical Diagnosis

The traditional triad for diagnosis of MKS was originally proposed as occipital encephalocele, cystic renal dysplasia, and polydactyly [3]. However, variability in phenotype and marked intrafamilial heterogeneity has made determination of the core features for diagnosis more complicated. With time, and notably, awareness of the hepatic features of MKS, the clinical diagnosis was revised to encompass the minimal criteria of a typical central nervous system (CNS) malformation (usually, an occipital encephalocele), cystic renal dysplasia, and congenital hepatic fibrosis (CHF)/hepatic ductal plate malformation (Fig. 10.1a–d). Other, more variable MKS features include the skeletal abnormalities of polydactyly, shortening and bowing of the long bones, cleft lip/palate and tongue, heterotaxy manifestations, eye anomalies, and abnormal development of the male genitalia [6, 12, 13]. As the clinical spectrum widens with the application of genetic testing, it is unclear whether any of these features will be invariant in gene-based diagnostic series. In general, postnatal survival is rare and appears to occur only when features are intermediate between MKS and JSRD; many affected fetuses perish in utero. MKS may comprise milder variants that overlap with other syndromes, particularly those with longer postnatal survival such as JSRD, intermediate forms between Joubert syndrome (JS) and MKS such as JS with encephalocele and/or CHF, and nephronophthisis with CHF [14].

Central nervous system. The most common CNS malformation reported thus far is occipital encephalocele, but the spectrum of neuroanatomic anomalies has expanded far beyond this initial description.

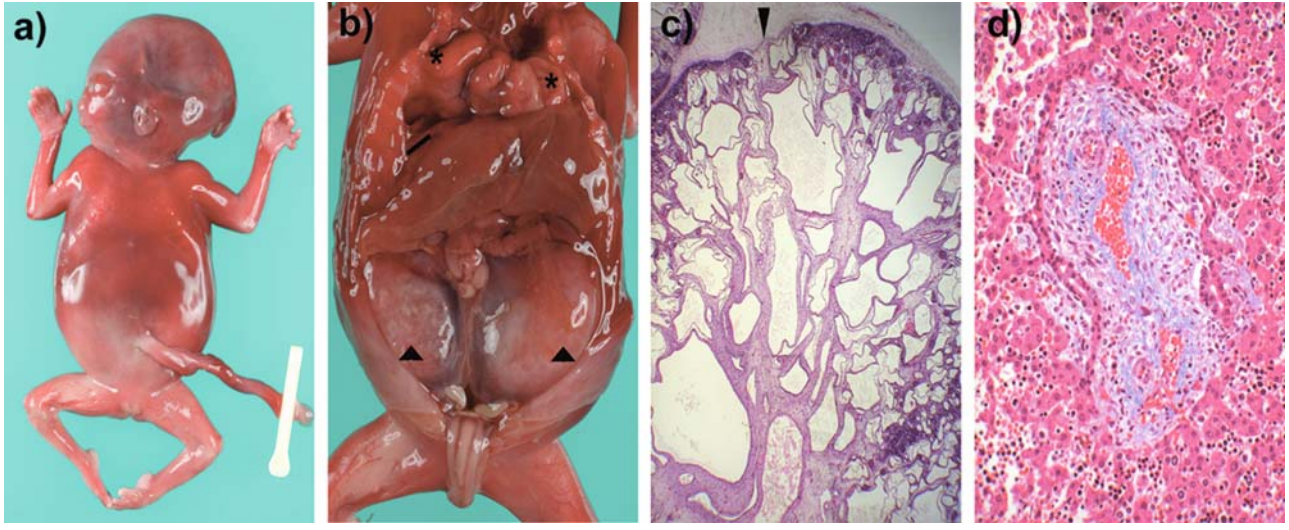

Fig. 10.1. Clinical and pathologic features of Meckel syndrome. (**a**) Classic phenotype of Meckel syndrome is demonstrated in this 19-week fetus and includes posterior occipital encephalocele, bilateral postaxial polydactyly of the hands, enlarged abdomen (secondary to large kidneys), and features associated with oligohydramnios such as flattened nose, micrognathia, low-set ears, limb contractures (*left knee*), and bilateral talipes equinovarus. (Photo courtesy of Joseph Siebert, PhD.) (**b**) In situ relationships of viscera are demonstrated at autopsy after the chest and abdominal walls have been reflected and the intestines removed. Bilaterally enlarged kidneys (*arrowheads*) fill the abdomen and elevate the diaphragm (*line*), thereby diminishing the size of pleural cavities, which in combination with oligohydramnios can lead to hypoplastic lungs (*asterisks*). (Photo courtesy of Joseph Siebert, PhD.) (**c**) The immature kidney in MKS has numerous cysts that involve all parts of the nephron including small glomerular cysts and larger cysts in the deep cortex and the medulla (*bottom*) that are surrounded by a cuff of condensed mesenchyme. The nephrogenic zone is focally interrupted (*arrowhead*) (hematoxylin and eosin, 40×). (**d**) Ductal plate malformation. An excess of bilaminar slightly dilated tubular structures nearly encircle the portal region and are partially incorporated into the portal mesenchyme (trichrome, 100×).

Neuropathological studies have identified CNS malformations resulting from prosencephalic dysgenesis, the formation of an occipital exencephalocele, and rhombic roof dysgenesis, with additional proliferation and migration defects [15, 16]. Prosencephalic dysgenesis encompasses agenesis of olfactory bulbs and tracts (arhinencephaly), hypoplasia of optic nerves and chiasm, microphthalmia, midline commissuration defects (including hypoplasia/agenesis of the corpus callosum), pituitary aplasia, and ventral induction defects, such as fused thalami and holoprosencephaly. In most studies, the encephalocele is associated with a defect in the occipital bone. In some cases, however, the brain may be extruded through an enlarged posterior fontanel rather than through an occipital cranial defect and may be better designated as an exencephalocele [15, 16]. Often, a second defect of the basal occipital bone may be present with or without extrusion of brain [13, 15, 16]. In one review, anencephaly was diagnosed in 10% of cases [16]. Unfortunately, terminology for neural tube defects is not standardized, making it difficult to summarize the literature. Rhombic roof dysgenesis may result in the absence of tectum or brain stem, cerebellar vermis hypoplasia, the Dandy–Walker malformation, the molar tooth pattern of mid-hindbrain malformation [17], and aqueductal stenosis [15, 16]. Proliferation and migration defects are also prevalent, seen microscopically as heterotopias, polymicrogyria, pachygyria, and neuroepithelial rosettes [13, 15, 16].

Overall, MKS manifests a broad spectrum of CNS malformations resulting from both dorsal and ventral induction defects, along with abnormal neuronal proliferation and migration.

Genitourinary. Renal cystic dysplasia is a constant feature in MKS as currently defined. However, it has become apparent that there is a spectrum of renal outcomes for patients with MKS. In typical MKS, the kidneys frequently become enlarged early in gestation, and by midtrimester, bilateral (often massive) renal enlargement is accompanied by oligohydramnios (Fig. 10.1b). Renal enlargement may range from a 5- to 50-fold increase in weight over gestational aged-matched normal kidneys [6, 12, 13, 18]. Cysts of variable size are located from the peripheral cortex (smallest sized cysts) to the medulla (largest), where the thick-walled fibromuscular cysts may reach several millimeters, with very little normal parenchyma remaining (Fig. 10.1c) [6, 9, 12, 13, 18, 19]. Other genitourinary malformations include renal hypoplasia/aplasia, horseshoe kidney, atretic or duplicated ureters, and atretic or hypoplastic bladder. External genital abnormalities are seen in males, including hypoplastic or ambiguous genitalia, and rarely true sex reversal [4, 6, 13].

Hepatic. Liver abnormalities are the third constant feature of MKS. The liver is often enlarged, although macroscopic fibrosis or cysts are rarely observed; microscopic evaluation is typically needed to achieve complete assessment. The hepatic features of MKS are primarily due to a failure or a disruption of normal fetal biliary duct development, known as the ductal plate malformation (Fig. 10.1d) [13].

Normal bile duct development, via fetal ductal plate "remodeling," is perturbed in MKS. At about 8–10 gestational weeks in normal embryonic development, primitive liver cells (hepatoblasts) around the mesenchyme that surrounds the branching portal vein condense and acquire a biliary phenotype, forming a cylindrical sleeve, termed the ductal plate. Varying length segments of the ductal plate are duplicated at about 9–12 gestational weeks, generating slit-like lumens that undergo progressive dilation, starting at the earliest formed ductal plates associated with the larger portal vein branches near the hilum. Ingrowth of mesenchyme surrounding the portal vein into the hepatoblasts incorporates the "tubules" into the future portal triad where they become the future bile ducts; excess ductal plate cells not involved in tubule formation gradually disappear, presumably by apoptosis [20]. Progressive ramification of the biliary tree continues toward the periphery throughout fetal life; the most distal branches of the intrahepatic biliary tree are not completely remodeled until approximately 4–6 postnatal weeks [20–23]. Hepatic artery branches develop by vasculogenesis within portal mesenchyme in conjunction with tubular incorporation.

Complete or partial arrest of this remodeling process results in the ductal plate malformation (DPM), characterized by complex and often dilated duct-like structures that partially encircle the portal mesenchyme; this appearance is often misinterpreted as ductal proliferation in pathology reports [21–24]. The DPM also seems to be associated with portal vein ramification abnormalities, yielding numerous small and closely apposed "sprouts" rather than widely spaced "branches."

Lack of ductal plate regression is often associated with the development of portal fibrosis, termed congenital hepatic fibrosis (CHF), which has variable histopathological appearances, and can lead to portal hypertension, cholangitis, and hepatic failure, although more latent clinical outcomes are known [20–23].

The invariant presence of hepatic fibrocystic disease in MKS was not widely recognized until a case series noted hepatic fibrosis and cysts [4], and Salonen carefully evaluated the Finnish cohort [6]. In spite of relatively recent appreciation of the DPM as a diagnostic criterion for MKS, it has been identified in all cases examined thus far, provided adequate liver tissue is available for histopathological evaluation by an experienced pathologist.

The identification of causative MKS genes as ciliary genes led to recent demonstration of the absence of the primary cilium in ductal plate cells of some, but not all, fetal MKS cases [25]. Aberrant lineage-specific staining of hepatoblasts and ductal plate cells suggests defective hepatic and/or biliary differentiation in MKS. The presence of the DPM links MKS to a wider group of congenital hepatorenal fibrocystic diseases with defects in ciliary assembly that include autosomal recessive polycystic kidney disease (ARPKD), nephronophthisis (NPH) with liver fibrosis, Bardet–Biedl syndrome, Jeune asphyxiating thoracic dystrophy, and the COACH variant of Joubert syndrome [20, 26–28].

Skeletal/limbs. Polydactyly is typically postaxial (on the lateral or fifth digit side of the hand or the foot), variably involving between one and four limbs. Although it is often highlighted as a core feature in MKS, its absence does not preclude diagnostic assignment to MKS. Occasionally, polydactyly may be preaxial (on the thumb side of the hand or the great toe side of the foot) [29]. Also, bowed and shortened long bones are sometimes present, suggesting overlap with other ciliopathies such as Jeune asphyxiating thoracic dystrophy and short-rib polydactyly syndromes that have similar skeletal features. Clubfeet with flexed legs may be fixed or due to positional effects from oligohydramnios [13].

Other. Intrauterine growth retardation, an enlarged placenta, and oligohydramnios are frequently present [3]. Fibrocystic changes in the pancreas and epididymis are also seen [13]. Distinctive facial features may in part be due to intrauterine molding effects, resulting in micrognathia, sloping forehead, low-set ears, broad cheeks, full lips, wide mouth, or a broad flattened nose (so-called Potter facies or sequence). Cleft lip and/or palate, with palatal involvement more typical than lip, is also observed. Tongue abnormalities including aplasia, lobulation, and tumors have been described [13]. Heterotaxy manifestations include situs inversus, accessory spleens, asplenia, intestinal malrotation, and uterine abnormalities. Congenital heart malformations also occur [13, 30]. Ocular manifestations, including microphthalmia, sclerocornea, microcornea, cataract, aniridia, optic nerve and optic chiasm hypoplasia [15], chorioretinal coloboma [30, 31], and retinal dysplasia [32], have been described [13].

Prenatal diagnosis. Prenatal ultrasound can identify an affected fetus starting at the end of the first trimester in an at-risk pregnancy, where enlarged cystic dysplastic kidneys are often visualized together with CNS, skull, and limb abnormalities [33, 34]. Distinctive renal findings may include abnormal cortico-medullary differentiation in addition to kidney cysts [35]. Later, oligohydramnios and abdominal prominence can lead to the diagnosis, although other structures such as polydactyly

and encephaloceles may be difficult to visualize [36]. Imaging by fetal MRI may further clarify the malformations, particularly involvement of the CNS and the skull [34, 37–39]. MKS is an important cause of bilateral cystic renal dysplasia discovered during routine ultrasound surveillance, although the presence of oligohydramnios can complicate diagnosis, especially in the second trimester [40], and overlap with other cystic renal dysplasia–polydactyly syndromes may lead to diagnostic dilemmas in an index case [41]. Genetic testing of a known familial mutation of an MKS gene can also be carried out during a pregnancy for definitive prenatal diagnosis.

Molecular Genetics of MKS

MKS is a genetically heterogeneous disorder as more than six loci and five distinct genes have been identified thus far (see Table 10.1 for a list of MKS genes and their associated clinical features). Three loci were originally identified in families with MKS: MKS1, mapped to chromosome 17q21–q24 [42] with mutations subsequently identified in the *MKS1* gene [43]; MKS2, mapped to chromosome 11q13 [44]; and MKS3, mapped to chromosome 8q24 [45], with mutations identified in the *TMEM67/MKS3* gene [46]. Three additional loci have been identified in individuals with MKS as well as JSRD. One of these, *CEP290*, causes MKS [47, 48], plus other phenotypes including JSRD [49, 50], Bardet–Biedl syndrome [51], Leber congenital amaurosis [52, 53], and isolated nephronophthisis (NPH) [54]. Nonsense and missense mutations in *RPGRIP1L* may also cause an MKS picture [55, 56], and *CC2D2A* mutations may similarly cause both MKS and JSRD [29, 57]. Severe loss-of-function mutations in *NPHP3* may result in embryonic lethality, an MKS-like condition, and renal–hepatic–pancreatic dysplasia [14].

In Finland, it is apparent that *MKS1* is the predominant gene involved in classic forms of MKS, as 26 of 40 MKS families (65%) had mutations in this gene [43]. In 70% of Finnish pedigrees bearing a common chromosome 17q23 haplotype at the MKS1 locus, a 29-base-pair intron 15 deletion termed MKS1-Fin_{major} was identified. This mutation induces aberrant splicing of the RNA [43]. MKS1-Fin_{major} is also observed in non-Finnish pedigrees, indicating an early introduction of this founder mutation into the European gene pool [30, 31, 43, 58]. *MKS1* and *CC2D2A* appear to account for almost 90% of MKS in the Finnish population, implicated in 68 and 21% of cases, respectively [29]. The exact contributions of the various MKS loci in other populations are much less clear. Claims that *MKS1* (5 of 17 families, 30%) and *MKS3* (5 of 17 families, 30%) together account for the majority of

Table 10.1
Molecular genetics of Meckel syndrome (MKS)

Locus name	*Gene symbol*	*Locus*	*Protein name*	*Types of mutations*	*CNS*	*Liver*	*Col*	*Renal*	*PD*	*Key clinical features*	*Proportion of MKS*
MKS1	*MKS1*	17q23	MKS1	Splice FS	++ OE, HC, MC, PRD, DWM, CVH	++	++	++	++	LBB, CL/CP, PC, EC, Situs inversus/ heterotaxy CAGUT, CHD, IUGR	7–30% [30, 58]; 68% of Finnish [29]
MKS2	*MKS2*	11q13	Unknown	NA	++ OE, PRD, MC, CVH	++	?	++	+	CL/CP, CAGUT, CHD, PD, microph-thalmia	?
MKS3	*TMEM67/ MKS3*	8q21.13–q22.1	Meckelin	Splice, Nonsense, Missense	++ OE, HC, MC, PRD, DWM, CVH, ND	++	+	++	+	CL/CP, Heterotaxy EC	7–30% [30, 58]

MKS4	*CEP290*	12q21.3	Centrosomal protein of 290 kDa	Nonsense, FS, splice	++ OE, CVH, HC, DWM, PRD, MC	++	?	++	+	Situs inversus CL/CP CAGUT Lung hypoplasia CHD Microphthalmia	?
MKS5	*RPGRIP1L*	16q12.2	RPGR-interacting protein 1-like protein	Missense, Nonsense	++ OE, PRD	++	–/?	+	+	CL/CP, LBB, microphthalmia	?
MKS6	*CC2D2A*	4p15.3	Coiled-coil and C2 Domains-containing protein 2A	Splice	++ OE, HC, PRD	++	–/?	++	+	Preaxial PD, CAGUT, lung hypoplasia, CL/CP	21% of Finnish [29]
Renal–hepatic–pancreatic dysplasia (Meckel-like syndrome)	*NPHP3*	3q22	Nephrocystin-3	Splice, FS, Missense, Nonsense	+ OE-like, HC, DWM	++	?	++	+	CHD, CAGUT, Situs inversus, PD	?

–, Not described; +/–, rare; +, present in some cases; ++, common; ?, unknown.

CAGUT, congenital anomalies of the genitourinary tract; CHD, congenital heart defect; CL/CP, cleft lip and/or cleft palate; CNS, central nervous system; Col, coloboma; DWM, Dandy–Walker malformation; EC, epididymal cysts; FS, frameshift mutation; HC, hydrocephalus; IUGR, intrauterine growth retardation; LBB, long bone bowing; MC, microcephaly; NA, not applicable; ND, no abnormalities detected/reported; OE, occipital encephalocele; PRD, prosencephalic dysgenesis (arhinencephaly, anencephaly, hypoplasia of the corpus callosum, holoprosencephaly, fused thalami, and other midline brain defects); PC, pancreatic cysts/dysplasia; PD, polydactyly.

cases of MKS in European populations [58] require further population-based studies to confirm. In a larger and mixed non-Finnish multi-ethnic cohort, just 14% of MKS pedigrees were identified as being caused by either *MKS1* (8 of 120 families; 7%) or *MKS3* (8 of 120 families; 7%) [30]. In a mixed non-European cohort, only 5 of 14 families (35%) mapped to the MKS1, MKS2, or MKS3 loci [59]. Tallila et al. collated all the series of genotyped MKS cases where mutations were identified, originating mostly from Europe, the Middle East, and Africa, and estimated the relative contributions of MKS genes as follows: *MKS1,* 52%; *MKS3,* 20%; *CEP290*, 13%; *RPGRIP1L*, 2%; and *CC2D2A*, 13% of cases [29]. Overall, it appears likely that additional genes for MKS remain to be identified, perhaps through discovery of genes for overlapping, allelic disorders.

Aside from the common *MKS1* founder mutation, series investigating both *MKS1* and *MKS3* mutations are notable for truncating mutations due to splice-site, nonsense, and frameshift mutations [30, 43, 46, 58, 59]. The mutations in pedigrees caused by *MKS1* typically predict a truncated MKS1 protein [30]. In contrast, *MKS3* mutations in non-consanguineous pedigrees typically have a truncating mutation accompanying a missense mutation; homozygous or compound heterozygous *MKS3* missense mutations are uncommon [30, 43, 46, 58, 59]. The predominance of splice-site mutations with variable quantities of intact or truncated protein may account for, in part, the high degree of inter- and intrafamilial variability observed in MKS case series.

Determining genotype–phenotype correlations has proved challenging, as the inclusion of cases using clinical criteria creates ascertainment bias toward more classical and extreme presentations. Nonetheless, cases due to *MKS1,* when compared to *MKS3,* appear to have a more severe phenotype and most notably, a higher prevalence of occipital encephalocele and skeletal manifestations [30, 58]. Thus far, cases due to *MKS3* mutations tend to have both a greater variety of CNS malformations and those that are considered less severe, such as the Dandy–Walker malformation and the molar tooth sign of JSRD [30, 58, 60]. In fact, at least one reported case due to an *MKS3* mutation had normal brain morphology [30]. Similarly, the complete and most severe heterotaxy defect situs inversus appears confined to *MKS1* mutations; in contrast, milder examples of disrupted laterality, such as intestinal malrotation, bicornuate uterus, and spleen anomalies (accessory spleens/polysplenia, asplenia), are seen in *MKS3* mutations [30]. A higher prevalence of skeletal anomalies consisting of shortening and bowing of the long bones (sometimes known as the campomelic variant of MKS) is observed with *MKS1* mutations [30, 58, 59]. Polydactyly, typically postaxial, is far more common and severe in cases due to

MKS1 than *MKS3,* with greater likelihood of affecting both upper and lower limbs [30, 58]. Preaxial polydactyly may occasionally be seen in cases due to *CC2D2A* [29]. The data are less clear for cleft palate [30]. When evaluated specifically, ocular coloboma have been reported in 11 of 17 cases due to *MKS1* mutations [31] and less frequently in those due to *MKS3* mutations [30]. In general, overlap between the clinical features related to these two genes is to be expected, as the MKS1 and MKS3 gene products physically interact with a protein complex required for ciliary formation and together promote epithelial branching morphogenesis crucial for the formation of multiple organs during embryonic and fetal development [61].

Outcome and Management of MKS

Typically, MKS is considered a lethal disorder either in the prenatal or the perinatal period but milder, less classical examples of the MKS spectrum such as JSRD have been recognized in recent years, and survival is less easy to predict. As one example, a previously assigned diagnosis of MKS in Hutterites has been reassigned as JSRD based on the recognition of the molar tooth sign [62, 63]. Another overlapping disorder compatible with longer postnatal survival is renal–hepatic–pancreatic dysplasia with Dandy–Walker malformation [64, 65]. In fact, the genes *MKS3*, *CEP290, RPGRIP1L*, and CC2D2A can all cause either an MKS or a JSRD phenotype [26, 27, 47–49, 55–57]. In theory, intrafamilial variability predicts the potential for one affected sibling to have a lethal form of MKS and another sibling to have milder manifestations of JSRD.

In most classic cases of MKS, palliative care is the most appropriate approach. If survival beyond the newborn period occurs in atypical cases, management options should be pursued in accord with the wishes of the family. In practice, consideration of aggressive management options may occur in the context of overlapping disorders such as JSRD. In addition, the increasing recognition of the genetic causes of MKS will inevitably lead to the identification of milder variants where treatment options will also need consideration. Neurosurgical management may include surgical closure and reduction of the encephalocele to reduce the risk of meningitis, but this often results in hydrocephalus requiring shunting. Management of renal failure can include supportive medical measures (diet, fluids, medications), peritoneal dialysis, hemodialysis, and/or renal transplantation, assuming sufficient potential for survival. Similarly, hepatic management can include supportive medical measures, monitoring for complications of portal

hypertension and liver failure, and treatments that may include portosystemic shunting or liver transplantation.

JOUBERT SYNDROME AND RELATED DISORDERS

Overview

Historical. Joubert syndrome (JS) was first described in 1969 by Dr. Marie Joubert and colleagues [66] in four siblings from a large French–Canadian family with consanguinity traced 11 generations to a common ancestor. Based on this and subsequent reports, autosomal recessive inheritance has been demonstrated. Key features include the relatively non-specific findings of hypotonia, developmental delay/intellectual disability, and cerebellar ataxia, which usually manifests with increasing age. The distinctive mid-hindbrain malformation characterized by hypoplasia of the midline cerebellar vermis known as the "molar tooth sign" (MTS) [67] is a radiologic sign now considered pathognomonic for the disorder (Fig. 10.2a–d) [68, 69]. An irregular breathing pattern in the newborn period (tachypnea and/or apnea) and abnormal eye movements (oculomotor apraxia; OMA) are often observed [66, 70, 71]. The term "Joubert syndrome and related disorders" (JSRD) has been applied to those conditions that have the MTS in common but may also have other distinctive features [72]. One such JSRD is the condition known by the acronym COACH (Coloboma, Oligophrenia/developmental delay, Ataxia, Cerebellar vermis hypoplasia, Hepatic fibrosis) with a core feature of congenital hepatic fibrosis [73, 74], which will be highlighted in this chapter.

Prevalence. Prevalence estimates for JSRD in the United States have been in the order of 1:100,000 [75], but this is likely an underestimate given the broad spectrum of features, particularly in those with milder manifestations.

Clinical Diagnosis

Classic Joubert syndrome. The features necessary for a diagnosis of classic Joubert syndrome include the following [66, 70, 71, 75, 76]: (1) The molar tooth sign on axial views from cranial MRI imaging comprised of these three findings: cerebellar vermis hypoplasia (CVH), deepened interpeduncular fossa, and thick, elongated superior cerebellar peduncles (Fig. 10.2c, d) [67, 77]; (2) intellectual impairment/developmental delay, of variable severity; (3) hypotonia in infancy; and (4) one or both of the following (not required but supportive of the diagnosis): irregular breathing pattern in infancy

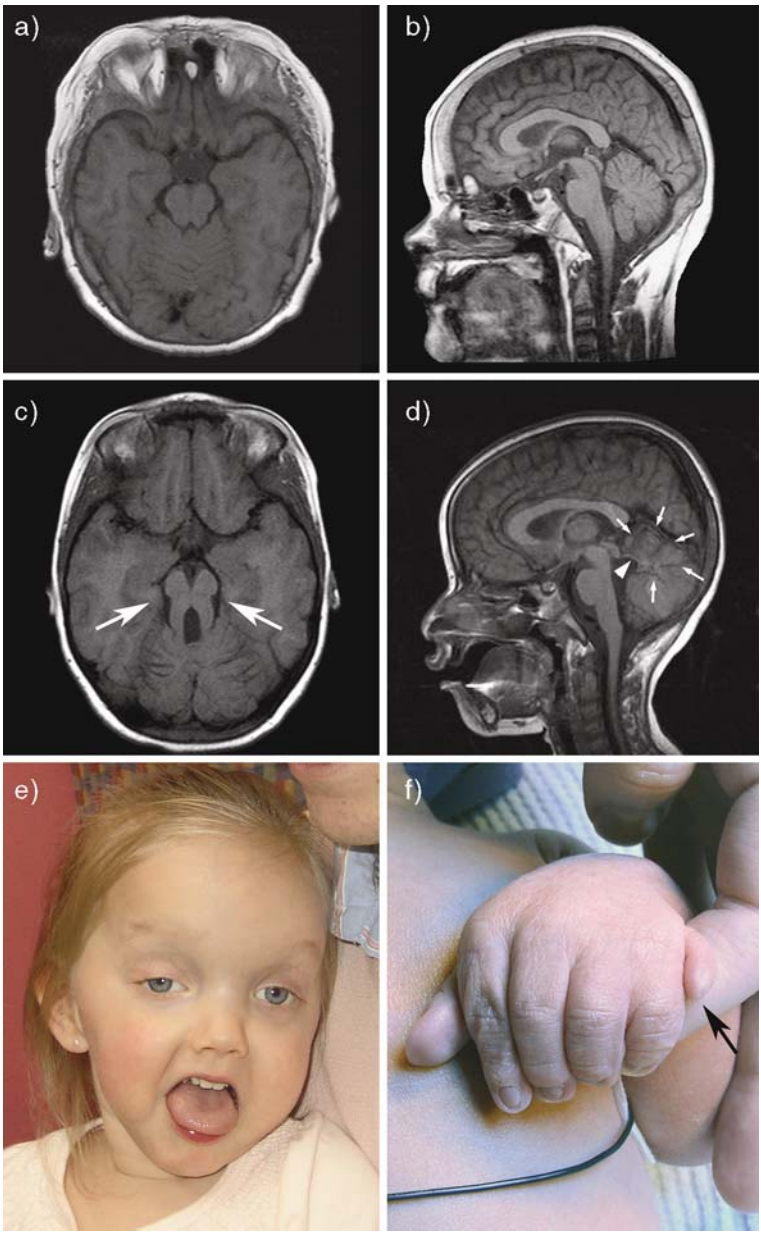

Fig. 10.2. The molar tooth sign and clinical features in an individual with JSRD/COACH syndrome. The typical appearance of the cerebellar vermis on (**a**) axial and (**b**) mid-sagittal imaging in a normal individual. (**c**) In a patient with JSRD, the molar tooth sign (MTS) is apparent (between *arrows*), reflecting a deepened interpeduncular fossa, thickened, elongated superior cerebellar

(episodic apnea and/or tachypnea, sometimes alternating) and abnormal eye movements (nystagmus, OMA).

Dysmorphic facial features are often described and may include broad forehead, arched eyebrows, eyelid ptosis, wide-spaced eyes, open mouth configuration, and facial hypotonia (Fig. 10.2e) [76, 78]. Some individuals also have polydactyly of the hands and/or feet (Fig. 10.2f).

Joubert syndrome and related disorders (JSRD). JSRD encompass classic JS as described above as well as conditions with associated features such as other central nervous system anomalies (including occipital encephalocele, corpus callosal agenesis), ocular coloboma, retinal dystrophy, renal disease (including cystic dysplasia or nephronophthisis, NPH), polydactyly, and hepatic fibrosis. When the ocular and renal systems are involved, the syndromes are collectively known as cerebello-oculo-renal syndromes (CORS) [69, 79]. An association between renal and retinal involvement has been observed in some individuals [70, 72, 73, 80]. Hepatic involvement, when coupled with renal cystic disease, has prompted inclusion of JSRD as a congenital hepatorenal fibrocystic disease [28, 81].

Central nervous system. In neuropathological studies of brains from individuals with JSRD, the radiological sign of the MTS has been shown to correspond to hypoplasia and midline clefting of the cerebellar vermis, often with inferior aplasia and superior dysplasia of the vermis, and abnormalities of the nuclei of the pons, cerebellum, and medulla [82, 83]. Absence of decussation of the corticospinal and superior cerebellar tracts has been demonstrated by diffusion tensor imaging [84], and abnormal activation patterns during motor tasks were shown by functional magnetic resonance imaging [84, 85], suggesting that the anatomical malformation has complex and striking functional outcomes. In addition to the MTS, approximately 10% of individuals with JSRD demonstrate fluid collections in the posterior fossa resembling the Dandy–Walker malformation [86]. Hydrocephalus is

Fig. 10.2. (continued) peduncles, and vermis hypoplasia. (**d**) The sagittal image from the same individual with JSRD demonstrates an abnormally positioned and elevated fourth ventricle (*arrowhead*) with hypoplastic cerebellar vermis (surrounded by five small *arrows*). (**e**) Facial features in a girl with COACH syndrome at 27 months of age showing broad forehead, arched eyebrows, strabismus, eyelid ptosis (on subject's right in particular), and open mouth configuration indicating reduced facial tone. (**f**) Left hand of an infant with JSRD with postaxial polydactyly (*arrow*). (Facial photograph used with permission of the family.)

uncommon in JSRD, with only rare patients requiring a shunt procedure for symptomatic increase in intracranial pressure [87]. Notably, occipital encephaloceles or meningoceles have been observed [55, 66, 70, 72], suggesting overlap with MKS. Other brain anomalies have included agenesis of the corpus callosum [57, 88], polymicrogyria [72, 89], and cerebellar heterotopias [70].

Cognitive impairment in JSRD is highly variable, with many children exhibiting moderately severe disability [71]. The average age of independent sitting was 19 months and the average age of walking was 4 years for those who developed these skills in one series [76]. Clinical features related to the complex cerebellar malformation include ataxia, which typically becomes apparent as children develop ambulation, and ocular, oral-motor, and speech dyspraxia. Seizure disorders have been reported in some children with JSRD, although no consistent radiologic features are predictive. A few children with autism have been reported [90, 91], although other surveys suggest that classic autism is not typical in JSRD [92]. More common are behavioral problems, often impulsivity, perseveration, and temper tantrums [93].

Hepatic: COACH syndrome. Hepatic involvement in JSRD is likely underreported, as manifestations of liver disease are usually not apparent at birth. However, with current management guidelines recommending routine screening for liver dysfunction, more children are being identified with hepatic involvement presymptomatically, and a recent estimate in a large cohort is that 9% have clinically apparent liver disease (23/251 families) [27]. COACH syndrome [MIM 216360] was first proposed in 1989 by Verloes and Lambotte in three individuals (including two siblings) to describe the combination of cerebellar vermis hypoplasia, oligophrenia (developmental delay/intellectual disability), ataxia, colobomas, and congenital hepatic fibrosis [74]. In 1999, Satran et al. [73] suggested that COACH syndrome represented a subtype of JSRD with the distinctive finding of liver involvement, and in 2004, Gleeson et al. [72] provided further evidence that the CVH in COACH syndrome represented the MTS, thereby affirming its status as a JSRD. Craniofacial features may be indistinguishable from those in the classic form of Joubert syndrome (Fig. 10.2e). A recent review of 26 previously unreported subjects with COACH syndrome demonstrated the similarities between these patients and 43 individuals reported in the medical literature with a constellation of findings consistent with COACH syndrome, based on evidence of liver disease, developmental delay/cognitive impairment, and CVH (specifically, the MTS when appropriate imaging was available) [27]. Thus, COACH syndrome is often considered equivalent to JSRD with congenital hepatic fibrosis [26, 27], although this designation has not been uniformly adopted.

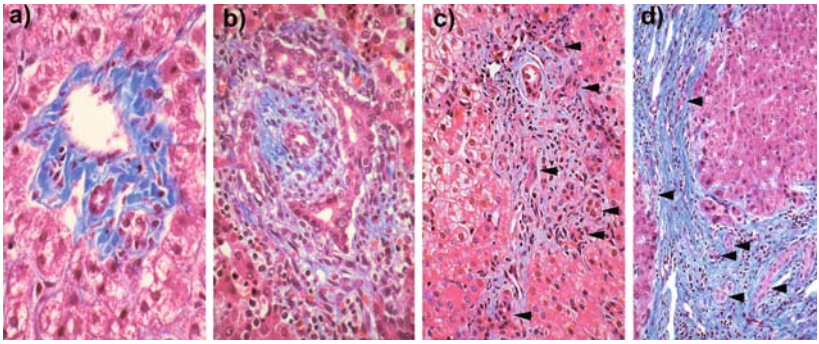

Fig. 10.3. Liver pathology in individuals with JSRD/COACH syndrome. (**a**) The normal mature portal triad contains a portal vein (*top*), a hepatic artery (*middle*), and a nearby and similarly sized bile duct (*bottom*) (trichrome, 200×). (**b**) Ductal plate malformation. The abnormal portal area in this late second trimester fetus has several ectatic and irregularly shaped bile duct structures within the portal connective tissue that surrounds the hepatic artery (trichrome, 200×). (**c**) The liver biopsy of an 11-month-old boy with JSRD/COACH syndrome shows numerous duct profiles (*arrowheads*) within a mildly fibrotic expanded portal area (trichrome, 200×). (**d**) At 10 years, the liver from this girl with JSRD/COACH syndrome was removed for transplantation and showed extensive portal-to-portal bridging fibrosis that created an irregular jigsaw pattern. A few bile ducts (*arrowheads*) are still evident within the fibrous bands (Trichrome, 150×).

The liver disease in JSRD/COACH is most typically described as congenital hepatic fibrosis (CHF) with histopathological features that include an excess of bile duct-like profiles and portal fibrosis (Fig. 10.3a–d). For patients in whom serial liver biopsies are available, the duct abnormalities have become less prominent over time, while the fibrosis has progressed with portal–portal bridging [27]. These observations suggest that, as in other organ systems, there is a spectrum of liver disease, and it is likely related to the ductal plate malformation (DPM) described in MKS, albeit often less severe [20, 22, 28]. Occasionally, cholangitis has been described in COACH subjects, with cirrhosis and chronic hepatitis reported [74, 94–97].

The clinical course of liver disease in COACH syndrome can be variable. Some JSRD/COACH patients have presented with evidence of portal hypertension, including hematemesis, esophageal varices, or portosystemic shunting, and occasionally life-threatening bleeding events [26, 27, 98–100]. Others have presented with elevated or fluctuating levels of serum transaminases [alanine transaminase

(ALT) and aspartate transaminase (AST)] or γ-glutamyl transferase (GGT). Physical examination findings may include hepatomegaly with or without splenomegaly, and many are asymptomatic. Radiologically, increased echogenicity and cysts may be apparent on liver ultrasound, and dilated intrahepatic bile ducts on liver MRI or magnetic resonance cholangiopancreatography [101]. Treatments have included medical management such as oral ursodiol and surgical intervention including sclerotherapy for varices, splenectomy, and portal shunt placement. In some cases, hepatic fibrosis has slowly progressed to end-stage liver failure and required liver with or without renal transplantation, typically in the second decade of life or beyond [26, 27, 99, 102, 103].

Renal. Renal involvement is relatively common in JSRD, having been reported in up to 30% of subjects in early surveys [70], and potentially more with long-term follow-up. Two different forms of kidney disease have traditionally been reported: cystic dysplasia and juvenile NPH [104]. Cystic dysplasia may be identified prenatally or congenitally by ultrasound changes that show multiple cysts of many different sizes in immature kidneys with fetal lobulations [70, 71, 73, 105]. This finding is characteristic of the JSRD known as Dekaban–Arima syndrome, which also includes congenital amaurosis (retinal blindness) and occasional encephalocele [72, 73, 80, 105, 106]. The other, more common renal disorder in JSRD is juvenile NPH, characterized by tubulointerstitial nephritis and tubular cysts concentrated at the cortico-medullary junction [107]. The presentation is typically urine-concentrating defects in the first or the second decade of life manifested by polydipsia, polyuria, anemia, and growth failure, with a rise in serum creatinine around 9 years and progression to end-stage renal disease by ∼13 years of age [104, 107, 108]. The less common infantile and adolescent forms of NPH have progression to ESRD by 1 and 19 years, respectively. Increased renal echogenicity is typically visualized on ultrasound early during disease progression, with small, scarred kidneys observed only later in the course of the disease. Of note, mutations in at least nine genes have been identified in individuals with NPH, about 20% of whom have extrarenal manifestations [104], including cerebellar malformations, oculomotor apraxia (OMA), and retinal dystrophy (this latter combination of NPH and retinitis pigmentosa is known as Senior–Løken syndrome) [54, 72, 73, 107, 109]. Although traditionally, cystic dysplasia and NPH have been considered distinct entities, in at least one report, the cystic kidneys from deceased individuals with Dekaban–Arima syndrome demonstrated pathologic changes typical of NPH, rather than the dysplasia considered characteristic of this JSRD subtype [110]. It is likely that the renal disease in JSRD is part of a continuum of manifestations with a common

etiology involving impairment of ciliary proteins leading to tubular dysfunction.

In rare cases, individuals have been described with JS and congenital renal disease characterized by enlarged kidneys and microscopic cysts distributed throughout the cortex and the medulla in the context of infantile hypertension, in part resembling the renal disease of autosomal recessive polycystic kidney disease (ARPKD). Several of these patients have *MKS3* mutations akin to those with COACH syndrome [95].

In two separate series, 42 and 46% of COACH patients had renal disease [26, 27], whereas a review of prior reports of COACH syndrome revealed 77% (33/43) with renal disease [27]; however, the average age of subjects was older in the literature review, and ascertainment bias may also be a factor. The spectrum of renal disease in COACH is similar to other JSRD.

Ophthalmologic. There is a broad spectrum of ocular findings in JSRD. Abnormalities of ocular motility are very common and include most typically nystagmus, which can be horizontal, torsional, and/or rotatory, and OMA, characterized by difficulty in smooth pursuits, with dysconjugate eye movements and head thrusting to compensate for poor saccade initiation [93, 111]. Nystagmus and OMA are often present at birth and may improve with age. Other common ocular anomalies that may require medical or surgical treatment include strabismus, amblyopia, and ptosis. Third nerve palsy and Duane anomaly have also been observed [93]. There are two basic forms of retinal disease: severe congenital blindness with a flat electroretinogram (ERG) recording known as Leber congenital amaurosis [111, 112] and a later-onset pigmentary retinopathy that may initially manifest with night blindness in childhood and have variable progression [70, 71]. The congenital ocular developmental defect of coloboma is present in a subset of individuals with JSRD and typically involves the choroid and the retina [70] and rarely the iris. Many children with unilateral or bilateral colobomas also develop liver disease, as described under COACH syndrome [27, 72, 74]. Ocular colobomas are not a necessary feature of COACH syndrome [102], and in fact, 71% of individuals with COACH syndrome in a large cohort had colobomas, while 64% of those culled from the literature had this finding [27]. However, retinal dystrophy is not typical in COACH syndrome, in contrast to other JSRD, occurring in 0–8% of subjects [27].

Skeletal/limbs. The skeletal findings in JSRD have ranged from cone-shaped epiphyses to polydactyly. The cone-shaped epiphyses have most often been observed in children with Mainzer–Saldino syndrome (cerebellar ataxia with NPH and retinal dystrophy) [113]. Polydactyly,

observed in 16% of subjects in one survey [70], is often postaxial (Fig. 10.2f), although preaxial polydactyly of the hands or great toes has been observed. Polydactyly is rarely seen in COACH syndrome (0–5%), somewhat unexpected, given the overlapping phenotypic features and genetic causes with MKS, in which polydactyly is common [26, 27]. The finding of mesaxial polydactyly (occurring between the middle digits and often associated with Y-shaped metacarpals) has been described in individuals with the oral–facial–digital type VI syndrome (OFD VI, also known as Varadi–Papp syndrome), a JSRD with oral frenulae, lingual lobules or hamartomas, and typical craniofacial findings such as wide-spaced eyes and midline lip groove [72, 114]. Small thoracic cavities with rib abnormalities in the spectrum of Jeune asphyxiating thoracic dystrophy have been described in children with the MTS and a probable JSRD (personal observations), suggesting overlap between these ciliary conditions. With age, some children with JSRD develop scoliosis due to abnormal tone.

Other. Some children with JSRD have pituitary hormone dysfunction such as isolated growth hormone or thyroid hormone deficiency, or more extensive panhypopituitarism, with some males demonstrating secondary micropenis [56, 68, 115]. Normal progression through puberty has been described in both sexes. Anecdotal evidence exists for a significant risk to develop obesity, particularly with increasing age and onset of adolescence (personal observation). Situs defects have also been described [116].

Prenatal diagnosis. There are several options for prenatal diagnosis in pregnancies to couples who have had a prior affected child. If the disease-causing mutations have been identified, prenatal diagnosis by DNA testing is possible. For other at-risk pregnancies, prenatal imaging via ultrasound and/or fetal MRI is the best and most practical diagnostic option. Extracranial anomalies such as polydactyly or renal cysts and major structural CNS malformations such as encephalocele may allow prenatal diagnosis of JSRD as early as the first trimester where a prior history is present [117] or may suggest the diagnosis in the absence of a prior history. Early diagnosis is more difficult when extracranial abnormalities are not present, because CVH cannot be reliably diagnosed until 18–20 weeks of gestation [118], and the MTS has not been observed prior to 27 weeks of gestation [119]. In the absence of a family history, prenatal diagnosis is possible but challenging, given the spectrum of outcomes for isolated CVH identified prenatally [120]. An imaging protocol for prenatal diagnosis has been proposed; however, its sensitivity and specificity for JSRD have not been systematically evaluated [121].

Molecular Genetics of JSRD

Seven causative genes and two additional loci. Mutations in the *AHI1, NPHP1, CEP290, TMEM67/MKS3, RPGRIP1L, ARL13B*, and *CC2D2A* genes have been identified in subjects with JSRD (Table 10.2). Two additional loci have been identified by linkage analysis, but the causative genes remain unknown. One of these loci, JBTS1, was mapped to 9q34 in two consanguineous Arab families in which several affected individuals developed retinal dystrophy, but renal disease has not been described [88, 122, 123]. The second locus, JBTS2 (also known as CORS2) on chromosome 11, was simultaneously identified in consanguineous families of varying ethnicities [79, 124]. The phenotype is variable, with some developing renal disease, others retinal dystrophy, and encephaloceles, colobomas, and polydactyly also described [88]. It is possible that the JBTS2 locus is the same as the MKS2 locus associated with MKS, which has been mapped to a nearby region on 11q13 [69].

AHI1. Mutations in the *AHI1* (Abelson helper integration site 1) gene have been identified in JSRD [89, 125–129]. In one survey of 117 subjects, 11% had causative *AHI1* mutations [126], whereas another survey of 137 subjects identified 7.3% with mutations in this gene [128]. Retinal dystrophy is a common finding, occurring in ∼80% of those with *AHI1* mutations [69]; and renal disease consistent with NPH has been observed in some subjects [126, 127]. However, no subjects with JSRD and *AHI1* mutations have exhibited features of encephalocele, polydactyly, or liver fibrosis. Other CNS anomalies including polymicrogyria, corpus callosum anomalies, and frontal lobe atrophy have been described in some individuals with *AHI1* mutations [89].

NPHP1. The first gene associated with JSRD, *NPHP1* (nephronophthisis 1) was previously identified as causing juvenile NPH [104, 130, 131]. *NPHP1* resides within a region of ∼290 kb flanked by large inverted repeat elements on chromosome 2q13 that is homozygously deleted in JSRD or NPH [132]; a few individuals have a deletion coupled with a point mutation in *NPHP1* [133]. Some individuals with the common deletion have congenital OMA known as Cogan syndrome [134], and others have Senior–Løken syndrome with retinal impairment [135, 136]. The deletion appears to be identical in the different disorders. The *NPHP1* mutation detection rate for the purely renal disorder is approximately 20–30% [104, 107], in contrast to a rate of approximately 1–3% for individuals with JSRD based on several case series [69, 132, 135]. Clues as to how deletion of the *NPHP1* gene can lead to diverse phenotypes are emerging; several individuals with NPH who also had neurological impairment or MTS had homozygous deletions

Table 10.2
Molecular genetics of Joubert syndrome and related disorders (JSRD)

Locus name	*Gene symbol*	*Chromosomal locus*	*Protein name*	*Types of mutations*	*MTS*	*Liver*	*Col*	*RD*	*Renal*	*PD*	*OE*	*Key clinical features*	*Proportion of JSRD*
JBTS1	Unknown	9q34.3	Unknown	NA	++	–	–	+	–	–	–	Retinal	?
JBTS2	*CORS2*	11p12–q13.3	Unknown	NA	++	–	+	+	+	+	+	Variable	?
JBTS3	*AHI1*	6q23.3	Jouberin	Nonsense, FS, splice, (missense)	++	–	+/–	++	+	–	–	Retinal	7–11% [126, 128]
JBTS4	*NPHP1*	2q13	Nephrocystin–1	Large homozygous deletion (missense)	+/–	–	–	+	++	–	–	Renal (retinal)	1–3% [69, 132, 135]
JBTS5	*CEP290*	12q21.3	Centrosomal protein of 290 kDa	Nonsense, FS, splice, (missense)	++	+	+	++	++	–	+	Retinal, renal	~10% [137, 138]
JBTS6	*TMEM67/MKS3*	8q21.1–q22.1	Meckelin	Missense, splice	++	++	+	–	+	+/–	+	Liver involvement	8–10% [27, 60]

JBTS7	*RPGRIP1L*	16q12.2	RPGR-interacting protein 1-like protein	Missense, FS, splice, nonsense	++	+	+/–	+/–	++	+	+	Renal	2–4% [27, 55, 69, 139]
JBTS8	*ARL13B*	3q11.2	ADP-ribosylation factor-like 13B	Missense (non-sense)	++	–	–	+	–	–	+	Very rare	<1% [140]
JBTS9	*CC2D2A*	4p15.3	Coiled-coil and C2 domains-containing protein 2A	Missense, nonsense, splice, FS	++	+	+	+	+	–	+	Variable	8–9% [27, 57]

–, Not described; +/–, rare; +, present in some cases; ++, common; ?, unknown.

Col, coloboma; FS, frameshift mutation; MTS, molar tooth sign; NA, not applicable; OE, occipital encephalocele; PD. polydactyly; RD, retinal dystrophy.

of *NPHP1* in combination with a heterozygous change in *AHI1* or *CEP290*, suggesting that the addition of multiple genetic defects may cause more severe phenotypes [133].

CEP290. The large *CEP290* gene has been associated with multiple clinical disorders ranging from isolated Leber congenital amaurosis to JSRD and MKS. The majority of affected individuals with JSRD due to *CEP290* mutations display retinal dystrophy or congenital blindness, and many also exhibit renal disease consistent with NPH or renal cortical cysts [137, 138]. *CEP290* was identified as causative in 7 of 96 individuals with JSRD (7%) in one series [137] and in about half of cases of JSRD with both retinal and renal involvement [49, 50, 53, 54, 69, 116, 133]. Findings in some affected individuals have included ocular colobomas, encephaloceles, septal heart defects, hepatic disease, and situs anomalies. Mutations in this gene have also been reported in individuals with Senior–Løken syndrome (retinal dystrophy plus NPH), without neurological impairment [49, 54, 133], and in those with MKS [47, 48]. Mutations in *CEP290* have been identified in at least 21% of patients with isolated Leber congenital amaurosis in two European series [52, 53]; in these individuals, a common intronic point mutation results in the production of some residual wild-type protein, hypothesized to account for a milder phenotype than do the complete loss-of-function mutations found in JSRD and other more severe phenotypes.

TMEM67/MKS3. The *TMEM67/MKS3* (transmembrane protein 67/Meckel syndrome, type 3) gene encodes a 995-amino-acid protein (Fig. 10.4) that is known to interact with MKS1 and plays a role in primary cilium formation [61]. Several groups have identified mutations in this gene in subjects with JSRD [26, 27, 60, 95]. In a large cohort of 232 unselected families with JSRD, 19 had mutations identified in the *MKS3* gene (8%), with the majority of these manifesting hepatic disease. In fact, for those with a clinical diagnosis of COACH syndrome, the prevalence of *MKS3* mutations was 83% (19/23) of subjects in one series [27] and 57% (8/14) in another [26]. In contrast to the types of *MKS3* mutations identified in MKS, which are typically compound heterozygous missense and truncating mutations or homozygous splice-site mutations [30, 46, 58, 60], the disease-associated mutations in JSRD/COACH tend to be missense mutations or the combination of a missense mutation and a splice-site or nonsense mutation distributed throughout the gene, with very few mutations overlapping with those seen in MKS [26, 27] (Fig. 10.4). This pattern suggests that more severe loss-of function mutations are more likely to be associated with the lethal MKS syndrome. The identification of only a single *MKS3* mutation in several COACH subjects suggests the possibility that mutations

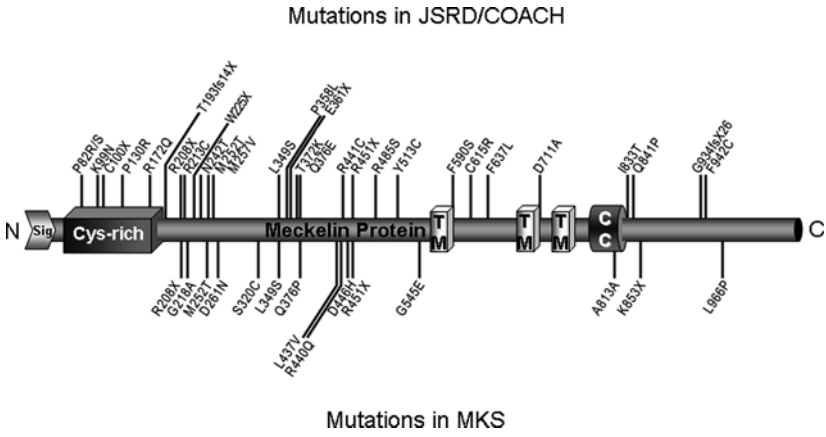

Fig. 10.4. Amino acid changes in the Meckelin protein resulting from *MKS3* mutations identified in individuals with JSRD and MKS. The horizontal bar represents the 995-amino-acid protein Meckelin with associated protein domains. Mutations identified in individuals with JSRD or COACH syndrome are indicated above the bar, and mutations identified in individuals with MKS are indicated below the bar. Traditional one-letter codes for amino acids are used, with X indicating a stop codon resulting from a nonsense mutation and fs indicating a frameshift mutation resulting from deletion or duplication with downstream stop codon "X" at the subsequent number of codons. C, carboxy terminus of protein; CC, coiled-coil domain; Cys-rich, cysteine-rich domain; N, amino terminus of protein; Sig, signaling peptide; TM, transmembrane domain.

in other JSRD- or MKS-associated genes may be contributing to the disorder, a phenomenon known as oligogenic inheritance known to occur in JSRD and related ciliary disorders [26, 27]. For JSRD individuals with ocular colobomas, regardless of liver status, 53% had mutations in *MKS3* [27]. Thus, for a patient with JSRD and either liver disease or coloboma or both, initial testing of the *MKS3* gene is recommended.

RPGRIP1L. Mutations in the *RPGRIP1L* gene were first identified in patients with the renal form of JSRD [55, 56]. The phenotypic spectrum includes predominantly renal disease (typically, NPH), with some affected individuals manifesting occipital encephaloceles and polydactyly, and rarely retinal disease or colobomas; a few individuals have had scoliosis, clubfoot, or pituitary hormone deficiency [55, 56, 115, 139]. Hepatic fibrosis has been described in a subset of individuals with JSRD due to *RPGRIP1L* [27, 115]. In addition, a few COACH families have had mutations in *RPGRIP1L* [55, 115]. The *RPGRIP1L* gene also causes MKS, with a tendency toward more severe mutations, whereas

mutations predicted to have a milder effect on protein function are more typical in JSRD [56, 115]. Overall, estimates of the prevalence of *RPGRIP1L* mutations in the cerebello-renal form of JSRD range from ~9% [115] to 12% [139], with estimates of 1–4% in unselected cohorts [27, 55, 69, 139].

ARL13B. This small gene encodes a protein that is a member of the Ras GTPase family and localizes to the primary cilia of cerebellar neurons, kidney, and retina. Two families have been identified with mutations in this gene, and their phenotype is most typical of classic JS, with two affected individuals also exhibiting occipital encephalocele and one exhibiting pigmentary retinopathy [140].

CC2D2A. The *CC2D2A* gene was first identified in an extended consanguineous Pakistani family with autosomal recessive cognitive impairment and retinitis pigmentosa [141]; later review of cranial MRI scans from two of the affected children revealed the MTS, thereby establishing the phenotype as JSRD [57]. The phenotype in affected individuals has ranged from classic JS to JS with encephalocele to the COACH phenotype with coloboma, liver, and kidney involvement [57]. This gene has also been identified as causative in MKS [29]. Overall, it is estimated to cause almost 10% of JSRD [27, 57].

Genetic heterogeneity of JSRD. These seven genes account for an estimated 50% of causative mutations in JSRD [27, 69] (Table 10.2). The observation of consanguineous families that do not map to any of the known loci suggests the presence of additional JSRD genes that have not been identified [88, 142]. It is clear that the same gene can cause multiple different phenotypes and that several different genes can be associated with the same clinical features. For example, three genes (*MKS3*, *RPGRIP1L*, and *CC2D2A*) account for over 95% of mutations in the COACH population [27]. Clinical features can vary between affected siblings within the same family, apparent even in the original family described [66]; this intrafamilial variability supports the existence of genetic modifiers and epistatic effects. The emerging genotype–phenotype correlations that have been described may simplify the quest for a causative gene in an affected individual, although the etiology will remain elusive in up to half of all patients.

Outcome and Management of JSRD

At the time of diagnosis, a detailed cranial MRI to evaluate for the MTS is essential, as well as other evaluations that have been outlined [75]. Given the heterogeneity in JSRD and the relatively high frequency of associated medical conditions, it is difficult to make generalizations about outcomes in this population. At birth, some infants have had

severe manifestations of altered respiratory control requiring mechanical ventilation and/or tracheostomy in rare cases. For children with significant feeding difficulties related to hypotonia or dyscoordinated oromotor function, nasogastric feeding tubes or gastrostomy placement has been necessary to prevent aspiration and provide adequate caloric intake. Rarely, seizures that have proved difficult to control have compromised long-term survival; these are more likely in those with significant CNS malformations. However, the vast majority of infants and children diagnosed with JSRD survive the neonatal period and many demonstrate improvement with time in their respiratory and feeding behaviors [68]. A program of comprehensive developmental assessments with appropriate interventions including special education programs, physical, occupational, and speech therapy, and adaptive equipment as needed, has had significant benefits for many children with JSRD.

Because of the risk of later development of retinal, renal, and hepatic complications, ongoing monitoring is essential. Recommendations for surveillance and management in JSRD have been developed [75] and are available online (http://www.joubertsyndrome.com/), with the recognition that an evaluation and management plan must be individualized for each child's unique medical needs. Briefly, in addition to annual pediatric, neurological, and developmental assessments, annual ophthalmologic evaluation is recommended to monitor eye movement abnormalities, strabismus, and ptosis, with surgical correction if indicated. An annual retinal exam (with electroretinogram if indicated) is necessary to identify the onset of pigmentary retinopathy. To identify renal disease in its early stages, annual evaluation for reduced urine-concentrating ability or anemia can be accomplished by a first-morning void urinalysis for specific gravity and complete blood count; annual BUN, creatinine, abdominal ultrasound scan, and blood pressure measurement may be other useful studies to identify and monitor renal disease, which may require dialysis and renal transplantation if progressive. Annual liver evaluation by measurement of liver laboratory tests (transaminases, albumin, bilirubin, prothrombin time) and ultrasound as appropriate can detect hepatic abnormalities, with further evaluation and monitoring as needed. Surgical interventions for esophageal varices or portal hypertension and liver transplantation may be necessary for those with COACH. Individuals with JSRD are at risk for sleep apnea, both central (especially in infancy) and obstructive (related to tongue enlargement, hypotonia, and obesity), and may require medical or surgical interventions if detected by polysomnography.

CONCLUSIONS: MKS AND JSRD ARE CILIOPATHIES

Perhaps it is not surprising that the overlap in clinical features between MKS and JSRD is also reflected by the overlap of their genetic basis. Although both groups of disorders have recessive inheritance, it is clear that the heterogeneity in clinical presentation is likely the result of modifying genetic factors, or oligogenic effects [51, 133]. In addition, the gene products associated with both MKS and JSRD that have thus far been evaluated are known to localize to the primary cilium and/or the basal body apparatus that has been identified in almost all cell types [81, 143]. Primary cilia are multiprotein complexes containing microtubules, adherens proteins, and other cytoskeletal components and play a role in intraflagellar transport, cell division, tissue differentiation, establishment of body axis, growth, and mechanosensation involved in cellular signaling processes [143]. These unique organelles are essential for signal transduction processes that underlie many aspects of vertebrate development, including the PDGF receptor alpha, hedgehog, and Wnt signaling pathways [144]. The expanding group of human disorders known as ciliopathies share overlapping clinical manifestations that reflect the critical role that cilia play in the growth and differentiation of the involved tissues, ranging from the neural tube to renal, biliary, and retinal epithelium to the developing limb bud [143].

The major challenges in ciliopathy research remain identification of the causative genes and translation of research findings into practical clinical guidelines and treatments for affected individuals and families. Great strides have been made in this field over the past 5 years as more genes have been discovered. However, the genetic etiology of a significant proportion of individuals with MKS and JSRD remains to be discovered. Fortunately, genotype–phenotype correlations are emerging, and as more genes are identified, this information will allow refinement of management recommendations and targeted therapeutics for the medical complications that impact, in particular, those with JSRD. Discovery of new genes for MKS and JSRD may provide further information about the function of the primary cilium, the basal body, and the centrosome, and may also lead to valuable insights into mechanisms of cerebellar, renal, retinal, and hepatobiliary development.

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11 Bardet–Biedl and Jeune Syndromes

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CONTENTS

Summary

Congenital fibrocystic liver diseases (CFLD) are a heterogeneous group of diseases that include a spectrum of features ranging from hepatic fibrosis, intrahepatic biliary tract dilatation to extrahepatic biliary tract dilatation, and liver cysts. CFLD frequently occur in association with renal disease such as autosomal recessive and autosomal dominant polycystic kidney disease (ARPKD, ADPKD) and nephronophthisis (NPHP). Recent insight into the molecular mechanisms underlying both disorders has demonstrated an important role for the primary cilium, a cellular sensory organelle. Cholangiocyte cilia play a regulatory role in bile formation through osmosensory, chemosensory, and mechanosensory functions while dysfunction of cholangiocyte cilia can result in cystic liver disease. Mutations in genes encoding components of the primary cilium can result in pleiotropic phenotypes such as that seen in Bardet–Biedl syndrome (BBS) and Jeune's asphyxiating thoracic dystrophy (JATD). In the

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following chapter, we present an overview of the clinical features of both these disorders and provide a summary of recent advances in the molecular genetics underlying both of these disorders.

Key Words: Congenital fibrocystic liver disease, Primary cilium, Bardet–Biedl syndrome, Jeune's asphyxiating thoracic dystrophy

INTRODUCTION

Overview

Congenital fibrocystic diseases (CFD) of the liver are a heterogeneous group of disorders that are characterized by a spectrum of biliary dysgenesis that includes congenital hepatic fibrosis, bile duct dilatation, and cyst formation [1]. Hepatic cysts are lined by specialized biliary epithelial cells known as cholangiocytes. The concept of cholangiociliopathies first evolved with the observation that patients with CFD frequently have other systemic features including renal diseases such as autosomal recessive polycystic kidney disease (ARPKD), autosomal dominant polycystic kidney disease (ADPKD), or nephronophthisis (NPHP) [2, 3]. Ciliary dysfunction has been shown to underlie the pathogenesis of these cystic disorders following the identification and localization of fibrocystin and nephrocystins, the genes mutated in ARPKD and in NPHP, respectively, to the primary cilium of cholangiocytes and renal tubular epithelial cells [4–9]. Cilia, projecting from the cholangiocyte cell surface under normal physiological conditions, regulate bile formation through mechanosensory, osmosensory, and chemosensory cues [10–12]. Defects in cholangiocyte ciliary structure and/or their integrated transducing function lead to a decrease in intracellular calcium and increased cAMP, causing cholangiocyte hyperproliferation, abnormal cell–matrix interactions, and altered fluid secretion/absorption which can result in hepatic cystogenesis [12].

Besides the association with cystic kidneys, CFD also occur as part of the pleiotropic phenotypes of Bardet–Biedl syndrome (BBS), Alström syndrome (AS), Meckel syndrome (MKS), Ivemark syndrome (HPRS), and Jeune's asphyxiating thoracic dystrophy (JATD) [13]. Mutations in genes encoding primary ciliary components including their basal body counterparts have since been identified in all these pleiotropic diseases, thereby denoting them as "ciliopathies." Since their discovery, several studies in both lower flagellated organisms such as *Chlamydomonas* [14] and *Caenorhabditis elegans* [15], as well as in transgenic mouse models expressing mutated ciliary genes, have successfully highlighted the crucial role of cilia during organogenesis and ciliary dysfunction in disease.

The aim of this chapter is to provide an overview of the pleiotropic phenotypes of two ciliopathies, namely BBS and JATD, and to highlight recent advances in the molecular genetics underlying both ciliopathic disorders.

Structure and Function of Cilia

In order to explain how a mutation in a single ciliary gene can result in heterogeneous phenotypes, it will be necessary to understand the structure and function of the cilium. Projecting from the cell surface, cilia are microtubule-based hair-like projections with motile and sensory functions which are expressed on almost all vertebrate cells and show remarkable conservation from protozoa to humans (Fig. 11.1) [16]. The ciliary backbone comprises the microtubular backbone and develops from and is anchored to a specialized centriole called the basal body which acts as a microtubule organizing center for its ciliary counterpart. The ciliary axoneme consists of nine doublet microtubules that originate at the triplet microtubules of the basal body centriole and extend the length of the cilium. Cilia fall into two broad categories: those that are motile and those that are not. Most motile cilia have an additional central microtubule pair ("9+2" microtubule arrangement) which are important for establishing fluid flow across their surface whereas primary cilia are usually immotile and lack a central pair ("9+0" microtubule arrangement). Cilia are assembled and maintained by intraflagellar transport (IFT) proteins which utilize microtubular motor proteins, kinesin-II. These proteins mobilize ciliary components such as receptors and dynein motors to carry proteins back to the basal body [17]. Components of the ciliary signaling machinery that have been identified include ligands and effectors of the hedgehog, Wnt, and platelet-derived growth factor receptor (PDGFR) signaling pathways. Following signal reception, the ciliary signal controls the balance between cellular differentiation, cell division, and apoptosis through the regulation of these key developmental pathways [18–20]. In the liver, cholangiocyte cilia are positioned to detect changes in bile flow, composition, and osmolality and have been shown to possess mechanosensory, chemosensory, and osmosensory functions [10–12, 21] (Fig. 11.2). Defective cholangiocyte ciliary function can lead to cholangiocyte hyperproliferation and hepatic cystogenesis, thereby highlighting the physiological importance of normal ciliary function in the cholangiocyte. The cholangiociliopathies encompass a heterogeneous phenotype whereby massive hepatic cysts are a feature of ADPKD, while major features of ARPKD and MKS include biliary dysgenesis, hepatic fibrosis, and cystogenesis. In contrast, BBS and NPHP are associated with

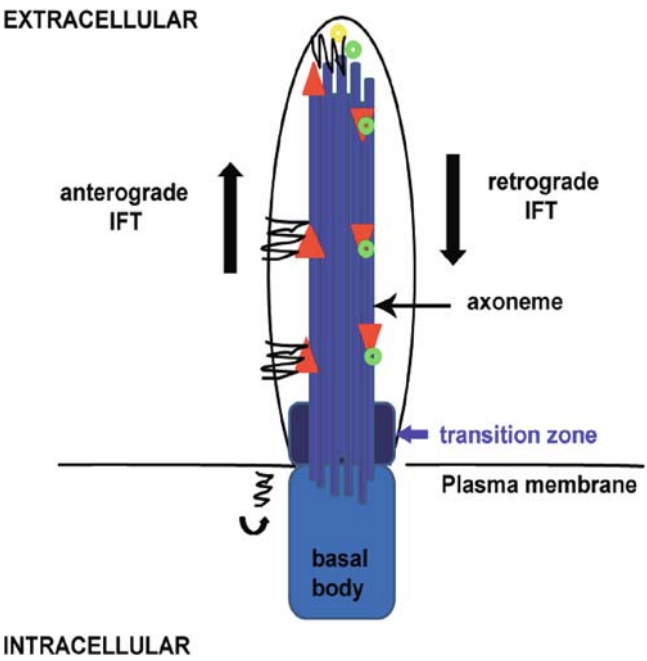

Fig. 11.1. Structure and function of the primary cilium. The primary cilium is a hair-like projection that extends from the plasma membrane into the extracellular space. Comprising the microtubular backbone, the ciliary axoneme develops from and is anchored to a specialized centriole called the basal body which acts as a microtubule organizing center for its ciliary counterpart. The ciliary axoneme consists of nine doublet microtubules that originate at the triplet microtubules of the basal body centriole and extend the length of the cilium. Localized within the ciliary membrane are various cilia-specific receptors, ion channels, and signaling molecules (e.g., *Shh*) which may be transported bidirectionally by intraflagellar transport (IFT) proteins.

congenital hepatic fibrosis and are attributed to malformation of the ductal plate during biliary tract development whereby the role of cholangiocyte ciliary signaling is currently undefined [22–24].

BARDET–BIEDL SYNDROME

Overview

A visually impaired obese girl with intellectual disabilities was first reported by Laurence and Moon in 1865 [25]. Following this initial observation, in 1920 George Bardet described the triad of retinitis pigmentosa, polydactyly, and central obesity. Two years later Arthur Biedl

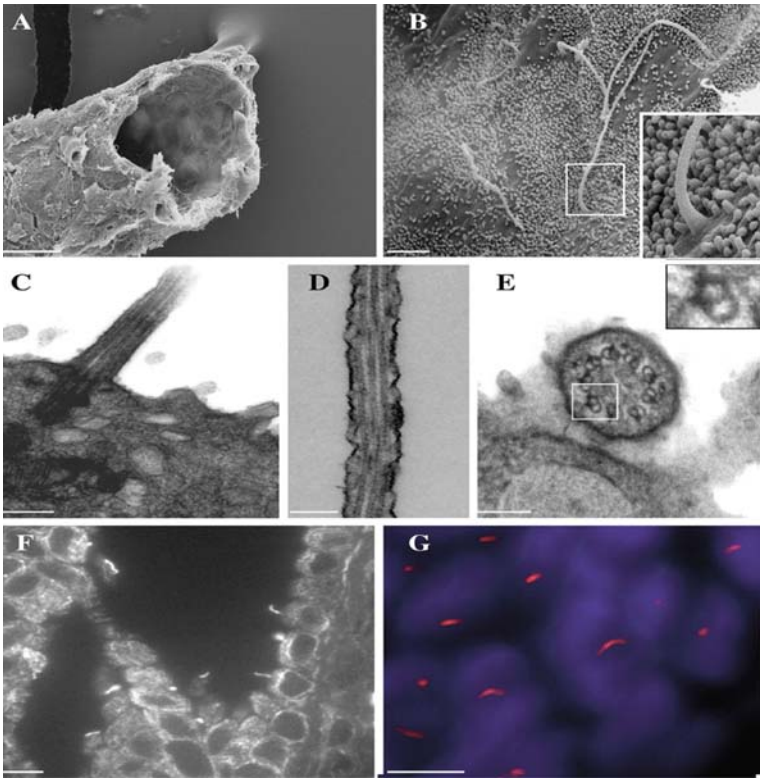

Fig. 11.2. Cholangiocyte cilia. (**a**) Low power scanning electron micrograph of bile duct. (**b**) High-power scanning electron micrograph of ciliated cholangiocytes lining the bile duct. Inset demonstrates higher power magnification of cholangiocyte cilium projecting from the cholangiocyte cell membrane. (**c**) Transmission electron micrograph demonstrating a longitudinal view of the cholangiocyte cilium. (**d**) Higher power magnification demonstrating the longitudinal arrangement of the axonemal microtubules. (**e**) Transmission electron micrograph demonstrating the "9+0" microtubular pattern of the cholangiocyte primary cilium. (**f**) Cilia are stained with alpha-acetylated tubulin antibody, a cilial marker. (**g**) Immunofluorescence confocal microscopy of murine primary cholangiocyte cilia. In (**g**), cilia have been stained with acetylated tubulin and rhodamine-conjugated secondary antibodies, respectively. DAPI (*blue*) was used to stain nuclei. (**a–g**) have been reprinted from Masyuk et al. [2006] with permission from publisher.

contributed two more cardinal features of hypogonadism and mental retardation to the syndrome that would later be known as Bardet–Biedl syndrome [26, 27]. It is inherited predominantly in an autosomal recessive pattern although the coexistence of multiple mutant alleles in some

patients causes it to behave as an oligogenic disorder. Diagnostic criteria for BBS were redefined in 1999 by Beales et al. and include learning difficulties, retinitis pigmentosa, obesity, male hypogonadism, and renal anomalies as primary criteria [28]. Secondary features of BBS include speech delay, strabismus, cataracts, brachydactyly, syndactyly, nephrogenic diabetes insipidus, ataxia/poor coordination, congenital heart disease, left ventricular hypertrophy, situs inversus, anosmia, and hepatic fibrosis [28]. The diagnosis of BBS is often made in late childhood when patients present with recently diagnosed renal failure occurring in the context of visual impairment and a history of hyperphagia and obesity. The incidence of BBS is estimated to be 1 in 100,000 individuals in European populations but is higher in consanguineous populations (e.g., North Africa and the Middle East) or geographically isolated areas. Clinical manifestations of BBS will be reviewed in the following sections. In addition, we will highlight recent advances underlying the molecular mechanisms which account for phenotypic heterogeneity in patients with BBS.

Clinical Features of BBS

In the following section, we describe in detail the cardinal manifestations of BBS (Fig. 11.3) and summarize the secondary features of BBS that have since been described in Table 11.1.

Rod–Cone Dystrophy

Rod–cone dystrophy, also known as retinitis pigmentosa (RP), represents one of the six primary clinical features of BBS (Fig. 11.3). Occurring in over 90% of patients with BBS, RP often begins in childhood [28, 29] and frequently in the context of other BBS features such as postaxial polydactyly and renal disease. Marked phenotypic variation can occur in BBS where postaxial polydactyly may represent the only accompanying feature with RP [30]. Similarly, a spectrum of RP can occur whereby bone spicule formation may be the only feature of RP with atrophic changes often predominating [31, 32]. Early in the disease process, maculopathy with or without peripheral retinal degeneration can be observed in BBS and can range from a diffuse to a bull's eye pattern [32, 33]. Vascular attenuation may often accompany maculopathic changes and may be severe [32]. By the second to third decade of life, legal blindness affects about 75% of affected individuals [28, 34, 35]. Moderate to severe visual field loss may occur and has been reported to occur at an annual loss of up to 3° per year during adolescence [36]. Accompanying electroretinographic findings often include severely reduced or extinguished responses with the

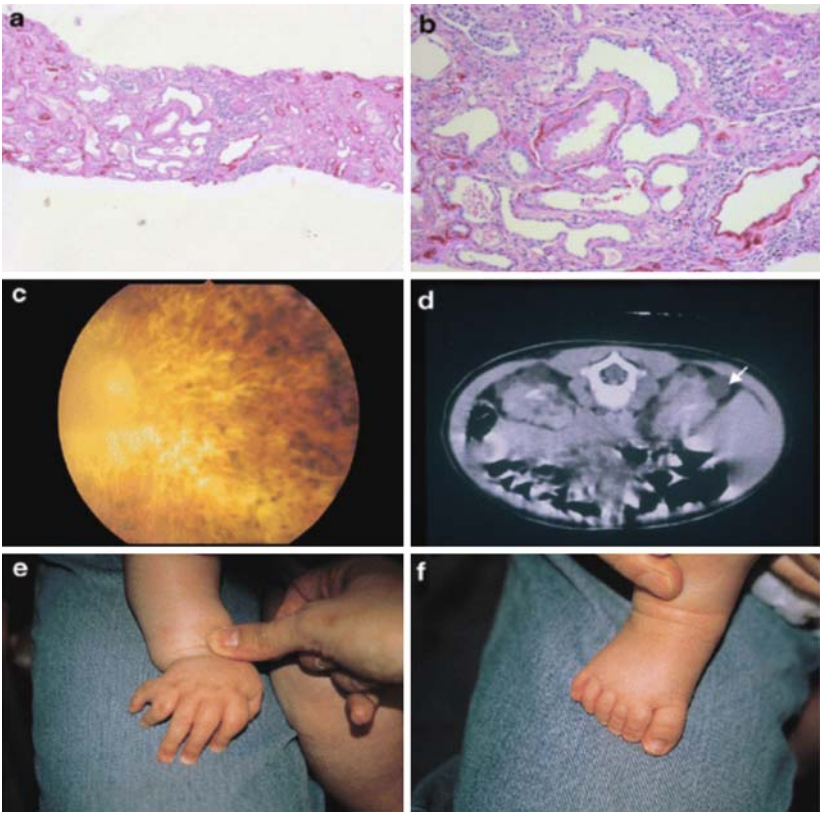

Fig. 11.3. Cardinal manifestations of Bardet–Biedl syndrome. (**a**, **b**). Histological findings of renal parenchymal cysts in a renal biopsy from a patient with Bardet–Biedl syndrome. (**c**) Fundoscopy of the same patient demonstrating pigmentary changes in the peripheral retina, consistent with a diagnosis of retinitis pigmentosa. (**d**) Fluid-filled cysts in the kidney (*white arrow*) are observed in a CT of the abdomen in a patient with BBS. (**e**, **f**) Polydactyly of the hands (**e**) and feet (**f**) in a patient with BBS.

pattern being described as rod–cone in some and cone–rod in others [36]. Recent evidence in a murine *Bbs4* transgenic model suggests that defects in the transport of phototransduction proteins occur from the inner to the outer segments of photoreceptors before cell death [12, 37]. Additionally, defects in synaptic transmission from the photoreceptors to secondary neurons of the visual system occurs in *Bbs4* null mice, thereby suggesting multiple functions for *Bbs4* in photoreceptors [12]. Other ocular findings described in BBS include strabismus, cataracts, astigmatism as well as iris and chorioretinal colobomata which are also features of Biemond syndrome [31].

Table 11.1
Diagnostic criteria for BBS

Features	*Prevalence (%)*	*Related features*
Primary features		
Rod–cone dystrophy	93	Astigmatism, strabismus, cataracts, color blindness, macular oedema and degeneration, optic atrophy
Postaxial polydactyly	69	May include all four limbs (21% patients) or hands only (9%), feet only (21%). Brachydactyly (46%) and syndactyly (9%)
Truncal obesity	72	Mean BMI (males, 31.5 kg/m^2; females 36.6 kg/m^2)
Hypogonadism	98	Hypogenitalism, cryptorchidism (9% males); oligomenorrhoea
Renal anomalies	24 (52 investigated)	Renal parenchymal cysts (10%); calyceal clubbing (10%); scarring (12%); renal dysplasia (5%); unilateral agenesis (4%); renal calculi (2%); vesicoureteric reflux (9%), bladder obstruction (4%); hydronephrosis (4%); horseshoe kidney (2%); ectopic kidney (2%)
Secondary features		
Speech disorder	54	
Developmental delay	50	Delay in walking (52%); delayed pubescence (31%, males)
Behavioral abnormalities	33	Emotional immaturity, outbursts, disinhibition, depression, lack of social dominance, obsessive compulsive behavior
Ataxia	40	
Diabetes mellitus	6	

Congenital heart defects	7	Aortic stenosis, patent ductus arteriosus, cardiomyopathy
Liver disease		Hepatic fibrosis
Hearing loss	21	Predominantly conductive but also sensorineural
Dysmorphism		Deep set eyes, hypertelorism, long philtrum, thin upper lip, anteverted nares, prominent forehead with male onset early balding
Situs inversus	Unknown	
Hirschsprung's disease	Unknown	
Polyuria, polydipsia		Non-renal aetiology
Dental crowning		High arched palate, hypodontia, small roots
Anosmia	60	

Modified from Tobin et al. [62].

Obesity

Obesity is a consistent central feature of BBS reported to occur in 72% of cases with the mean body mass index (BMI) in females estimated to be 31.5 mg/m^2 while in males is 36.6 mg/m^2 [28, 38]. While little is known about the biochemical and physiological abnormalities leading to energy imbalance in BBS, it is thought that the obesity associated with BBS is caused by a combination of increased food intake and decreased energy expenditure. Both human and murine studies have supported this concept. Lower levels of physical activity have been demonstrated in subjects with BBS compared to healthy control subjects despite comparable body mass indices [39], while recent findings in murine models of BBS have demonstrated that the development of obesity in BBS is associated with increased food intake and decreased locomotor activity [40]. Defects in leptin action have been shown to underlie the development of obesity in three murine models of BBS with null mutations in *Bbs2*, *Bbs4*, and *Bbs6* [40]. Leptin under normal physiological circumstances suppresses appetite and increases energy expenditure by activating leptin receptors on specific neurons. Within the hypothalamic arcuate nucleus, leptin activates a catabolic pathway via the propiomelanocortin (POMC) neurons and inhibits an anabolic pathway via the neuropeptide Y (NPY) neurons. In murine *Bbs*, high circulating levels of leptin have been demonstrated and exogenous leptin administration fails to decrease body weight and food intake there by suggesting that leptin resistance likely underlies the development of obesity in *Bbs* [40]. Furthermore, *Pomc* gene expression is reduced in *Bbs*-null mice, thereby suggesting that *Bbs* proteins lead to leptin resistance by impairing POMC function which may be due to underlying aberrant ciliary signaling mechanisms. Future investigations will be required to delineate the molecular mechanisms underlying leptin resistance in murine models of *Bbs*.

Postaxial Polydactyly

Polydactyly is one of the cardinal features of *bbs* and may involve all four limbs (21% cases) or the hands or feet alone. Recent studies evaluated fin bud patterning in zebrafish *bbs* morphants and demonstrated altered Sonic hedgehog (*Shh*) expression and subsequent changes to fin skeletal elements [41]. Similarly, Tobin and colleagues demonstrated *shh* signaling defects downstream of *ptc1* and defective *gli3* processing in *bbs6* and *bbs8* morphant zebrafish [42]. While polydactyly has not been a feature of murine *Bbs* models [37, 43–45], *Shh* is expressed in the posterior vertebrate limb bud mesenchyme and mediates functions

of the zone of polarizing activity (ZPA) that plays a critical role in tetrapod limb/fin bud patterning [46]. Therefore, BBS proteins likely mediate limb patterning via ciliary-dependent *Shh* signaling.

Renal Manifestations

Renal malformations frequently occur in patients with BBS, and renal failure can be a major cause of morbidity [35, 36]. Heterogeneous in nature, renal manifestations of BBS encompass a wide range of disorders that include renal dysplasia which is characterized by malformation of the renal parenchyma, cystic tubular disease such as nephronophthisis which often presents with anemia, polyuria, and polydipsia in late childhood [47]. Less frequently, glomerular disease has been reported in BBS, rarely presenting with focal segmental glomerulosclerosis with histopathological findings of splitting of the glomerular basement membrane [48, 49]. Similarly, lower urinary tract malformations such as detrusor instability of the bladder while reported are less common than upper tract malformations in patients with BBS [28, 35, 47, 48]. Antenatal ultrasonography often reveals large hyperechoic kidneys with loss of corticomedullary differentiation and in the presence of polydactyly should prompt a differential diagnosis of BBS or Meckel's syndrome [49, 50]. Progressive renal impairment frequently occurs in BBS and can lead to end-stage renal failure necessitating renal transplantation in up to 10% of patients [51].

Hepatic Involvement in Bardet–Biedl Syndrome

Hepatic fibrosis and cystic dilatation of the biliary tract has been rarely reported in patients with BBS (Table 11.2) [52–58]. Histological findings include portal fibrosis with narrow bands of fibrous tissue bridging portal tracts producing a micronodular cirrhosis. In addition, both intrahepatic and extrahepatic cystic dilatations of the biliary tract have been observed [54].

Craniofacial Dysmorphism in BBS

Until recently, previous reports of craniofacial dysmorphism have been controversial among patients with BBS [28, 38, 59]. A recent elegant study by Tobin et al. utilizing three-dimensional dense surface modeling (DSM) of facies of 83 patients with BBS confirmed that certain craniofacial defects are present in BBS patients and include nasal bridge hypoplasia, nasal shortening/reduced bulbosity at the nasal tip, relative upward displacement of the nose and upper lip, mid-facial hypoplasia, and mild retrognathia [60]. Similar findings in the mouse and zebrafish

Table 11.2
Liver manifestations of patients with Bardet–Biedl syndrome

Reference	*Age*	*Clinical presentation*	*Histopathology*	*Outcome*
Pagon et al. [51]	12 years	Elevated AST, hepatomegaly	Portal fibrosis with micronodular cirrhosis	Deceased
Ross et al. [52]			Portal fibrosis	
Meeker et al. [53]	28 years	Right hypochondrium pain	Dilatation of common bile duct	Alive
Tsuchiya et al. [54]		Cholangitis	Cystic dilatation of bile ducts	
Dekaban et al. [55]			Portal fibrosis	
Proesmans et al. [56]			Portal fibrosis	
Delaney et al. [57]			Portal fibrosis	
Esmer et al. [61]	18 months	Hepatomegaly, elevated transaminases	Portal fibrosis and BDP	Deceased
	3 months	Jaundice, hepatomegaly, elevated transaminases	Portal fibrosis, neonatal hepatitis, bile duct dilatation, and proliferation	

AST: aspartate aminotransferases; BDP: bile duct proliferation.

supported these findings, thereby providing further evidence for craniofacial dysmorphism as yet another manifestation of BBS [60]. Aberrant neural crest cell migration secondary to defective Sonic hedgehog signaling was subsequently shown to underlie the craniofacial defects in *bbs* morphant zebrafish, thereby highlighting the crucial role of BBS proteins in *Shh* signaling and tissue morphogenesis.

Hirschsprung's Disease in BBS

Hirschsprung's disease is a disease of the enteric nervous system, characterized by absence of enteric nerves in the distal colon (Fig. 11.4). While the incidence in patients with BBS is unknown, Hirschsprung's disease has been reported among patients with BBS [28, 62]. Tobin et al. recently identified defective vagal neural crest cell migration from the vagal region, through the branchial arches and into the gut in *bbs* morphant zebrafish, thereby confirming a role for BBS proteins in directing cilia-bearing vagally derived neural crest cells to their correct location in the enteric mucosa [62].

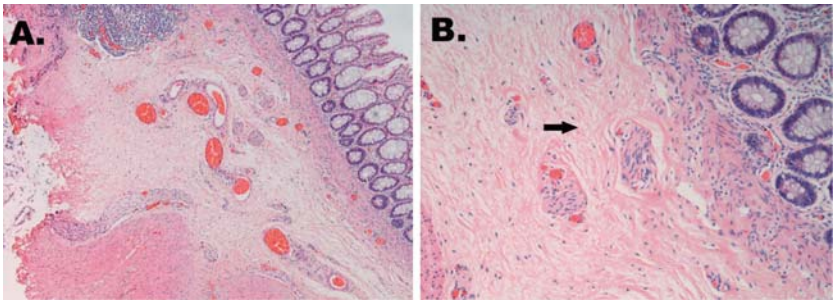

Fig. 11.4. Hirschsprung's disease in Bardet–Biedl syndrome. (**a, b**). Haematoxylin and eosin-stained section of a rectal biopsy from a patient with Bardet–Biedl syndrome. Histological demonstration of aganglionic colonic tissue. (Courtesy of N. Sebire, Great Ormond Street Hospital, UK).

Molecular Genetics of BBS

Homozygosity mapping in highly consanguineous families with BBS allowed the progressive recognition of a high level of nonallelic genetic heterogeneity with 15 causative genes identified to date (*BBS1-12, MKS1, CCDC28B*, and *CEP290*) (Table 11.3) [58–60, 63–76]. Mutations in any one of these genes can result in a BBS phenotype, although no consistent genotype–phenotype correlation has been established to date. In addition, (*MKS1*, *CCDC28B*, and *CEP290*) mutations

Table 11.3
BBS genes and their putative functions

Gene	*Chromosomal location*	*Cellular localization*	*Putative function*	*References*
BBS1	11q13	Basal body/cilium	Cilial Function	Mykytyn et al. [64]
BBS2	16q21	Basal body/cilium	Cilial function/flagellum formation	Nishimura et al. [63]
BBS3	3p12–q13	Basal body/cilium	Vesicle trafficking	Chiang et al. [65]
BBS4	15q23	Pericentriolar/basal body	Microtubule transport	Mykytyn et al. [62]
BBS5	2q31	Basal body/cilium	Cilia function/flagellum formation	Li et al. [69]
BBS6/MKKS	20p12	Basal body/cilium	Cilia function/flagellum formation	Kim et al. [70]
BBS7	4q32	Basal body/cilium	IFT particle assembly	Blacque et al. [71]
BBS8/TTC8	14q31	Basal body/cilium	IFT particle assembly	Blacque et al. [71]
BBS9/B1	7p14.3	Unknown	Unknown, expressed in bone cells	Nishimura et al. [72]
BBS10	12q21.2	Unknown	Unknown	Stoetzel et al. [73]
BBS11/TRIM32	9q31–34.1	Unknown	E3 ubiquitin ligase	Chiang et al. [75]
BBS12	4q27	Unknown	Type II chaperonin	Stoetzel et al. [76]
MKS1	17q23	Basal body/cilium		
CEP290	12q21.3	Centrosome/cilium	G-protein trafficking	
CCDC28B	1q35.1	Centrosome/basal body		Badano et al. [78]

Modified from Tobin et al. [62].

are associated with the Meckel and Joubert syndromes. Following identification of a mutation in *BBS8* causing BBS and the localization of BBS8 to the centrosome and basal body, it was proposed that BBS results from dysfunction of the basal body. Supporting a role for BBS8 in ciliary function was the finding that BBS8 interacts with pericentriolar material 1 (PCM1), a protein important for ciliary assembly. Furthermore, the nematode homologue, *bbs8*, was shown to contain regulatory sites, known as X-box elements for *daf-19*, a ciliogenic transcription factor which regulates genes involved in ciliary assembly and intraflagellar transport [67]. While the precise functions of BBS proteins have yet to be elucidated, it has recently been shown that seven of the most evolutionary conserved BBS proteins (BBS 1, 2, 4, 5, 7, 8, and 9) form a stable complex called the BBSome that localizes predominantly at the ciliary base and mediates vesicular transport to the cilium [77] (Fig. 11.5). Organization of the cilium as an extracytoplasmic organelle requires extension of the plasma membrane and intracellular vesicular trafficking which is modulated by proteins which are members of the Arf and Rab family. Disruption of Rabin8, a GTP nucleotide exchange factor specific for Rab8, leads to loss of BBS4 from centriolar satellites and impaired cilia formation [77]. Rabin8 is therefore thought to recruit members of the BBSome to the basal body from neighboring centriolar satellites and activate Rab8 to promote docking of vesicles to the ciliary membrane. This likely promotes movement of Rab8 GTP and BBS proteins into the cilia where they further promote ciliary assembly by regulating intraflagellar transport. *BBS6*, *10*, and *12* are thought to function as chaperonins which may facilitate protein folding and altogether account for one-third of all cases [76]. Whether these proteins affect folding of key IFT proteins or other proteins involved in ciliary assembly requires further investigation.

Mutations in *BBS1* and *BBS10* account for 20% of the mutational load in families of European descent, while each of the other genes account for <5%, some of which are mutated in only a few families or a single family [73, 75]. A further complication is the finding that, in some cases, inheritance departs from classic autosomal recessive inheritance and involves three mutated alleles in two genes defining oligogenic inheritance, with severity modulated by an allele of a modifier gene [78]. Supporting this concept has been the recent identification of both homozygous and heterozygous mutations in *MKS1*, the gene mutated in Meckel syndrome. In some families with BBS, the mutations in *MKS1* may occur in isolation or concomitant with homozygous mutations in *BBS1* or *BBS10* [74]. As a result, it has been postulated that MKS may represent the severe end of the BBS phenotype. Furthermore, heterozygous mutations in *MKS3* or *CEP290*,

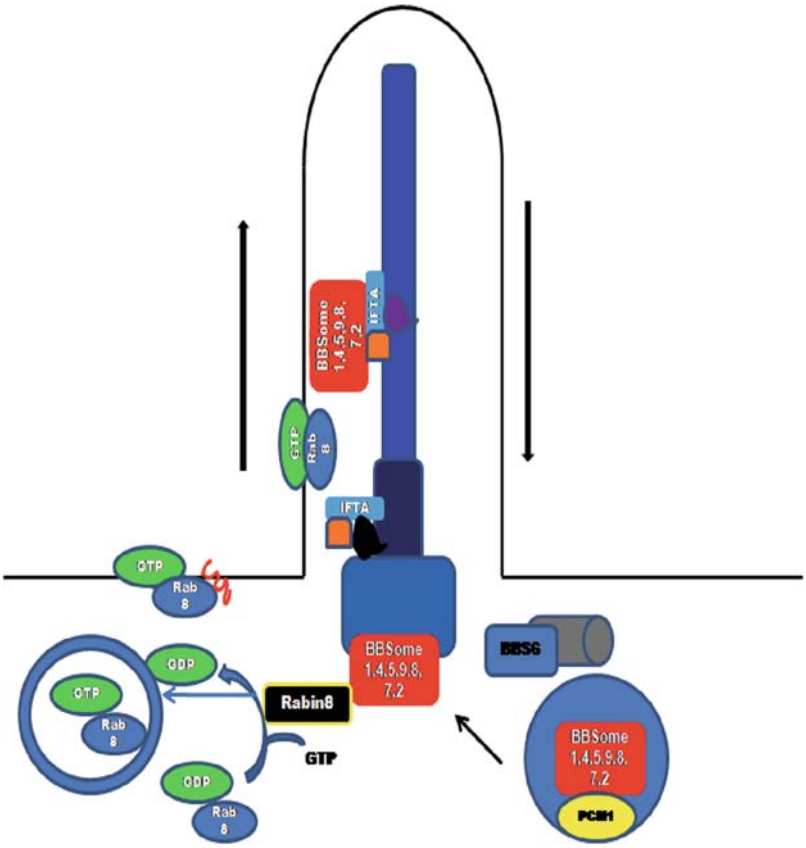

Fig. 11.5. The BBSome and vesicular trafficking to the primary cilium. The BBSome complex is comprised of BBS 1, 2, 4, 5, 7, 8, and 9 which interact with pericentriolar material 1 (PCM1) before the complex moves to the basal body. Following activation by the BBS1-associated Rabin8, the GTP-bound Rab8, small GTPase is proposed to facilitate docking and fusion of the vesicles bearing transmembrane proteins near the ciliary membrane. Initially, the BBSome is thought to dock onto the intraflagellar (IFT) transport machinery on the transitional fibers and from there moves into the cilia with $Rab8^{GTP}$. BBS proteins may associate with cargo, the ciliary membrane, and with BBS3 either directly or indirectly during IFT. BBS6 is a basal body protein which may facilitate the assembly of the BBSome complex.

which have been identified in other ciliopathies, have similarly been reported in patients carrying homozygous mutations in *BBS1* and *BBS9* [74]. Functional studies in model organisms, such as the zebrafish, have shown that these genes may exert an epistatic effect on BBS recessive genes, a phenomenon which has previously been shown with a

transcript encoding a BBS-interacting protein [67]. Therefore, the heterogeneous presentation of BBS seems to represent not only the effects of a single gene but also other modifying genes, thereby highlighting the crucial role of total mutational load on ciliary function and disease presentation.

Taken together, the 15 known BBS genes account for ~80% of affected families, suggesting that additional BBS genes remain to be identified [73]. A systematic screen of the ciliary proteome should aid with the further identification of new genes involved in BBS.

JEUNE SYNDROME

Jeune asphyxiating thoracic dystrophy is a rare autosomal recessive chondrodysplasia that frequently is associated with infantile death as a result of a severely constricted thoracic cage associated with respiratory insufficiency from pulmonary hypoplasia [79]. There are two types, JATD Type 1 which maps to chromosome 15q13 and JATD Type 2 which is caused by mutations in IFT80 gene on chromosome 3q24 [80, 81].

Clinical Diagnosis of JATD

SKELETAL FINDINGS

Characteristic skeletal findings include a narrow thorax with short ribs, hypoplastic iliac wings, trident acetabular roofs (horizontal acetabular roofs with spur-like projections at lower margins of sciatic notches), and rhizomelic limb shortening (Fig. 11.6). Radiological confirmation of the diagnosis is essential [82, 83]. JATD Type 1 is characterized by the presence of radiologically irregular metaphyseal ends and histopathologically irregular cartilage–bone junction with patchy distribution of the physeal bone hypertrophy. JATD Type 2 is characterized by radiologically smooth metaphyseal ends and histopathological diffusely retarded and disorganized physes with smooth cartilage–bone junctions. Histologically, hyperplastic proliferating cartilage and poor progression of endochondral mineralization have been noted. Postaxial polydactyly is also a feature and usually includes both hands and feet. Brachydactyly has also been reported. Hydrocephalus and nonspecific large ventricles may also be a feature [84]. Ellis van Creveld (EVC) syndrome lies in the differential diagnosis and it may not be possible to distinguish between the two based on radiological grounds alone [85]. Distinction from EVC lies in a few small distinguishing features, namely EVC uncommonly affects the feet, and the primary lesion in EVC is usually the heart whereas in JATD, it is renal. Nail dysplasia

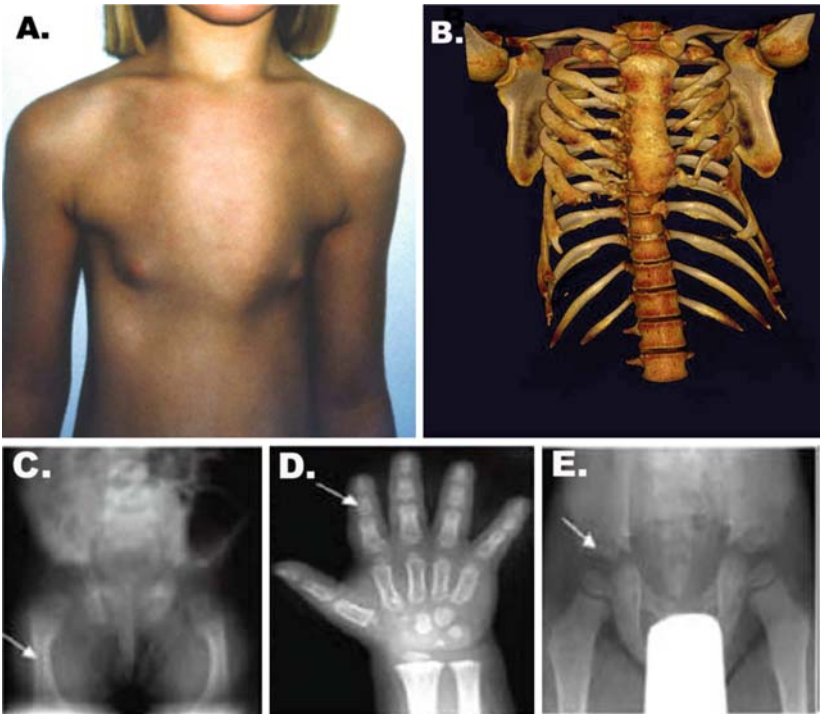

Fig. 11.6. Skeletal manifestations of Jeune's asphyxiating thoracic dystrophy. (**a**) JATD patient with a narrow thorax. (**b**) Three-dimensional reconstruction of same thorax on computed tomogram of the chest (Courtesy of Raoul Hennekam). (**c**) Mild femoral bowing in an infant with JATD. (**d**) Small middle phalanges are another manifestation of JATD. (**e**) Trident acetabular roofs in a patient with JATD (Courtesy of O. Amala, Great Ormond Street Hospital, UK).

and a peculiar upper lip also distinguish EVC from Jeune syndrome. Cranioectodermal dysplasia (also known as Sensenbrenner syndrome), a recessive disorder similar to EVC but with the addition of renal cysts, is possibly caused by mutations in one of the related centrosomal/IFT genes.

Respiratory Insufficiency

Neonatal respiratory insufficiency may occur as a result of pulmonary hypoplasia associated with a narrow thorax [86]. Symptoms may range from mild respiratory distress to asphyxia and death depending on the severity of the thoracic dystrophy [87]. Up to 80% of infants die in the neonatal period due to respiratory insufficiency. Lateral thoracic

expansion has been employed to expand the thoracic cage in patients with JATD to try to improve quality of life and extend life expectancy but have been met with variable success [88]. More recently, titanium plates have been employed by specialist centers and have reported improved outcomes for some patients who have satisfactory pulmonary function prior to surgery [89, 90].

Retinal Degeneration

Retinal degeneration has also been described in patients with JATD and has been described as similar to that which occurs in Leber's congenital amaurosis. Reported features include a retinitis pigmentosa with pigmentary changes as well as retinal aplasia [91, 92]. In cases of retinal degeneration, predominantly cone-type cells have been reported to remain [93, 94].

Cystic Renal Disease

Similar to other ciliopathies, renal disease in JATD is heterogeneous. However, cystic renal disease is most commonly reported and includes nephronophthisis. Less commonly, glomerulosclerosis has also been reported in JATD [95–98]. While the predominant cause of morbidity is progressive respiratory insufficiency, progressive renal failure has been reported and may lead to death in some cases of JATD [99].

Liver Disease

Liver involvement has been reported in Jeune syndrome (Table 11.4) [100–110]. Features reported include both clinical and laboratory evidence of liver disease. Liver involvement may be severe, manifesting as periportal fibrosis and bile duct proliferation leading to biliary cirrhosis with portal hypertension [100–108]. Neonates with JATD may present with cholestasis as the initial manifestation. However, patients with JATD and without neonatal cholestasis may also present later with hepatic fibrosis of an insidious onset. Therefore, it is recommended that patients with JATD should have their liver function monitored regularly. Pancreatic fibrosis may also accompany the hepatic fibrosis in patients with JATD [101, 102].

Natural History

Early death usually occurs in the majority of patients as a consequence of asphyxia with or without pneumonia. Those who survive do have progressive improvement in the relative growth of the thoracic cage. Renal failure is a serious complication of this syndrome and may be

Table 11.4
Spectrum of liver disease in patients with Jeune's asphxiating thoracic dystrophy

Reference	*Age*	*Clinical presentation*	*Histopathology*	*Outcome*[d]
Pirnar et al. [82]	1 month	Hyperbilirubinemia	None	Alive
Cremin et al. [105]	1 day	None	Portal fibrosis	Deceased
Russell and Chouskey et al. [106]	18 weeks	Hyperbilirubinemia	None	Alive
Shokeir et al. [95]	33 h	Hepatomegaly	Portal fibrosis and BDP[c]	Deceased
Edelson et al. [107]	13 months	Elevated AST and ALP[a]	Modest portal fibrosis and BDP	Deceased
Friedman et al. [108]	32 years	Elevated AST and ALP	None	Alive
Oberklaid et al. [99]	16 months	None	Portal fibrosis and BDP, mild	Deceased
	41 months	None	Portal fibrosis and BDP, moderate	Deceased
	5 years	None	Portal fibrosis and BDP, moderate	Deceased
	10 weeks	None	Portal fibrosis and BDP, moderate	Deceased
	5 weeks	None	Portal fibrosis and BDP, mild	Deceased
	3 years	Jaundice and vomiting	None	Deceased
	12 h	None	Portal fibrosis and BDP, moderate	Deceased
	4 years	None	None	Alive

	17 years	None	None	Alive
	12 h	None	Portal fibrosis and BDP, mild	Alive
Landing et al. [100]	1 day	None	Polycystic disease with smaller portal tracts	Deceased
	18 days	None	Polycystic disease with smaller portal tracts	Deceased
Turkel et al. [104]	Stillborn	None	Periportal fibrosis and BDP	Stillborn
	2 h	None	Periportal fibrosis and BDP	Deceased
	2 h	None	Periportal fibrosis and BDP	Deceased
	1 h	None	Periportal fibrosis and BDP	Deceased
	2 h	None	Periportal fibrosis and BDP	Deceased
	10 h	None	Periportal fibrosis and BDP	Deceased
	5 h	None	Cirrhosis	Deceased
Whitley et al. [101]	10 days	Direct hyperbilirubinemia	Biliary dysgenesis with portal fibrosis and BDP	Alive
Hudgins et al. [102]	1 year	Jaundice	Cirrhosis and BDP	Alive
	2 months	Elevated GGT[b]	Early biliary cirrhosis	Alive
Labrune et al. [103]	3 years	Jaundice	Portal fibrosis with architectural disorganization	Alive

	10 years	Hepatomegaly, portal hypertension	Biliary cirrhosis	Alive
	25 months	Jaundice, portal hypertension	Biliary cirrhosis, thin but normal bile ducts	Alive
Ozcay et al. [109]	6 years	Hepatomegaly	Portal tract fibrosis, BDP	Alive
Yerian et al. [110]	8 years	Portal hypertension	Subcapsular cirrhosis, mild fibrosis, portal vein changes	Alive

AST:aspartate aminotransferase; ALP: alkaline phosphatase; BDP: bile duct proliferation; GGT: gamma-glutamyl transpeptidase.

[a]SGOT, serum glutamic oxaloacetic transaminase, ALP, alkaline phosphatase.

[b]GGT, gamma–glutamyltransferase.

[c]BDP, bile duct proliferation.

[d]At time of presentation.

evident as early as 2 years of age. However, some cases do survive well into adulthood [108].

Molecular Genetics of Jeune Syndrome

Jeune's asphyxiating thoracic dystrophy is a genetically heterogeneous disorder. A genome-wide linkage search using autozygosity mapping in five consanguineous families with JATD, three from Pakistan, one from Southern Italy, and one from France, identified a novel JATD locus to 15q13 [80]. Furthermore, investigation of four European kindreds with no history of parental consanguinity showed evidence of marker homozygosity across a similar interval of 1.2 cM on chromosome 15. Families with both mild and severe forms of JATD are mapped to chromosome 15q13, but mutation analysis of two candidate genes, formin and gremlin, has failed to identify pathogenic mutations. Recent progress has been made, however, in our understanding of the pathogenesis of JATD when Beales and colleagues identified two missense mutations and an in-frame deletion in Ift80, the gene encoding the intraflagellar transport protein, thereby linking JATD to ciliary dysfunction [81]. Using a polyclonal antibody against murine *Ift80*, it was subsequently shown that *Ift80* does indeed localize to the basal body of cilia in a murine chondrocytic cell line. Zebrafish morphant for ift80 demonstrates downregulation of ptc1, a Sonic hedgehog (*Shh*) binding receptor [81]. Furthermore, aberrant *Shh* signaling appears to underlie the skeletal manifestations observed in mice mutant for another intraflagellar transport protein, Ift88 [111]. Phenotypic similarity is observed in mice null for Indian hedgehog (*Ihh*) compared to patients with JATD, in that they exhibit extremely short narrow rib cages. Similarly, mice carrying a mutation in *Pthrp*, a gene regulated by *Ihh* via Gli3 during chondrocyte differentiation, also have short ribs and sternum leading to a narrow rib cage. Following the identification of *Ift80* mutations, it is highly likely that further cases of JATD may be attributed to ciliary dysfunction in the future.

CONCLUSION

Recent insight into the molecular mechanisms underlying the pleiotropic phenotypes of both BBS and JATD has demonstrated an important role for the primary cilium, a cellular sensory organelle. Within the biliary tract, cholangiocyte cilia perceive intraluminal cues that regulate bile formation. The occurrence of fibrocystic liver disease in ciliopathic diseases, such as BBS and JATD, supports the hypothesis that cholangiocyte cilia are highly likely to play a crucial role

during development of the biliary tract. Future studies are required to identify the downstream signaling pathways which are regulated by cholangiocyte ciliary signaling and may shed important insight into the pathogenesis of CFLD which may lead to the identification of molecular targets for therapeutic modulation in this disease.

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12 Congenital Disorders of Glycosylation and Their Effects on the Liver

Erik A. Eklund, MD, PhD and Hudson H. Freeze, PhD

CONTENTS

Summary

Glycoprotein synthesis is one of the major duties of the liver. During the last decade a large number of inherited disorders in protein glycosylation have been identified that alter the architecture and function of the liver. Many congenital disorders of glycosylation (CDG) compromise the synthesis of a host of membrane and secreted

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glycoproteins often leading to steatosis, hepatic fibrosis, and abnormal bile duct architecture. Misfolded glycoproteins accumulate in the endoplasmic reticulum (ER) and improperly glycosylated plasma proteins are frequently unstable, leading to pro- or anti-coagulant status. CDG patients with liver pathology often present with a host of abnormalities in other systems, including the gastrointestinal tract causing protein-losing enteropathy, hypotonia, hypoglycemia, developmental delay, and seizures. In some cases, pathology is primarily confined to the liver. Effective therapy is available for only one of the more than 20 types of CDG. A simple and inexpensive serum transferrin glycosylation test can suggest a glycosylation disorder and should be used early on in a diagnostic workup of any patients with unknown liver-related pathology, especially if it occurs in combination with abnormalities in other organ systems. Many CDG patients probably remain undiagnosed.

Key Words: Congenital disorder of glycosylation (CDG), *N*-glycan, Steatosis, Hepatic fibrosis, Protein-losing enteropathy, Mannose, Ductal plate malformation

INTRODUCTION

This chapter deals with a rare group of congenital disorders, the congenital disorders of glycosylation (CDG), where the genetic defects compromise different steps in protein glycosylation. Their clinical presentation is very diverse and often effect several organ systems. As liver pathology is often present, the pediatric hepatologist should be aware of this group of disorders.

GLYCOSYLATION – INTRODUCTION

Complex carbohydrates are collectively termed glycans. They constitute the most complex group of molecules in living organisms and are found in all known cell types. In man, glycans are found in many versions, *N*- or *O*-linked to proteins, linked to various lipids, or as free molecules [1]. The enormous variation of glycan structures arises from the presence of a large number of monosaccharide building blocks that can be assembled as linear or branched structures through different linkages. Biologically important structures can range from single monosaccharides to polymers with a hundred monosaccharides. Up to 3% of the human genome is believed to encode proteins involved in the formation or binding of carbohydrates [2]. Due to the large overlap of enzyme specificity in some biosynthetic steps, mutations

in some of these genes seem non-pathogenic, whereas loss of other, non-redundant, functions leads to severe or lethal syndromes.

HISTORY – GLYCOSYLATION DISORDERS

Since the initial report in 1980 of a syndrome due to a deficiency in glycosylation [3], a vast number of syndromes emanating from defective *N*-glycosylation have been characterized. Initially, these disorders were named CDGS (carbohydrate-deficient glycoprotein syndrome(s)), referring to the hypoglycosylation of transferrin, a glycoprotein synthesized in the liver and involved in iron homeostasis, which was a biochemical characteristic of these patients. In 1999, the term CDG (congenital disorders of glycosylation) was introduced instead [4]. The CDGs are separated into two entities – Type I and Type II defects – based on their molecular characteristics (see below). Hitherto, each new defect in the *N*-linked pathway (or both *N*- and *O*-linked pathways as with the conserved oligomeric Golgi (COG) complex subunit deficiencies) has been designated by a name based on the type and order of discovery. For instance, the first discovered subtype, phosphomannomutase 2 (PMM2) deficiency, was designated CDG-Ia and so forth. It has since become evident that genetic disorders can also stem from deficiencies in the biosynthesis of other types of glycans such as *O*-linked (e.g., certain muscle dystrophies, hereditary multiple exostosis, familiar tumoral calcinosis, and Peters plus syndrome), combined *N*- and *O*-linked (e.g., COG subunit deficiencies and dolichol kinase 1 deficiency), and glycolipids (e.g., GM3-synthase deficiency [Amish infantile epilepsy]). Many of these are organ-specific and will not be discussed further in this chapter. However, because the spectrum of CDGs (i.e., congenital glycosylation defects) has expanded to all types of glycosylation, a new nomenclature was recently proposed [5]. In this review we will therefore refer to each syndrome based on the name of the deficient protein's gene along with (if applicable) the CDG subtype in parentheses.

N-LINKED GLYCOSYLATION

A large and important group of carbohydrates is the asparagine (Asn)-linked, or *N*-linked, glycans. Their biosynthetic pathway is complex and requires the concerted action of a plethora of glycosyltransferases, glycosidases, transporters, synthases, and ER/Golgi-related proteins. This complex system allows for the formation of a multitude of different final glycan structures, important in early steps in protein life, such as folding and intracellular transport/localization, as well as late

steps such as activity/function and degradation. A significant amount of carbohydrate-binding molecules (lectins) depend on correct glycosylation for appropriate binding, where a prime example is the selectins, involved in leukocyte extravasation.

All *N*-linked glycans stem from a common lipid-linked oligosaccharide (LLO) precursor, synthesized in the ER on a dolichol-phosphate (Dol-P) anchor. The mature LLO glycan is transferred co-translationally to consensus sequence Asn residues in the nascent protein and is further modified by trimming and re-building in the Golgi apparatus. Deficiencies in the genes involved in *N*-linked glycosylation constitute the molecular background to the CDGs, where Type I defects involve the synthesis and transfer of the LLO glycan and Type II defects impair the modification or processing of protein-bound *N*-glycans. To date there are 14 known Type I CDGs (CDG-Ia-In) and 8 Type II CDGs (CDG-IIa-IIh). The *N*-linked biosynthetic pathway is depicted in Figs. 12.1 and 12.2. They also indicate the location of *N*-glycosylation defects, most of which have CDG designations.

The formation of the LLO is initiated by the synthesis of a polyisoprenyl, Dol, from farnesyl, a precursor of cholesterol. Dol is then activated to Dol-P via the action of Dol kinase (DK1) in the ER membrane. Four patients have been diagnosed with DK1 deficiency (CDG-Im), which is lethal within the first year of life [6]. Using the corresponding nucleotide sugar donors UDP (uridine diphosphate)-GlcNAc and GDP (guanidine diphosphate)-mannose (Man), a pyrophosphate-linked seven-sugar glycan is formed on the cytoplasmic side of the ER. It is then flipped to the ER lumen (defect in RFT1 deficiency [CDG-In]) and completed with Dol-P-Man and Dol-P-glucose (Glc) to form the final structure $Glc_3Man_9GlcNAc_2$-P-P-Dol [7].

Disease-causing mutations have been described in the genes encoding GlcNAc-1-P transferase (DPAGT1 deficiency [CDG-Ij]) [8], Dol-P-Man synthase (DPM1 deficiency [CDG-Ie]) [9], mannosyltransferase (MT) I (HMT1 deficiency [CDG-Ik]) [10–12], MT II (*ALG2*

Fig. 12.1. (continued) and CDG-Ig, respectively. Using Dol-P-Glc as donor, three Glc residues are added to make mature LLO structure. Deficiency in the first glucosyltransferase (encoded by *ALG6*) causes CDG-Ic, whereas lack of the second transferase (encoded by ALG8) causes CDG-Ih. The LLO is now transferred to consensus sequence acceptor asparagines in the recipient protein. The transfer is catalyzed by oligosaccharyltransferase (OST) complex. Mutations in two of the subunits cause underglycosylation, but this leads to developmental delay without liver involvement. (Adapted with permission from Freeze and Aebi [102].)

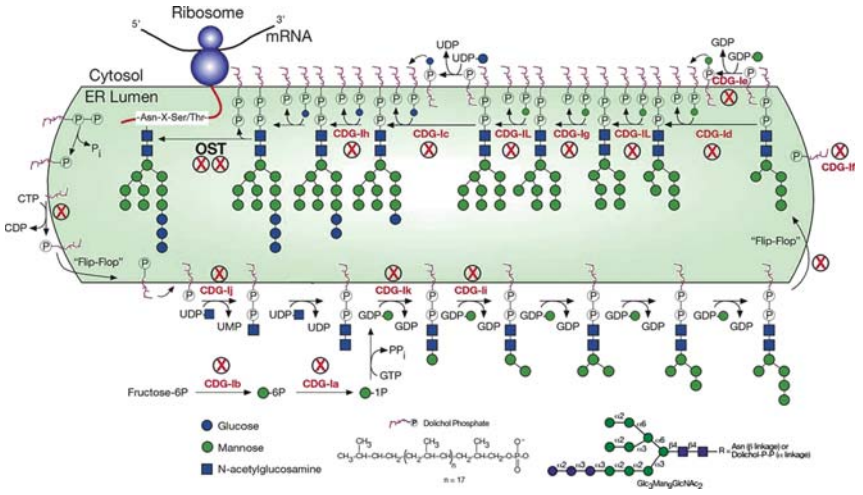

Fig. 12.1. *N*-glycan biosynthesis and addition of glycans to proteins: The figure depicts the biosynthesis and transfer of the LLO glycan. Defects in the pathway are shown as *red* "X" in the circle. Guanidine diphosphate Mannose (GDP-Man) is made from the glycolytic intermediate, fructose-6-P by conversion into Man-6-P via the action of phosphomannose isomerase, the enzyme deficient in CDG-Ib. Man-6-P is converted into Man-1-P and subsequently to GDP-Man via a synthase. Phosphomannomutase catalyzes the conversion of Man-6-P to Man-1-P and is deficient in CDG-Ia. The precursor glycan is assembled on the lipid dolichol (Dol), which is synthesized from an intermediate (farnesyl-pyrophosphate) in the cholesterol biosynthetic pathway. It is activated to Dol-P via the action of dolichol kinase; its deficiency causes a defective glycosylation. GlcNAc-1-P residue is added to the lipid phosphate by GlcNAc-1-phosphotransferase, using uridine diphosphate GlcNAc (UDP-GlcNAc) as donor. This step is deficient in CDG-Ij. Addition of a β1,4-GlcNAc is followed by addition of a β1,4-linked Man, catalyzed by mannosyltransferase-1, deficient in CDG-Ik. GDP-Man is used as the donor sugar nucleotide. Consecutive additions of an α1,3-Man, an α1,6-Man, an α1,2-Man on the α1,3-Man branch, and finally another α1,2-Man on the α1,3-Man branch follow. These reactions all occur on the cytoplasmic side of the ER using GDP-Man as donor nucleotide sugar. The second mannosylation step is deficient in CDG-Ii. Next, the hepta-oligosaccharide is flipped into the lumen of the ER; a defect in RFT1 prevents flipping into the lumen resulting in deficient *N*-glycosylation. Additions of an α1,3-Man to the α1,6-Man branch, an α1,2-Man to this α1,3-Man, an α1,6-Man to the core α1,6-Man residue, and finally an α1,2-Man to the last α1,6-Man occur. The donor substrate is Dol-P-Man and the reactions are facilitated by a protein, MPDU-1, whose mode of action is uncertain, however, it is deficient in CDG-If. The synthesis of Dol-P-Man is deficient in CDG-Ie. These last mannosylation reactions are catalyzed by three different enzymes (MTVI (M5→M6), MTVII (M6→M7 and M8→M9), and MTVIII (M7→M8)), which are deficient in CDG-Id, CDG-I*L*,

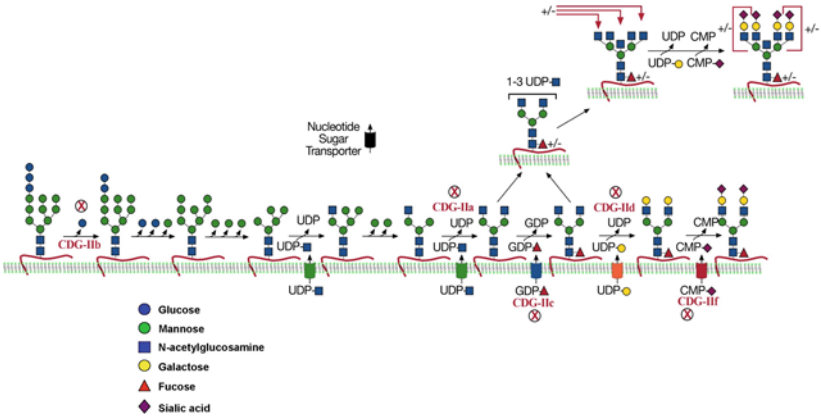

Fig. 12.2. Processing of *N*-glycans and location of defects in the *N*-glycosylation pathway: Defects in the pathway are shown as *red* "X" in the circle. After transfer, the protein-bound oligosaccharide is trimmed by various glycosidases before it enters the Golgi apparatus. The first step, removal of the ultimate Glc residue, catalyzed by glucosidase-I is deficient in CDG-IIb. The remaining Glc and one Man residue is removed in the Golgi. Within the Golgi, another mannosidase removes three more Man residues generating a $GlcNAc_2Man_5$ structure. Some *N*-glycans stop processing at this stage and are referred to as high Man structures. Alternatively, GlcNAc can be added to the α1,3-branch catalyzed by GlcNAc transferase II (MGAT1). This enzyme is deficient in CDG-IIa. Next two Man residues are removed from the α1,6-branch using α-mannosidase-II. After the Man removal, the α1,6-branch is substituted with one to four additional GlcNAc residues and this structure serves as substrate for various reactions including fucosylation, galactosylation, and sialylation. Fucosylation is decreased in CDG-IIc, galactosylation in CDG-IId, and sialylation in CDG-IIf. (Adapted with permission from Patterson and Freeze [103].)

deficiency [CDG-Ii]) [13], MT VI (NOT56L deficiency [CDG-Id]) [14], MT VII/IX (DIBD1 deficiency [CDG-I*L*]) [15], MT VIII (ALG12 deficiency [CDG-Ig]) [16], glucosyltransferase-I (ALG6 deficiency [CDG-Ic]) [17], and glucosyltransferase-II (ALG8 deficiency [CDG-Ih]) [18]. Deficiency of a protein with unclear activity, involved in the utilization of Dol-P-Glc and Dol-P-Man (MPDU1), also causes a CDG (MPDU1 deficiency [CDG-If]) [19].

The completed LLO structure is finally transferred to consensus sequence Asn residues in the growing peptide chain, catalyzed by the oligosaccharyltransferase complex. Patients with non-syndromic mental retardation carrying mutations in the oligosaccharyltransferase were recently described [20]. The synthesis of GDP-Man is crucial for proper

N-glycosylation, as it serves as donor substrate for the formation of Dol-P-Man and the initial $Man_5GlcNAc_2$-P-P-Dol structure. GDP-Man synthesis is linked to glycolysis via the interconversion step fructose-6-P ←→ Man-6-P; phosphomannose isomerase (PMI) deficiency causes CDG-Ib, the only partially treatable CDG-I subtype [21]. Man-1-P is then formed from Man-6-P via PMM2, whose deficiency causes CDG-Ia [22]. Man-1-P serves as substrate in the GDP-Man synthesis. PMM2 deficiency (CDG-Ia) is the most common CDG subtype identified so far.

Once transferred to the protein chain, many glycans undergo a variable amount of "processing" that converts them into a large variety of different structures (Fig. 12.2). They are first trimmed by specific glycosidases. This process is vital since the lectin chaperones calnexin and calreticulin, involved in protein quality control, bind to the $Glc_1Man_9GlcNAc_2$ structure and assure proper folding. One patient has been described with lack of ER glucosidase-I activity (GLS1 deficiency [CDG-IIb]) [23]. Removal of the next two Glc residues is catalyzed by ER glucosidase-II. Interestingly, the non-catalytic β-subunit of this enzyme [24] is the protein hepatocystin, deficient in a form of autosomal dominant polycystic liver disease [25]. Removal of the Glc residues and the first Man residue occurs in the ER. The glycoprotein then travels to the Golgi, where a multitude of different structures with different biological activities are formed. Mannosidase-I creates a $Man_5GlcNAc_2$ structure on the protein that, *nota bene*, has a different structure than the one made on LLO. Next, addition of a GlcNAc residue forms $GlcNAc_1Man_5GlcNAc_2$, the substrate for α-mannosidase-II (αManII). αManII then removes two Man residues, creating the substrate for GlcNAc transferase II, which adds a GlcNAc to the second Man branch. This structure serves as substrate for additional galactosylation, fucosylation, and sialylation reactions. Addition of one or two more GlcNAc residues to the trimannosyl core followed by their elongation can yield tri- and tetra-antennary molecules, respectively. Not all structures are fully modified, some remain as high-mannose structures, others as hybrids (one unmodified Man branch and one modified), but the majority of plasma glycoproteins become fully modified to complex type glycans. In addition to glucosidase-I, disease-causing mutations have been found in the corresponding genes to GlcNAc transferase II (MGAT2 deficiency [CDG-IIa]) [26], the GDP-Fuc transporter (SLC35C1 deficiency [CDG-IIc]) [27], a β-1,4-galactosyltransferase (B4GALT1 deficiency [CDG-IId]) [28], some COG complex subunits (COG1 deficiency [CDG-IIg] [29], COG7 deficiency [CDG-IIe] [30] and COG8 deficiency [CDG-IIh] [31, 32]), and the CMP-sialic acid (NeuAc) transporter (SLC35A1 deficiency [CDG-IIf]) [33]. However,

more than 100 genes in total are needed to complete the *N*-linked glycan biosynthesis explaining why many more defects will likely be found.

CLINICAL PRESENTATION OF "CLASSICAL" CDG

In "classical" CDG (moderate to severe PMM2 deficiency [CDG-Ia]), inverted nipples and abnormal subcutaneous fat pads are present at birth [34]. The patient develops axial hypotonia, psychomotor retardation, and ataxia due to cerebellar atrophy and some develop epilepsy. Feeding is usually a problem; the patient presents with failure to thrive. Some patients have hypertrophic obstructive cardiomyopathy, microcystic kidney disease, hypoglycemia, and skeletal defects. The liver function is often affected leading to elevated aminotransferases, steatosis, variable fibrosis, and sometimes liver failure. Coagulopathy frequently occurs due to deficiencies in liver-synthesized coagulation factors. Depending on what factor(s) deficiency dominates, a patient can experience both bleeding and thrombotic events. Stroke-like episodes are rather common, also probably due to the coagulopathy. Biochemically, there is often an increased level of aminotransferases, but normal bilirubin and γ-glutamyltransferase (GGT) levels. Night blindness and *retinitis pigmentosa* are also common. The life span of a PMM2-deficient (CDG-Ia) patient is expected to be near normal if he/she manages to survive childhood where mortality can reach 20%.

Unfortunately, many clinicians only suspect CDG when the patient presents with signs of "classical" CDG, leading to delayed or missed diagnosis in an increasing number of patients. Over the last years, it has become evident that CDG patients can present with relatively mild phenotypes, lacking the stigmata of classical CDG such as inverted nipples and fat pads. As new subtypes are detected, the apparent clinical spectrum of phenotypes gets wider. In PMI deficiency (CDG-Ib) there is no neurological involvement at all, making this a totally separate entity. We and others, therefore, recommend that *CDG should be excluded in all patients with symptoms from two or more non-related organs when no other clear explanation is at hand*.

CDG AND LIVER DISEASE – GENERAL FEATURES

Liver pathology is a common feature in CDG, especially in Type I subtypes [35, 36]. Many clinical reports on CDG patients describe elevated aminotransferases. Aminotransferase elevation is not confined to a specific subtype, but seems to be a rather general finding. The alanine aminotransferase (ALT) levels seem to peak during bouts

of fever and when patients are put on anti-convulsive therapy [37]. Glycoproteins synthesized in the liver are often decreased, especially the ones of the coagulation system, e.g., protein C, anti-thrombin III (AT-III), and factors VII, IX, and XI [38]. In some studies liver biopsies have been performed in CDG patients (Table 12.1) with or without signs of liver pathology (hepatomegaly or increased aminotransferases) [37, 39–41], and in a few cases the liver has been investigated under the microscope postmortem [42, 43]. In some cases the liver histology has been normal or only mildly pathological in the presence of a moderately increased ALT value [37]. In many patients, however, the microscopic features were clearly abnormal. Common findings are steatosis with periportal fibrosis and swollen hepatocytes. Rarely, there are signs of inflammation. In some cases changes typical of fibrocystic disease, such as ductal plate malformations and dilated bile ducts, are seen, which will be discussed in detail below. Several studies have also investigated ultrastructural changes in CDG using electron microscopy. A prominent feature found in hepatocytes from different CDG subtypes is lysosomal inclusions. Resemblance to the inclusions seen in Niemann–Pick disease type C (myelinosomes) has prompted some authors to speculate that defective glycosylation of the NPC2 protein is causing these inclusions by halting cholesterol trafficking [39, 44]. The inclusions are, however, only seen in the hepatocytes and not in the Kupffer cells, in contrast to Niemann–Pick type C [35]. It is interesting to notice that several lysosomal enzymes are increased in the plasma of CDG patients [45], indicating that their intracellular trafficking, determined by correct glycosylation (high-mannose structures) and addition of lysosomal targeting signal (Man-6-P) to the glycan chain is also perturbed. Also, lipid droplets within hepatocytes are preferentially found in the periportal region [39]. Further, a couple of studies describe accumulation of lipofuscin within the hepatocytes. Similar findings have been made in neuronal ceroid lipofuscinosis, where one cause seems to be hypoglycosylation of tripeptidyl peptidase, affecting its folding, trafficking, and stability [46].

FEATURES OF THE RESPECTIVE CDG SUBTYPES

PMM2 Deficiency (CDG-Ia)

Most patients diagnosed with CDG belong to this group, and over 800 patients are known worldwide. The estimated occurrence of PMM2 deficiency is, however, 1/20,000, suggesting that the number of actual cases probably is considerably larger [36]. The defective gene is *PMM2*

Table 12.1
CDG subtypes and their liver individual pathologies

Gene defect	*No of patients*	*CDG designation*	*Hypertransaminasemia*	*Coagulopathy*	*Microscopic finding*	*EM finding*
PMM2	>800	CDG-Ia	+	++	Steatosis, mild to moderate fibrosis	Lysosomal inclusions in hepatocytes
MPI	>30	CDG-Ib	++	++	As CDG-Ia + ductal plate malformations or dilated bile ducts. Progressive	n.k.
ALG6	>50	CDG-Ic	+	+	Normal	Minor lysosomal inclusion
NOT56L	7	CDG-Id	+/–	+/–	Ductal plate malformation in one case	n.k.
DPM1	7	CDG-Ie	+/–	+/–	n.k.	n.k.
MPDU1	6	CDG-If	–	–	n.k.	Moderate lysosomal inclusions (1 pt)
ALG-12	8	CDG-Ig	+/–	+	n.k.	n.k.
ALG-8	5	CDG-Ih	+	+	Cystic, dilated bile ducts, and cholestasis in one case	n.k.
ALG-2	1	CDG-Ii	n.k.	+	n.k.	n.k.
DPAGT1	1	CDG-Ij	–	–	n.k.	n.k.
HMT1	4	CDG-Ik	+/–	+	n.k.	n.k.
DIBD1	2	CDG-I*L*	+	+	n.k.	n.k.
DK1	4	CDG-Im	–	–	n.k.	n.k.

RFT1	1	CDG-In	n.k.	n.k.	n.k.	n.k.
N33/TUSC3	2	None designated	–	–	n.k.	n.k.
IAP	3	None designated	–	–	n.k.	n.k.
MGAT2	4	CDG-IIa	–	Due to deficiency in glycoprotein Ib	Normal	Normal
GCS1	1	CDG-IIb	+/–	n.k.	Dilated bile ducts, fibrosis, steatosis	Enlarged macrophage lysosomes, lamellar inclusions
SLC35C1	>10	CDG-IIc	–	–	n.k.	n.k.
B4GALT1	1	CDG-IId	+	+	n.k.	n.k.
COG7	4	CDG-IIe	++	+	Intrahepatic cholestasis and fibrosis	n.k.
SLC35A1	1	CDG-IIf	–	–	n.k.	n.k.
COG1	1	CDG-IIg	+/–	n.k.	n.k.	n.k.
COG8	2	CDG-IIh	+/–	+/–	n.k.	n.k.

n.k.: not known or not discussed in the literature; –: denied in the literature; +/–: few cases or only very mild; +: most patients; ++: severely affected or clinically significant phenotype.

[22], encoding phosphomannomutase 2 (PMM2), the enzyme responsible for conversion of Man-6-P → Man-1-P [47]. PMM2 deficiency leads to diminished production of GDP-Man and Dol-P-Man, both essential precursors for LLO biosynthesis. The most common mutation is R141H, with a carrier frequency in a Caucasian population (Danish) of about 1/60 [48]. The R141H mutation gives rise to an enzyme with no activity and has never been found to be homozygous in a patient, indicating that some residual PMM2 activity is required for fetal survival. Diagnosis of PMM2 deficiency is made by a positive transferrin hypoglycosylation test followed by an enzymatic assay of PMM in either fibroblasts or white blood cells. Considerable residual PMM activity in fibroblasts (>30%) may be a potential pitfall, as a patient may be considered normal upon analysis [49, 50].

The first patients diagnosed with PMM2 deficiency (CDG-Ia) were phenotypically alike (the "classical" presentation of CDG), presenting with inverted nipples, abnormal subcutaneous fat pads, psychomotor retardation, ataxia due to cerebellar hypoplasia, *retinitis pigmentosa*, and short stature [3]. Today we know that a PMM2-deficient patient can present with a wide spectrum of signs and symptoms. Phenotypic expression ranges from only very mild psychomotor retardation [49–51], to patients with hypertrophic cardiomyopathy [52], gastrointestinal problems [37], or hypoglycemia [53] as leading symptoms, to severely affected individuals with profound psychomotor retardation, intractable seizures, and grave dysmorphisms.

Many PMM2-deficient patients show signs of liver pathology with elevated aminotransferases, especially in the first 3 years of life [36]. In PMM2-deficient patients, the coagulation factors, both promoters (factors VII, IX, and XI) and inhibitors (AT-III, protein C, and protein S), are often deranged. Depending on the profile, a patient can be at risk for both bleeding events and thromboses. A recent study indicated that the risk of such events is much higher in patients younger than 15 years of age [38]. There seems to be a connection between the levels of ALT and how deranged the coagulation pattern is. Why the liver pathology in PMM2-deficient patients does not progress after adolescence is not known, but probably reflects the fact that the demands on the glycosylation system are highest in times of stress, such as in extreme growth, fever, and other types of illness.

There are several published PMM2-deficient cases in which a liver biopsy was performed. Under the light microscope the histologic picture is dominated by steatosis, mild to moderate fibrosis, sometimes with porto-portal bridging, and swollen hepatocytes [35, 37, 41]. Follow-up biopsies have indicated that the process is rather non-progressive. Electron microscopy shows that there are lysosomal

inclusions indicative of accumulation of undegraded material [35, 37, 41], possibly due to errors in protein folding and secretion [35] or to lack of glycosylation of lysosomal enzymes [45]. It is of interest to note that the inclusions seen in the hepatocytes do not occur in other types of cells such as fibroblasts. This is probably due to the extremely high demand on the hepatocytes to produce and secrete a vast amount of glycoproteins into the serum, and that the residual PMM2 activity in other cells is enough to supply the cells with Man-1-P to cover their own glycoprotein synthesis [35].

There is no current treatment for PMM2 deficiency. In PMM2-deficient fibroblasts, supplementation with Man to the growth medium corrects the abnormal LLO profile [54], but clinical trials with Man given to PMM2-deficient patients were unsuccessful [55–58]. Because the neurological defects seen in PMM2-deficient patients are chronic and likely arise *in utero*, they cannot be treated [59]. Liver and intestinal related problems, such as coagulopathy, fibrosis, and protein-losing enteropathy, however, more dynamic and should theoretically be treatable. Experiments with Man-1-P compounds that could penetrate the cell membrane and replenish the Man-1-P pool [60, 61] were successful in cell cultures, but the compounds' instability makes their use in animal models or patients challenging. Currently, our laboratory is conducting high-throughput screening of chemical libraries searching for compounds that increase the amount and flux of Man-6-P into the depleted glycosylation pathway. Several candidates have been identified but none are ready for testing in patients.

PMI/MPI Deficiency (CDG-Ib)

This disorder stems from a deficiency in the biosynthetic step before Man-1-P synthesis in glycosylation, the interconversion of Man-6-P and fructose-6-P. This enzymatic step thus links glycosylation to glycolysis. Man-6-P can also be produced in cells by the action of hexokinase on free Man, which is why this disorder is treatable (discussed below). The molecular basis of PMI deficiency was elucidated in 1998 [21], but the first clinical description of PMI-deficient patients, diagnosed, retrospectively, was in 1980 [43]. The PMI-deficient patients differ from other CDG patients in that they are mentally spared or, in rare cases, present with only minor neurological symptoms. Their main problems are in the gastrointestinal system and include protein-losing enteropathy, recurrent hypoglycemia, hepatic fibrosis, and sometimes bleeding or thromboses [34]. Chronic diarrhea and cyclic vomiting have also been described. Celiac disease can incorrectly be diagnosed due to partial villous atrophy seen in the duodenum [36], and some patients have

been put on gluten-free diets for long periods, without improvement in the symptoms [62]. More than 20 patients have been described in the literature, but this figure probably grossly underestimates the true prevalence. Since this is a treatable disorder (with alimentary Man) that is potentially fatal, its correct and swift diagnosis is vital. Children with the symptoms mentioned above, or combinations thereof, should be tested for hypoglycosylation of transferrin and the enzymatic activity of fibroblast or leukocyte PMI should be measured.

Several studies describe microscopic and electron microscopic investigations of liver biopsies from PMI-deficient patients [36, 37, 40, 43, 62]. In addition to the findings seen in PMM2-deficient (CDG-Ia) patients, livers from PMI-deficient individuals often show signs of bile duct pathology such as ductal plate malformations [36, 40, 43] and hamartomatous collections of bile ducts (similar to Meyenburg complexes) [37]. In some cases, prior to the correct diagnosis of PMI deficiency, the diagnosis established had been Caroli's disease and congenital hepatic fibrosis (CHF) [36, 43]. Very few studies describe long-term follow-up of Man treatment in PMI-deficient patients [40, 62]. It is interesting to note that the Man treatment, even if initiated early, does not seem to fully improve the liver histology, with clinical evidence of continued progression in the fibrotic process in some patients [40]. One should perhaps look upon the changes seen in the livers from PMI-deficient patients as two separate entities: one early (static with bile duct pathologies as a result of embryonic/fetal mishaps and hence irreversible) and one late (dynamic with ongoing accumulation of mis- and hypoglycosylated proteins in the ER and lysosomes). The latter would be reversible upon introduction of functional glycosylation, whereas the former would be persistent and possibly even progressive. This would explain why patients with no signs of bile ductule dystrophies in the liver biopsy recover completely upon Man treatment whereas patients with already existing ductal plate malformations do not fully recover. Coagulation factor deficiencies, protein-losing enteropathy, and hypoglycemic events are totally abolished by the Man treatment in all patients [40, 62]. It is hard to explain why bile duct pathology is not seen in PMM2-deficient patients, nor in all PMI-deficient patients, even though their abnormal transferrin isoelectric focusing (IEF) profiles are very similar. It is interesting to note that oligosaccharides released from LLO or glycoproteins synthesized by PMI-deficient fibroblasts are normal when compared to, for example, PMM2-deficient cells, in which truncated species are seen under selected culture conditions [21]. Possibly the lack of whole chains, and not the gain of certain protein-linked oligosaccharides that are teratogenic, is part of the explanation, but there are no solid data to support

this notion. A very low PMI activity during fetal life could potentially cause the toxic accumulation of Man-6-P (formed from the action of hexokinase on Man from the mother). MPI-knockout mice die embryonically, where Man-6-P accumulation is thought to be causative [63]. Whether this is the true etiology needs to be further elucidated.

The treatment of PMI deficiency should be started as soon as the diagnosis is made and the recommended dose of Man is 100–150 mg/kg bodyweight four times daily [36]. Side effects are flatulence and loose stools and individual dosing sometimes is required. There is also a risk of hyperglycemia and the HbA_{1C} should be followed regularly. It is not known how long the treatment needs to be continued, but it seems that the requirements for Man treatment decrease with age. It is important that the supplement is in monosaccharide form, since poly-Man (mannans) are indigestible fibers, which are only metabolized by anaerobic bacteria in the human colon.

Autosomal Dominant Polycystic Liver Disease (ADPLD)

Another interesting disorder with liver cysts springing from the bile duct epithelium is ADPLD. Two causative gene defects of this disorder have been identified. One defective gene in this disease was recently shown to be the protein kinase C substrate 80 K-H (*PRKCSH*) gene, which encodes the protein hepatocystin [25], the non-catalytic β-subunit of α-glucosidase-II [24]. This enzyme complex is responsible for the formation of the $Glc_1Man_9GlcNAc_2$ structure that is involved in the binding of the chaperones calnexin and calreticulin, crucial for protein folding [64]. This clearly suggests that correct glycosylation is important in the formation and eventually persistence of proper bile ductules and could potentially be part of the explanation for the finding of dilated and proliferating bile ductules in CDG patients. Maybe ADPLD should be classified as a CDG with organ specificity? Whether other glycosylation-related disturbances exist in this disorder is not known. Furthermore, deficiency of the α-glucosidase-I (CDG-IIb), removing the outermost α1,2-Glc residue from the protein-bound oligosaccharide, also caused bile duct proliferation and dilatation, pointing to a correlation between cyst formation and errors in the early steps of trimming of the oligosaccharide, prior to chaperone binding [23, 65]. The other gene known to cause ADPLD is Sec63 [66] – the resulting protein (Sec63p) being involved in trafficking of proteins in and out of the ER. Although not directly involved in the glycosylation process, it is involved in the early processes leading up to protein folding, implying that all mutations disturbing the protein folding process could potentially cause liver cystic disease.

ALG6 Deficiency (CDG-Ic)

ALG6 deficiency is the second most common CDG subtype with more than 30 known patients. Its molecular etiology is the deficiency of the first glucosyltransferase that adds an α1,3-Glc residue to the growing LLO chains [17]. The phenotype is typically milder than classical PMM2 deficiency (CDG-Ia), often with only minor psychomotor retardation and a spared cerebellar anatomy [34]. Why these patients' disease is milder is not completely known. The defects which occur relatively late in the LLO formation appear to have a smaller impact on brain formation, possibly because these chains are transferred to the proteins in a larger amount than shorter chains. Features such as low LDL, deficiency in coagulation factor XI, and protein-losing enteropathy are often present and may persist into adolescence [67]. The diagnosis of ALG6 deficiency is made by the detection of truncated LLOs, lacking Glc, after a positive transferrin hypoglycosylation test has been performed. Typically, the plasma aminotransferases are increased. Very few ALG6-deficient patients have undergone a liver biopsy. In one, there was almost no signs of abnormal histopathology [37] and in three others, the electron microscope revealed inclusions in lysosomes, as in PMM2 deficiency, but to a much less extent [35]. There is no treatment available for this subtype.

NOT56L Deficiency (CDG-Id)

This is a very severe subtype of CDG, often with multiple facial dysmorphic features, intractable seizures, pronounced mental retardation, and optic nerve atrophy [42, 68–70]. Chronic diarrhea and food intolerance were also described in these patients. The defective gene encodes the MT that catalyses the first Dol-P-Man-dependent step in LLO formation, addition of the sixth Man residue to the growing LLO chain [14]. Only seven patients have been described and the diagnosis is based on a pathological LLO pattern together with mutational analysis. Six of the patients did not show, or were not investigated for, the presence of liver involvement. In the most severe case reported thus far, a postmortem revealed ductal plate malformations [42], expanding bile ductule pathologies to more CDG types than PMI deficiency. Whether this was co-incidental or is a true finding in this subtype remains enigmatic until more patients are diagnosed. Two patients were, however, diagnosed with coagulopathies indicating at least partial involvement of the liver [42, 71]. There is no known therapy for this subtype.

DPM1 Deficiency (CDG-Ie)

Mutations in the gene (*DPM1*) encoding the catalytic domain of Dol-P-Man synthase [9] are the molecular cause of this syndrome. Clinically, the presentation is extremely severe and is characterized by pronounced psychomotor retardation, muscular hypotonia, and often blindness and facial dysmorphisms such as hypertelorism. One publication, however, describes a milder phenotype, possibly due to residual activity of the synthase [72]. In many clinical aspects it resembles NOT56L (CDG-Id) and MPDU1 (CDG-If)deficiencies, and the LLO patterns are indistinguishable in these patient groups. These children sometimes have normal birth weight, length, and head circumference, but microcephaly is characteristic later on [9, 73, 74]. Seven patients have been reported to date. The diagnosis is based on enzymatic analysis of Dol-P-Man synthase and a defective LLO pattern with accumulation of $Man_5GlcNAc_2$ structures. There are no reports describing liver histology in these patients, but signs of mild hepatic pathology, including hepatomegaly, recurrent elevations in aminotransferases, and mild coagulopathy have been noted [9, 72, 74]. Currently there is no therapy for this subtype.

MPDU1 Deficiency (CDG-If)

The underlying defective gene in this subtype (*MPDU1*) encodes a protein whose function is not fully understood, but is involved in the utilization of Dol-P-Man and Dol-P-Glc. Phenotypically, it resembles CDG-Id and CDG-Ie. Interestingly, two patients presented with ichthyosis [19, 75]. To date there are six known patients and the diagnosis is based on a pathological LLO pattern (accumulation of $Man_5GlcNAc_2$ and $Man_9GlcNAc_2$ structures) and genetic analysis. There seems to be very subtle liver pathology in this subtype with consistently normal aminotransferases [19, 75]. There is no available therapy.

ALG12 Deficiency (CDG-Ig)

The deficient gene in this CDG subtype, *ALG-12*, encodes MT VIII, adding the eighth Man to the growing LLO chain [16]. Currently eight patients have been described. This syndrome presents with mental retardation, male genital hypoplasia, dysmorphic facial and skeletal changes, and hypogammaglobulinemia [16, 76–79]. It is occasionally very severe with early lethality [79], whereas others have a milder phenotype [77]. Liver pathology is not profound in this subtype, however, all known patients have biochemical signs of coagulopathy and intermittently increased aminotransferases seems common. No patient has undergone liver biopsy. This subtype has no available therapy.

ALG8 Deficiency (CDG-Ih)

Together with PMI deficiency, this is the only CDG subtype that *can* present without obvious neuronal involvement. It stems from mutations in *hALG8*, encoding the second glucosyltransferase in the LLO synthetic pathway [18]. The patients often present with a severe syndrome involving the liver, the intestine, and the kidneys, and an early, fatal outcome is often seen (coagulopathies, protein-losing enteropathy, kidney failure) [80]. The lack of severe central nervous system (CNS) symptoms can be explained, in most patients, by the fact that they succumbed before reaching an age where they could be properly monitored [80, 81]. However, the first known case was 3 years of age at the time of diagnosis and no neurological findings were present [18]. The lack of CNS symptoms in this case can possibly be explained by the relatively low underglycosylation ($Glc_1Man_9GlcNAc_2$ structures are transferred relatively well to proteins). The liver in one subject was examined postmortem using light microscopy [81]. Interestingly, a finding of "multiple cystic dilated intra- and extrahepatic bile ducts, cholestasis, bilateral microcysts in all parts of the liver. . ." was seen – a liver pathology consistent with many PMI-deficient patients [36], one ALG3-deficient patient [42], and the only GCS1-deficient patient [23], described below. As argued before, the defects in the late LLO synthesis, and early in the protein-bound trimming (final glucosylation and deglucosylation), seem to develop structural pathologies of the bile ducts, where possibly there is a common mechanism. A pathological LLO pattern (accumulating $Man_9GlcNAc_2$ and $Glc_1Man_9GlcNAc_2$ structures), along with genetic analysis, diagnoses ALG8 deficiency, and there are five known cases to date [18, 80, 81].

ALG2 Deficiency (CDG-Ii)

Only one *ALG2*-deficient patient has been described so far [13]. The defective gene (*ALG2*) encodes MT II, the enzyme responsible for addition of the second Man residue to the growing LLO chain, on the cytoplasmic side of ER. The patient was born apparently normal, but developed colobomas of the iris and cataracts in her second month of life and thereafter seizures (infantile spasms) and MRI findings were consistent with hypomyelinization. There was biochemical evidence of coagulopathy (prolonged activated partial thromboplastin time and decreased levels of factor XI) and borderline hepatomegaly, but no other reported liver symptoms. No treatment exists for this subtype.

DPAGT1 Deficiency (CDG-Ij)

The defective gene (*DPAGT1*) of this subtype encodes a GlcNAc-1-P transferase, catalyzing the addition of the first GlcNAc (as GlcNAc-1-P) to the Dol-P lipid to initiate the LLO chain. The only known patient presented with mental retardation, microcephaly, intractable seizures, muscular hypotonia, and esotropia, but there was no signs of liver involvement [8]. The diagnosis was based on LLO analysis (low amounts of LLO formed), enzymatic assays, and genetic analysis. No treatment is at hand for this subtype.

HMT1 Deficiency (CDG-Ik)

This is an extremely severe syndrome and only one of four known patients survived into the second year [10–12]. All patients exhibited intractable seizures, and hypotonia, cerebral atrophy, visual impairment, and coagulopathy were prominent features. One patient developed nephrotic syndrome and hypogammaglobulinemia [10], whereas another had pronounced dysmorphic features and cardiomyopathy [12], showing the large variability within the CDG subtypes. Hepatomegaly was reported in one case, but there is no description of a light microscopic investigation of the liver at the autopsy [82]. The defective gene (*HMT1*) encodes the first MT in the LLO biosynthetic pathway and the disease is diagnosed by analyzing short LLO species [83], enzymatic assays, and genetic analysis. There is no treatment for this subtype.

DIBD1 Deficiency (CDG-IL)

This subtype stems from a deficiency in a MT that catalyzes the addition of both the seventh and the ninth mannose residue onto the growing LLO chain (MT VII/IX). It is encoded by *DIBD1* and there are two known patients to date [15, 84]. Both had severe brain abnormalities including microcephaly [15], diffuse brain atrophy, cerebellar hypoplasia, delayed myelinization, and seizures [84]. One patient also showed failure to thrive, cystic renal disease, hepatosplenomegaly with coagulopathy, pericardial effusion, and inverted nipples [84]. No specific analysis of the liver parenchyma was performed. Their diagnoses were based on a pathological LLO pattern and genetic analysis. There is no treatment available for this subtype.

DK1 Deficiency (CDG-Im)

The deficient gene in this subtype (*DK1*) encodes the enzyme responsible for the formation of Dol-P, the lipid anchor of the LLO, but also involved in the formation of other glycans, such as GPI anchors and *O*- and *C*-Man. Four patients have been described so far, all with a very

severe phenotype and no one survived beyond nine months [6]. The most prominent feature was ichthyosis or hyperkeratosis of the skin and muscular hypotonia. One patient developed hypsarrhythmia, one had persisting hypoglycemia and two dilative cardiomyopathy. Nothing is reported regarding specific liver involvement and there is no current treatment.

RFT-1 Deficiency (CDG-In)

Several patients have been described with this subtype [7, 85; PMID 19701946, PMID19856127]. The phenotype includes sensorineural deafness, failure to thrive seizures, developmental delay, hypotonia, and hepatomegaly, but no specific liver pathology is described. The defective gene (RFT-1) encodes the enzyme that flips the $Man_5GlcNAc_2$-P-P-Dol structure from the cytosolic to the luminal side of the ER. There is no available treatment.

Deficiencies in the Oligosaccharyltransferase Complex (N33/TUSC3 or IAP Deficiencies)

Recently two families with non-syndromic mental retardation proved to carry mutations in two paralogue genes encoding subunits of the oligosaccharyltransferase [20], responsible for the transfer of the $Glc_3Man_9GlcNAc_2$ glycan to the nascent polypeptide chains. Since there was no liver involvement these syndromes are not discussed further.

Untyped Type I Defects (CDG-Ix)

The number of patients in this group is steadily increasing and contains all CDG patients with a diagnostic biochemical profile for Type I CDG, but in whom the defective gene has not yet been identified. It accounts for approximately 50% of the patients with a Type I transferrin glycosylation pattern, diagnosed between 2001 and 2003 in the United States (Eklund E, et al., unpublished). This group contains patients with all different types of symptoms, including hepatic malfunction, but will not be discussed further in this chapter.

MGAT2 Deficiency (CDG-IIa)

This syndrome is caused by a deficiency in the GlcNAc transferase-II [26], involved in the formation of complex type oligosaccharide [86]. Four patients are known [34], and a mouse model has been developed [87]. The patients present with marked psychomotor retardation, but without peripheral neuropathy. Muscular hypotonia and seizures have also been described. MGAT2-deficient patients are prone to bleeding,

in contrast to PMM2-deficient ones (who are usually pro-thrombotic), possibly because MGAT2-deficient platelets are devoid of glycoprotein Ib reactivity with the vessel walls [88]. Analysis of liver biopsies failed to reveal lysosomal inclusions in this subtype and no other liver pathology has been described [89]. The diagnosis of MGAT2 deficiency is based on glycan structure analysis and enzymatic assays, and no treatment is available.

GLS1 Deficiency (CDG-IIb)

Only one patient has been described with this syndrome [23]. The molecular defect is the loss of ER α-glucosidase-I, the enzyme that removes the outermost α1,2-Glc residue from the protein-bound oligosaccharide $Glc_3Man_9GlcNAc_2$. This ameliorates the next step in processing, removal of the second Glc (by α-glucosidase-II), which creates the substrate for calnexin and calreticulin binding, crucial for protein folding [64]. The patient presented with dysmorphic features (e.g., prominent occiput, short palpebral fissures, retrognathia, and high-arched palate), severe hypotonia, failure to thrive, seizures, and progressive hepatomegaly. She died at day 74 after having become stuporous. A liver biopsy was taken at day 34 and the liver was also investigated postmortem. Light microscopy revealed proliferation and dilatation of bile ducts surrounded by fibrotic septa (cholangiofibrosis), with numerous macrophages in the periphery. Furthermore, the lysosomes were enlarged in the macrophages and more numerous in the hepatocytes. There was marked steatosis, which, together with the fibrosis, increased from the time of the biopsy to the time of autopsy. EM revealed myelin-like structures in the parenchymal cells [23]. The microscopic picture thus revealed a fibrocystic process, similar to the one reported for PMI deficiency and a few other CDG subtypes. Interestingly, as has been noted above, mutations in the non-catalytic subunit of α-glucosidase-II [24] also cause a fibrocystic syndrome [25], possibly via the same mechanism. The diagnosis of this patient was established by the finding of a Glc_3Man structure in the urine, which arises from the action of an endomannosidase that is thought to serve as a backup to normal glycosidase cleavage. The defect was confirmed by enzymatic measurements [23]. As with the great majority of these disorders, there is no treatment for this subtype.

SLC35C1 Deficiency (CDG-IIc; Leukocyte Adhesion Deficiency Type II [LADII])

SLC35C1 (encoding a Golgi-localized GDP-fucose transporter) deficiency leads to diminished GDP-fucose transport and is

characterized by increased amounts of non-fucosylated glycans [27]. Patients present with severe mental retardation and immunodeficiency due to an adhesion defect of the leukocytes (probably due to absence of α-1,3-fucosylated sialyl-Lewis^x selectin ligands) [90]. Addition of fucose to the diet has shown a positive response in two patients, whereas others were non-responders, possibly due to the nature of the mutations (K_m or V_{max} mutations). No liver involvement has been described. Recently, an animal model of this subtype was identified and the diagnosis is based on oligosaccharide and mutational analyses [91].

B4GALT1 Deficiency (CDG-IId)

Mutations in *B4GALT1* result in deficiency of a galactosyltransferase involved in the formation of sialylated complex type oligosaccharides [92]. One patient has been described so far, suffering from a syndrome involving muscular hypotonia, mild developmental delay, coagulopathy, and myopathy [28]. He also had a Dandy–Walker malformation with macrocephalus and progressive hydrocephalus. No specific liver involvement was noted, but the patient had prolonged activated partial thromboplastin time and elevated aspartate aminotransferase (AST). Diagnosis was based on glycan and mutational analysis. There is no treatment available.

COG Subunit Deficiencies (CDG-IIe, -IIg, and -IIh)

The COG (conserved oligomeric Golgi) complex is an eight-subunit structure involved in ER–Golgi transport, presumably the retrograde Golgi to ER and Golgi to Golgi paths [93]. Chinese Hamster ovary (CHO) cell mutants in two subunits of the corresponding complex show multiple glycosylation defects, due to disturbed Golgi function [94]. Two Type II CDG siblings were identified with multiple glycosylation errors, who were deficient in COG subunit 7 [30]. This subtype was named CDG-IIe, and a new field in CDG research was opened. The children showed a very severe syndrome with dysmorphic features, hypotonia, and cholestatic liver disease. Biochemically, there was evidence of coagulopathy. Both children died within months of their birth [95]. One child underwent autopsy and light microscopy of his liver revealed fibrosis and intrahepatic cholestasis with tubular formation [95], thus resembling the liver pathology of some other CDG patients. Four additional patients with COG7 deficiency have since been described [96, 97], all with a similarly severe syndrome with early lethal outcome. All have carried the same homozygous mutation suggesting a common ancestral gene [93].

One patient has been described with a defect in COG subunit 1 (CDG-IIg) [29]. This child presented with generalized hypotonia, dysmorphic features (including small hands and feet, anti-mongoloid eyelids with temporal region hypoplasia), and failure to thrive. She also developed mild hepatosplenomegaly, with slightly elevated aminotransferases. No liver biopsy was performed. The patient is only slightly developmentally delayed and a brain magnetic resonance imaging at 21 months revealed only slight atrophy of the cerebellum and the cerebrum.

Deficiency of COG subunit 8 (CDG-IIh) has been described in two patients [31, 32]. They had growth retardation, hypotonia, failure to thrive, seizures, psychomotor retardation, and polyneuropathy in common. One patient showed elevated aminotransferases and coagulopathy [32]. No liver biopsies were performed. There are no current treatments for any of the COG subunit defects.

SLC35A1 Deficiency (CDG-IIf)

The encoded protein for the defective gene in this subtype of CDG is a CMP-sialic acid transporter [33] and the only described patient suffered from enlarged, but few, platelets (macrothrombocytopenia), and neutropenia with total lack of the sialyl-Le^x epitope on polymorphonuclear cells [98]. However, as no other organs were involved this syndrome will not be discussed further.

Untyped CDGs (CDG-IIx)

As for CDG-Ix, many patients have tested positive for Type II defects of transferrin glycosylation (loss of less than whole glycan chains) or defects in both *N*- and *O*-linked glycosylation, but the causative gene is unknown. These patients are collectively termed CDG-IIx and many clinical variants exist. Some authors describe patients with CDG-IIx of particular interest concerning liver pathology [99–101]. Mandato et al. described a series of four patients with cryptogenic liver disease and mild hypoglycemia as the only sign of CDG [99]. The first patients (siblings) had elevated aminotransferases, mild coagulopathy, and reduced haptoglobin. A liver biopsy revealed mild periportal fibrosis and steatosis. Otherwise the siblings were normal. The combination of fibrosis and multiple biochemical defects prompted testing for CDG and the transferrin pattern was consistent with a combination of both Type I and Type II defects. Mass spectrometric analysis of transferrin showed an absence of entire *N*-glycan chains together with loss of individual sugar residues (mostly galactose and sialic acids) from the glycans. High performance liquid chromatographic (HPLC) analysis

of total serum *N*-glycans showed a similar loss of these monosaccharides. No causative mutation has been identified. The third case also presented with elevated aminotransferases and coagulopathy without signs of other disease. The liver biopsy showed steatosis and mild periportal inflammation (which had developed into periportal fibrosis at a follow-up biopsy). The fourth patient developed elevated aminotransferases during a bout of hypoglycemia and a liver biopsy also revealed steatosis. Recent examinations of these patients as young adults (18–22 years of age) (P. Vajro, personal communication) showed variable mild hypercholesterolemia, slightly elevated or normal AST, and occasional hypoglycemia; alkaline phosphatase (ALP) and creatine phosphokinase (CPK) were elevated in only one patient. These results show that mild, yet to be defined, defects in *N*-glycosylation of proteins can lead to liver-specific abnormalities and suggests that CDG should be excluded in the setting of cryptogenic liver pathology.

CONCLUSIONS

The liver is a highly efficient glycoprotein factory, and not surprisingly, glycosylation disorders take a heavy toll on the liver's ability to function. Whether it is the addition of entire *N*-glycan chains or their processing to more complex forms, many of the glycosylation disorders affect embryonic development, architecture, and functional output of the liver. Why the phenotype is highly variable between different Type I defects, all of which fail to add entire *N*-glycans to proteins, is not known. One impediment to more detailed understanding is the near absence of appropriate vertebrate models of the glycosylation disorders. Most relevant gene knockout mice die *in utero*, and organ- or temporal-specific conditional knockouts may not be more informative, since these cells would likely undergo apoptosis. Several laboratories are replacing normal alleles with known patient mutations in the highly homologous mouse genes. Mouse *Pmm2* and *Mpi* are the leading candidates with the latter having the advantage of mannose rescue. Another attractive option is use of zebrafish models to investigate the effects of decreasing the expression of a variety of glycosylation-relevant genes. Forward and reverse genetic approaches may both generate data relevant to liver pathology.

We cannot overemphasize the importance of testing transferrin glycosylation early in a patient's evaluation. This simple and inexpensive test is well worth the investment because, if done early on, it can detect a score of different glycosylation disorders. While therapy is only now available for one type, work is in progress to find treatments for the hundreds of PMM2-deficient patients, who have the most common form of CDG.

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13 Autosomal Recessive Polycystic Kidney Disease

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CONTENTS

Summary

Autosomal recessive polycystic kidney disease (ARPKD) is an inherited disorder involving cystic dilatation of the renal collecting ducts as well as varying degrees of hepatic abnormalities consisting of cysts, fibrosis, and portal hypertension. The ARPKD locus has been mapped to chromosome 6p21 and encodes a novel protein product named fibrocystin or polyductin. There are several modes of presentation depending on the age and the predominance of hepatic or renal involvement. With improvements in medical management and continued progress in end-stage renal disease therapy in young infants, further improvements in survival and rehabilitation can be anticipated.

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INTRODUCTION

Autosomal recessive polycystic kidney disease (ARPKD) is an inherited disorder involving cystic dilatation of the renal collecting ducts as well as varying degrees of hepatic abnormalities consisting of cysts, fibrosis, and portal hypertension. ARPKD has previously been referred to as "infantile polycystic kidney disease." This term has largely been discarded with the recognition that the disease can present at any time from the prenatal period through adolescence. Furthermore, other forms of polycystic kidney disease can present in the neonatal period. The incidence is estimated at 1 in 10,000 to 1 in 40,000 live births [1–3]. There are several modes of presentation depending on the age and the predominance of hepatic or renal involvement.

GENETICS AND PATHOGENESIS

Typical of an autosomal recessive disorder, heterozygotes are unaffected, the sexes are involved equally, and the recurrence risk is 25% with each subsequent pregnancy in couples at risk. The ARPKD locus has been mapped to chromosome 6p21 [4]. The extremely large gene, polycystic kidney and hepatic disease 1 gene (PKHD1), encodes a novel protein product named fibrocystin [5] or polyductin [6] that is made up of over 4,000 amino acids. A single genetic defect leads to varying degrees of renal or hepatic involvement [7–9]. Fibrocystin/polyductin is found in the cortical and medullary collecting ducts and the thick ascending limb of the kidney and the epithelial cells of the bile ducts [10]. A genotype–phenotype correlation has been described with ARPKD, with truncation mutations portending a much worse prognosis than those with substitution mutations [11–13].

Despite the advances in the molecular genetics of ARPKD, the pathogenesis is not well understood. On the basis of extensive in vivo and in vitro studies utilizing human tissue and animal models of PKD, it appears that at least three basic processes are operative in renal cyst formation and progressive enlargement: tubular cell hyperplasia, tubular fluid secretion, and abnormalities in tubular extracellular matrix, structure, and/or function [14–19]. The specific role of each of these factors,

singly or in combination, in ARPKD or autosomal dominant polycystic kidney disease (ADPKD) is an area of active investigation.

PATHOLOGY

In the infant and young child, the kidneys are increased in size, with pinpoint opalescent dots visible on the capsular surface which correspond to the cystic cortical collecting ducts [20]. Microscopically, the cysts are usually less than 2 mm in size and have been shown by microdissection, histochemical, and immunologic studies to be dilated collecting ducts [21–24] (Fig. 13.1). They are lined by low columnar or cuboidal epithelium. The glomeruli and other tubular structures appear to be decreased in number due to marked collecting duct ectasia and interstitial edema. The pelvi-calyceal system and renal vessels appear normal [25]. Microdissection studies and scanning electron microscopy demonstrate that obstruction of urinary flow is not a component of ARPKD [20, 21]. The severity of the renal disease is proportional to the percentage of nephrons affected by cyst. With increased patient survival, the development of larger renal cysts (up to 1 cm), fibrosis, and hyperplasia produces a pattern more like seen in ADPKD [26, 27]. Gang and Herrin describe increasing fibrosis and inflammation in later specimens from patients who had typical collecting duct microcysts during infancy [28]. It is unclear whether the fibrotic change is part of the natural course or due to environmental factors such as toxins, hypertension, end-stage renal failure, or hemodialysis.

Some degree of biliary dysgenesis and hepatic fibrosis is always present in ARPKD. Even in the newborn, the liver demonstrates microscopic abnormalities. The classic liver lesion is portal fibrosis surrounding an increased number of hyperplastic, ectatic biliary ducts with normal hepatocellular histology [29]. With time, hepatomegaly and portal hypertension become evident in many patients. Intrahepatic biliary ectasia may result in macrocysts and dilation of extrahepatic bile ducts, sometimes resulting in an enlarged gallbladder [30] or choledochal cysts [27]. Although the combination of collecting tubule and biliary ectasia with periportal fibrosis is unique to ARPKD, portal fibrosis and bile duct proliferation may be associated with other types of renal disease including ADPKD [28], juvenile nephronophthisis [31], renal dysplasia [32], Meckel syndrome [29], Jeune and Zellweger syndromes [29], Ivemark syndrome (renal–hepatic–pancreatic dysplasia) [29, 33], Bardet–Biedl syndrome [34], chondrodysplasia syndromes [29], trisomy 9 and 13 [29], glutaric aciduria Type II [29], and congenital hypernephronic nephromegaly with tubular dysgenesis [35, 36].

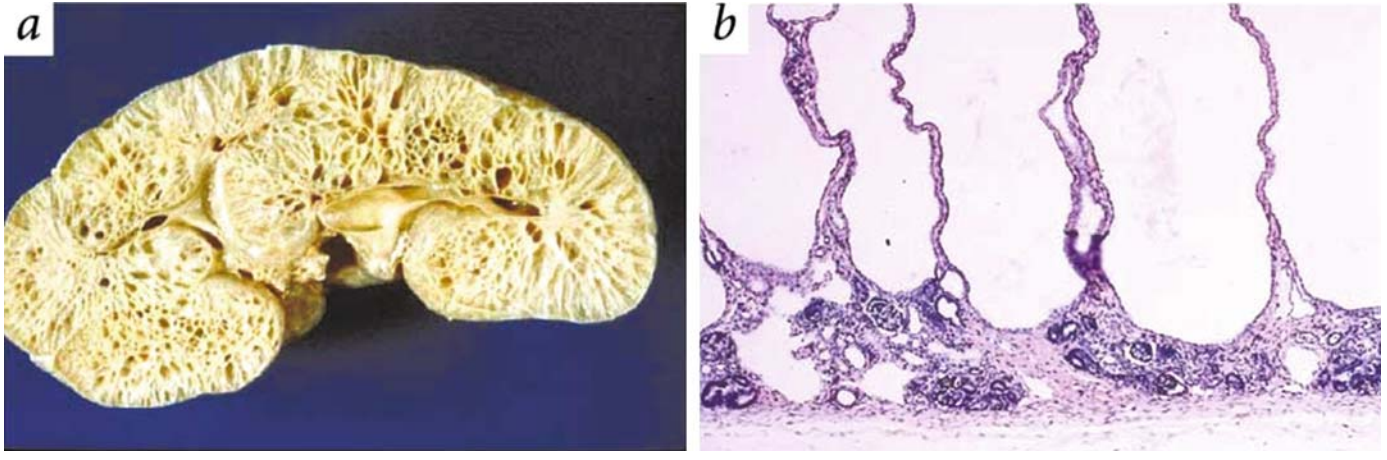

Fig. 13.1. a, A cross section of the kidney shows the typical pattern of fusiform, cylindrical channels that occupy most of the kidney parenchyma. These cysts are massively dilated terminal branches of collecting ducts. **b**, A photomicrograph of a section shows the radially oriented, fusiform cysts that are characteristic of ARPKD.

CLINICAL MANIFESTATIONS

There are several types of clinical presentation depending on the age of onset and the predominance of hepatic or renal involvement. One gene defect can be associated with varying degrees of disease.

Most patients with ARPKD present in infancy [25, 37–39]. Prenatal ultrasound may demonstrate the findings of oligohydramnios, large renal masses, or absence of fetal bladder filling [40]. Others present at birth with huge flank masses that can complicate delivery. Severely affected infants may demonstrate the oligohydramnios sequence with pulmonary hypoplasia, and Potter's phenotype which consists of deep set eyes, beaked nose, micrognathia, and low set ears [41]. There are often extremity contractures which are felt to be secondary to fetal restriction from lack of amniotic fluid [25]. Respiratory distress, an early complication, can be a symptom of pulmonary hypoplasia, pneumothorax, atelectasis, or a variety of common neonatal pulmonary disorders (including surfactant deficiency, bacterial pneumonia, meconium aspiration, or persistent fetal circulation). Infants with true pulmonary hypoplasia often die soon after birth secondary to pulmonary insufficiency. With marked oligohydramnios, renal function in the infant is likely to be compromised, although death from renal insufficiency in the newborn period is uncommon [38, 42]. With improvement in respiratory status, urine output often increases followed by a corresponding improvement in renal function [43].

Patients who survive the neonatal period usually have a decreased glomerular filtration rate, but studies have demonstrated subsequent improvement in renal function consistent with some degree of continued renal maturation [44]. GFR may be normal by 12 months of age and those patients with neonatal presentation who survive past 1 month of age do not develop renal insufficiency until late childhood or early adolescence.

ARPKD may also present in infants or older patients with abdominal enlargement secondary to enlarged kidneys or hepatosplenomegaly. Because of the involvement of the collecting tubule, almost all patients have a concentrating defect and can have symptoms of polyuria and polydipsia [25, 26, 37–39]. Although metabolic acidosis and hyponatremia have been reported, the serum electrolytes are generally normal [38, 39].

Hypertension is common in both infants and children and can be a presenting feature [25, 26, 44]. It can be present in patients with normal renal function and eventually affects almost all children with the disease [38, 44]. Although the pathophysiology of hypertension in ARPKD is not clearly understood, peripheral renin levels are not usually elevated

[37, 39]. If not aggressively managed, hypertension can result in cardiac hypertrophy and congestive heart failure, which may be a factor in progression of underlying renal insufficiency. In addition, endocardial fibroelastosis has been reported in a patient with ARPKD [45].

Occasionally, the infant or older child with ARPKD presents with an abnormal urinalysis which may consist of microscopic or gross hematuria, proteinuria, or pyuria [25, 38, 44]. Pyuria can be seen in the absence of demonstrable bacteriuria or documented infection [26]. Urinary tract infection has been reported as a frequent complication, but it is unclear whether children with ARPKD truly have an increased incidence of upper or lower urinary tract infections when compared with appropriately age-matched controls.

Deterioration of renal function occurs in most patients. The glomerular filtration rate is decreased in the first few weeks of life, then increases during the first 2 years, remains stable for several years, and then slowly declines. End-stage renal disease usually occurs after 15 years of age [37] but may occur earlier in those who present in the first year of life [44].

The older child may present with complications of hepatic fibrosis and portal hypertension [26, 42]. These include hepatosplenomegaly; bleeding esophageal varices; portal thrombosis; and hypersplenism causing thrombocytopenia, anemia, and leukopenia. Liver tests are usually normal. Although macroscopic liver cysts are uncommon, choledochal cysts have been reported [27]. A serious and potentially lethal complication in ARPKD patients with significant hepatic involvement is bacterial cholangitis which has been reported as early as a few weeks of age [38]. Hepatic complications progressively dominate the clinical picture of many patients with ARPKD following successful early renal replacement therapy such as dialysis and/or transplantation.

DIAGNOSIS

Prenatal ultrasound findings of enlarged kidneys, oligohydramnios, and absence of urine in the bladder can suggest the diagnosis of ARPKD but are not diagnostic [46]. Sonographic features of ARPKD may be noted as early as 24 weeks gestation but usually are not apparent until after 30 weeks gestation [2]. Both false positives and false negatives have been reported [47]. Increased maternal alpha fetoprotein has been reported, but is nonspecific. The identification of the genetic defect associated with ARPKD has provided the basis for genetic linkage or haplotype-based prenatal diagnosis in “at-risk” families. It is important to stress that haplotype-based prenatal analysis is an indirect method whose accuracy depends on the correct diagnosis in the index case. Accurate

phenotypic diagnosis in previously affected children is essential for reliable haplotype-based prenatal testing.

Until direct genetic testing for the ARPKD gene is available, the diagnosis is typically based on post-natal ultrasound findings. The ultrasound usually shows enlarged kidneys with diffusely increased echogenicity obscuring the corticomedullary junction [48]. A more specific ultrasound feature has been reported to be the presence of a hypoechoic subcapsular rim of parenchyma [49]. Macrocysts can be present but usually are less than 2 cm in diameter [48, 50]. Herrin also noted a decrease in renal size by ultrasound with age which is a differentiating feature from ADPKD where kidneys usually increase in size over time [51]. Renal macrocysts less than 2 cm and increased medullary echogenicity have been reported in older children [26, 43].

The abdominal ultrasound in the older child can show massive hepatosplenomegaly with hyperechoic liver. Macroscopic liver cysts are uncommon [48] but choledochal cysts have been reported [27]. Computed tomography with enhancement shows linear or radial opacification in enlarged kidneys [38]. Magnetic resonance imaging may demonstrate the microcystic dilated water-filled collecting ducts [52].

Diagnostic criteria have been developed [53]: Ultrasonographic features typical of ARPKD, including enlarged, echogenic kidneys with poor corticomedullary differentiation, with one or more of the following (a) absence of renal cysts in both parents when aged older than 30 years; (b) clinical, laboratory, or radiographic evidence of hepatic fibrosis; (c) hepatic pathology demonstrating characteristic ductal plate abnormality; (d) previously affected sibling with pathologically confirmed disease; or (e) parental consanguinity suggestive of autosomal recessive inheritance.

CLINICAL MANAGEMENT

Survival of neonates with ARPKD has improved in concert with overall developments in neonatal artificial ventilation and intensive care. It is currently impossible to predict which neonates with ARPKD require immediate artificial ventilation and have critical degrees of pulmonary hypoplasia incompatible with survival [38, 54]. In some instances, severe pulmonary distress may be secondary to potentially reversible fluid overload, neonatal lung disease, or restricted diaphragmatic motion secondary to massively enlarged kidneys. In selected cases, continuous renal replacement therapy or bilateral nephrectomy coupled with peritoneal dialysis may be initially required to allow optimal ventilation and thereby assess the long-term pulmonary prognosis of the patient.

Infants and young children without significant renal insufficiency must be followed closely. Supplemental bicarbonate therapy is required for those with metabolic acidosis. As most children with ARPKD have a concentrating defect, significant dehydration is a particular risk during intercurrent illness which may increase insensible water loss (fever), limit free water intake (nausea), or increase extrarenal water loss (vomiting, diarrhea). In patients with severe polyuria, thiazide diuretics may be of benefit to decrease distal nephron solute and water delivery. Hypertension can be treated with calcium channel blockers, beta blockers (in those without chronic lung disease or signs of congestive heart failure), and diuretics. Despite the fact that peripheral renin values are not usually elevated in hypertensive ARPKD patients, many patients respond well to angiotensin-converting enzyme inhibitors.

The presence of pyuria does not always indicate infection. Thus, as in any child with an abnormal urinalysis, clinical features and appropriately obtained urine cultures must guide antibiotic therapy. If urinary tract infection is documented, a voiding cystourethrogram and renal ultrasound should be performed to determine the possible presence of vesicoureteral reflux and rule out obstruction or superimposed upper tract structural abnormalities [28].

The treatment of chronic kidney disease in children with ARPKD has improved over the past decades. Aggressive management of renal osteodystrophy and anemia is indicated. The use of growth hormone and nutritional management including formula supplements has improved growth and overall health in children with chronic kidney disease.

Dialysis and/or renal transplantation is indicated when children with ARPKD reach symptomatic end-stage renal failure or if progressive uremia results in growth failure or developmental delay. Preemptive nephrectomy and institution of peritoneal dialysis may be necessary in the neonatal period if the large cystic kidneys preclude adequate respiratory function. Peritoneal dialysis is often the preferred method in the young child. Peritoneal dialysis in ARPKD is often successful even in the face of large kidneys and hepatosplenomegaly.

Kidney transplantation offers definitive renal replacement therapy in children with ARPKD. Successful kidney transplantation prolongs survival and often accelerates growth and development in young uremic children. At the time of transplantation, bilateral nephrectomy may be indicated to control hypertension, or in the case of massively enlarged kidneys, permit room for transplant placement.

Cholangitis may be a particularly difficult problem in the immunocompromised ARPKD transplant patient. In infants and children with predominant hepatic involvement, close monitoring for complications

of portal hypertension is mandated. Radiographic studies and gastroesophageal endoscopy may be necessary for evaluation of suspected esophageal varices that can subsequently be treated with sclerotherapy or variceal banding. Periodic monitoring may reveal the hematological profile of hypersplenism. Portacaval shunting may be indicated in some cases, but may adversely influence the subsequent success of liver transplantation which may be required in rare instances for advanced portal hypertension. Fever or elevation of liver tests at any time should lead to suspicion of cholangitis and result in complete evaluation and appropriate antimicrobial therapy.

In addition to the significant medical problems noted, the psychosocial stresses of ARPKD on the patient and family can be overwhelming. Social support measures and periods of respite care are often necessary. A team approach utilizing the skills of pediatric nephrologists in concert with other pediatric medical subspecialists, specialized nurses, dietitians, social workers, psychiatrists, and other support staff provides optimal comprehensive care for children with ARPKD.

PROGNOSIS

The largest cohort of patients described is from the ARPKD clinical database which includes information on 254 patients from 34 centers in North America [55]. In this longitudinal study, neonatal ventilation, earlier age of diagnosis of chronic kidney disease, and hypertension were strongly predictive of mortality. In addition, they found that the long-term prognosis for infants who survive the perinatal period is much better than generally perceived. A long-term outcome study of neonatal survivors with ARPKD reported 1- and 5-year patient survival rates of 85 and 82%, respectively, and renal survival at 5, 10, and 20 years of 86, 71, and 42%, respectively [11]. With improvements in medical management and continued progress in end-stage renal disease therapy in young infants, further improvements in survival and rehabilitation can be anticipated.

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14 Caroli Disease, Caroli Syndrome, and Congenital Hepatic Fibrosis

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CONTENTS

Summary

The cystic diseases of the liver are mostly autosomal recessive disorders with variable intrahepatic biliary dilatation and increased hepatic fibrosis either as part of the underlying defect or as a result of liver damage from recurrent episodes of ascending cholangitis. Caroli disease (CD) denotes congenital saccular intrahepatic dilatation of the biliary tree. Caroli syndrome (CS) is a more common disorder in which the bile duct dilatation is associated with congenital hepatic fibrosis (CHF). The clinical manifestations of CS are related to the biliary abnormalities and portal hypertension. Recurrent bacterial cholangitis, liver abscesses, intra- and extrahepatic stones, and increased risk of cholangiocarcinoma are the main complications of CD and CS. Treatment is supportive and directed toward treating the biliary tract infections and the complications of portal hypertension.

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Surgical treatment consists of liver resection or liver transplantation. The prognosis of CD and CS is variable depending on the severity of the disease, the presence of portal hypertension, and the presence of coexisting renal disease.

CHF is a hereditary developmental malformation that belongs to the family of hepatic ductal plate malformation (DPM), which resulted from persistence of excess embryonic bile duct structures in the portal tracts. It may be associated with other liver malformations such as in CS and it is usually associated with autosomal recessive polycystic kidney disease (ARPKD). The identification of the gene for ARPKD, PKHD1 (polycystic kidney and hepatic disease 1) had shown that most cases of ARPKD with CHF are genetically homogeneous. Clinically, symptoms vary and different manifestations of CHF have been described such as portal hypertensive CHF, cholangitic CHF, combined portal hypertensive and cholangitic CHF, and latent forms of CHF. Therapy of CHF depends on the clinical manifestation of the disease. Treatment is largely supportive and it is directed toward treating biliary tract infection and the complications of portal hypertension. Liver transplantation is indicated in patients with recurrent uncontrolled cholangitis and in patients with end-stage liver disease.

Key Words: Caroli disease, Caroli syndrome, Congenital hepatic fibrosis, Ductal plate malformation (DPM), Autosomal recessive polycystic kidney disease, Portal hypertension, Cholangitis, PKDH1 (polycystic kidney and hepatic disease 1) mutation

INTRODUCTION

The cystic diseases of the liver are predominantly autosomal recessive disorders with variable intrahepatic biliary dilatation and increased hepatic fibrosis either as part of the underlying defect or as a result of liver damage from recurrent episodes of ascending cholangitis [1]. These diseases are frequently associated with cystic renal diseases that determine the clinical presentation and the prognosis. In this chapter we will discuss the two forms of congenital dilatation of the intrahepatic biliary tree – Caroli disease (CD) and Caroli syndrome (CS), and congenital hepatic fibrosis (CHF).

CAROLI DISEASE AND CAROLI SYNDROME

In 1958, Jacques Caroli described two forms of congenital intrahepatic dilatation of the biliary tree [2]. The rare variant characterized by pure ductal ectasia without other hepatic abnormalities and is now referred to as CD and the more common type in which the bile duct dilatation is

associated with CHF is now called CS [3]. A defect in bile duct morphogenesis has been postulated to be the cause of ductal plate malformation (DPM) that leads to persistent embryonic bile ducts [4]. The level of involvement of the biliary tract by the DPM determines the anatomic manifestation of the cystic disease of the liver. DPM of the larger intrahepatic bile ducts leads to the development of CD, whereas CHF is a result of malformations at the level of the interlobular bile ducts [5]. The two variants are associated with renal cystic disease: CS is associated with autosomal recessive polycystic kidney disease (ARPKD) [6] or rarely with autosomal dominant polycystic kidney disease (ADPKD) [7, 8]. CD is rarely associated with ARPKD.

Genetics

The mode of inheritance of CD is almost certainly autosomal recessive although some authors believe that it is not hereditary [9, 10]. There are reports of siblings affected by this disease in the literature [11–14]. Wu et al. described [15], two siblings from Taiwan who were diagnosed at age 26 and 29 years with CD. Sonographic survey of the family was carried out but did not reveal the disease in the parents or the other two siblings. They concluded that CD is an autosomal recessive inherited condition and family history should be carefully evaluated. There is a rare report of an autosomal dominant inheritance of CD in a Japanese family [16]. The mode of inheritance of CS is autosomal recessive.

Epidemiology

Prior to 1998, over 200 cases of CD have been reported in the literature [1, 7] and the estimated incidence is about 1 in 1,000,000 births [10]. Males and females are equally affected and more than 80% of the patients present before 30 years of age [17, 18]. The incidence is of CS is not known but it seems that it is higher than the "pure form" of CD.

Pathogenesis

CS and CD are developmental anomalies secondary to an abnormality in remodeling of the developing ductal plate of the liver [1]. DPM refers to the persistence of excess embryonic bile duct structures in the portal tracts [4]. Intrahepatic bile ducts are derived from primitive hepatocyte precursor cells that ensheath the mesenchyme of the portal vein [19]. The normal development of intrahepatic bile ducts requires finely timed and precisely tuned epithelial–mesenchymal interactions, which proceed from the hilum of the liver toward its periphery along the branches of the developing portal vein. Lack of remodeling of the

ductal plate results in the persistence of an excess of embryonic bile duct structures remaining in their primitive ductal plate configuration [20]. DPM is a feature of all types of hepatic fibrocystic diseases and may affect all levels of the biliary system, ranging from the irregularly shaped bile ducts in portal tracts in CHF to the segmental, saccular dilatation of the larger intrahepatic ducts in CD. The molecular pathogenesis of CD and CS and the association to ARPKD are incompletely understood. The gene for ARPKD, PKHD1 (polycystic kidney and hepatic disease 1) is located on chromosome 6p12 and encodes a large protein called fibrocystin/polyductin (FPC), one of many proteins that are normally present at the primary cilia of the renal tubules and intrahepatic bile ducts [21]. Although exact function of FPC is not fully known, it is generally felt that like many of the other ciliary proteins, it plays a vital role in maintaining the structural integrity of organs such as kidney and liver by modulating important cellular functions, including proliferation, secretion, apoptosis, and terminal differentiation. Genetic abnormalities in PKHD1 may result in structural and functional abnormalities of FPC, leading to cystic phenotype [22, 23]. The renal cysts of ARPKD arise as ecstatic expansion of the collecting tubules. The liver cysts in CS or CD develop from bile ducts and remain in continuity with their structures of origin. FPC probably works in conjunction with cellular proteins involved in ADPKD disease, that is, polycystin-1 and polycystin-2, which are also located in the primary cilia [23].

Pathology

The biliary abnormality of CD consists of segmental, saccular dilatation of the large intrahepatic bile ducts [24–26]. The ducts are lined by biliary epithelium that may be hyperplastic or ulcerated. In CS these findings are associated with CHF lesions – broad and narrow septa of dense, mature fibrous tissue. In CD the bile duct abnormalities can be diffuse or localized, usually in the left lobe. In a reported series of 12 cases of unilobular liver disease, 9 were localized to the left lobe and 3 to the right lobe [26]. CD is rarely associated with renal lesions of ARPKD, while CS is typically accompanying CHF and associated with this form of renal disease.

Clinical Manifestations

The saccular dilatation of bile ducts in patients with CD predisposes to bile stasis and to the formation of biliary sludge and stones. Recurrent bacterial cholangitis often occurs and can be complicated by sepsis and formation of intrahepatic abscesses [27]. A literature review revealed recurrent acute cholangitis as the main mode of presentation in 64% of the patients with CD [28]. The clinical manifestations of these episodes

are fever, jaundice, and right upper quadrant pain [29]. The disease can be asymptomatic for decades or can present in early childhood. In a series of 33 patients with CD who were referred for surgical treatment (18 females), the initial symptoms and signs included right upper quadrant pain, fever, and jaundice. Only two patients had clinical evidence of cirrhosis and one patient suffered from variceal bleeding [30]. The clinical manifestations of CS are related to the biliary abnormalities and portal hypertension secondary to CHF [9, 25, 31]. The major manifestations of portal hypertension are hematemesis or melena secondary to bleeding from esophageal varices. The age of presentation of CS is highly variable with renal-presenting symptoms and cholestasis in infancy, while cholangitis and manifestations of portal hypertension (variceal bleeding, ascites) are more likely the presenting features in early childhood [32]. Symptoms may appear later in life, depending on the progression of the portal hypertension and the severity of the biliary disease. Presenting signs and symptoms of CD and CS include intermittent abdominal pain, pruritus, and an enlarged and hard liver. The findings of either splenomegaly or cytopenias secondary to hypersplenism are early indications of evolving portal hypertension and are more common in children with CS than in children with CD. Frequent episodes of cholangitis may lead to end-stage liver disease with the related clinical and laboratory findings. Enlarged kidneys are often palpable in patients depending on the degree of renal involvement. Jaundice may occur in the neonate during bouts of cholangitis or in patients who have advanced liver disease. Systemic hypertension is related to the degree of renal involvement.

The laboratory findings reveal mildly elevated aminotransferase levels. Hepatic synthetic function is preserved initially but may be affected by progressive liver damage secondary to recurrent cholangitis and biliary obstruction. Coagulopathy (elevated prothrombin time (PT)) can be secondary to liver dysfunction or may be due to vitamin K malabsorption in cholestatic patients. The blood count may show evidence of hypersplenism with leukopenia and thrombocytopenia. Anemia can be detected and is often secondary to occult variceal bleeding. Blood urea nitrogen (BUN) and creatinine may be elevated reflecting the degree of renal involvement. Elevation of γ-glutamyltransferase (GGT), alkaline phosphatase, and direct bilirubin can be noted during episodes of cholangitis or biliary obstruction.

Diagnosis

The diagnosis of CS and CD is usually based on the radiologic findings of communication between the intrahepatic cystic lesions with the biliary system. Abdominal ultrasound, computerized tomography

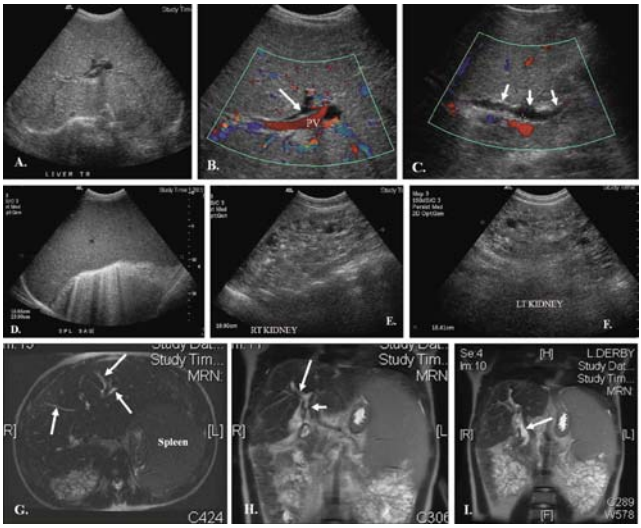

Fig. 14.1. (a–i). CS with ARPKD. 15-year-old female with ARPKD and hepatosplenomegaly. In (**a**), there is diffusely increased coarse parenchymal echogenicity throughout the liver with almost no visualization of the peripheral portal venous walls. In (**b**), there is central biliary ductal dilatation (*arrow*) (PV=portal vein). In (**c**), the extrahepatic biliary ducts are dilated and beaded (*arrows*). Massive splenomegaly is demonstrated in (**d**) with the spleen length measuring 23.8 cm. Both kidneys are enlarged (**e**), (**f**) and appear diffusely heterogeneous with tiny cysts interspersed with focal areas of bright echogenicity likely from the multiple specular reflectors from the enumerable cysts. There is no hydronephrosis. MRI images confirmed the intrahepatic ductal dilatation (*arrows*) on the axial SS THIN slice in (**g**) and on the coronal SS THIN slice in (**h**), both the intrahepatic duct (*large arrow*) and the extrahepatic ducts (*small arrow*) are dilated. In (**i**), the intrahepatic ducts are once again noted to be dilated, but in addition, there is marked dilatation and beading of the extrahepatic biliary ducts (arrow). The spleen is massively enlarged on all the MRI images.

scan (CT), endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography (PTC), and magnetic resonance cholangiopancreatography (MRCP) are the common modalities for evaluation of these lesions (Fig. 14.1). Although ERCP and PTC have a high sensitivity in the diagnosis of CD and CS, they are invasive procedures with risk of complication such as bleeding, bile leaks, or infection. MRCP generally allows differentiation between fibrocystic liver diseases and polycystic liver diseases [30]. The presence of dilated sacciform or tubular bile ducts on cholangiopancreaticography associated with a centrally located fibrovascular bundle (central dot sign)

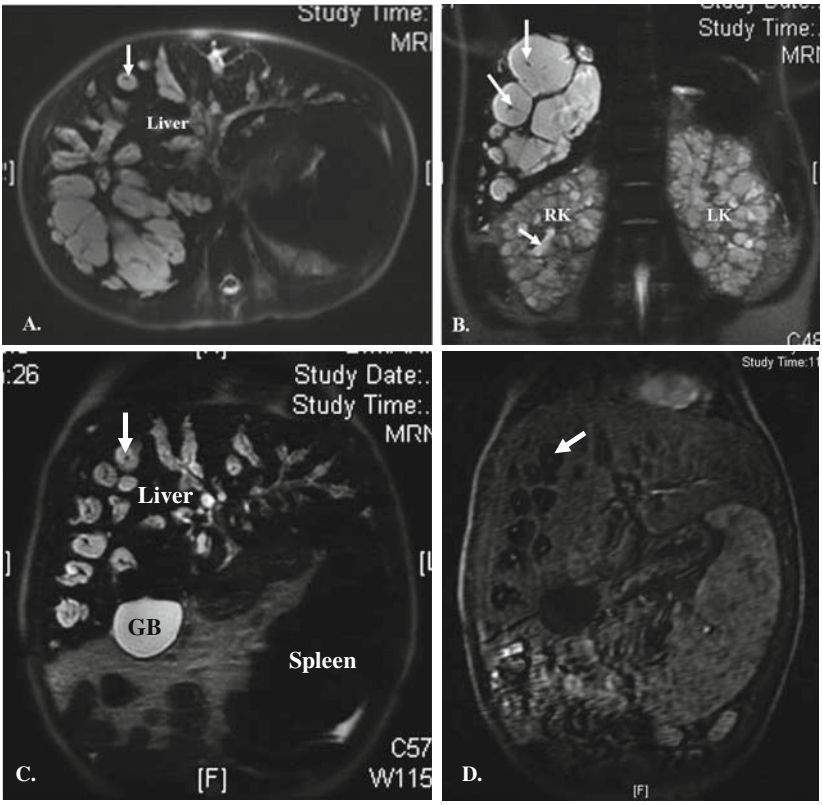

Fig. 14.2. (a–d). CS and ARPKD "central dot" sign. An 11-year-old female with CS and ARPKD. Axial (**a**) fat suppressed T2 axial MRI image demonstrating hepatomegaly with a lobulated border and very marked dilatation of the intrahepatic bile ducts (*arrow* points to "*central dot*"). In (**b**) the fat-suppressed coronal T2 image demonstrates additional tiny central defects within the dilated intrahepatic bile ducts, consistent with the "*central dot sign*" (*arrows*). Both kidneys are enlarged and there are multiple variable size cysts throughout the renal parenchyma bilaterally (RK = right kidney, LK = left kidney). Minimal fullness of the right renal pelvis (*arrow*) is noted. In (**c**) the coronal GE GRE thin-slab T2 MRI image and in (**d**) the coronal post-gadolinium-enhanced image, there are multiple defects within all of the visualized ducts arrows in (**c**) and (**d**) point to "central dot". The spleen is massively enlarged extending inferiorly to the bony pelvis and displacing bowel toward the right side of the abdomen (GB = gallbladder).

suggests CS (Figs. 14.2 and 14.3). The presence of associated signs of liver dysmorphia including right lobe atrophy and hypertrophy of segment IV may suggest associated CHF [33]. MRCP can demonstrate the accompanying renal abnormalities, the presence of splenomegaly,

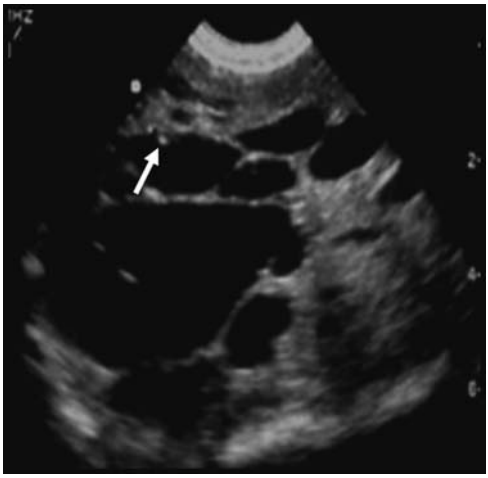

Fig. 14.3. "Central dot sign." Teenager with hepatic fibrosis and ARPKD. This ultrasound image of the liver demonstrates multiple intrahepatic cysts representing saccular dilatation of multiple biliary ducts that converge toward the porta hepatis ("central dot sign"). The *arrow* points to a portal radical which is completely surrounded by a dilated duct. Note that the hepatic parenchyma is hyperechoic and coarse throughout the non-cystic portions of the liver, consistent with fibrosis.

and varices as part of portal hypertension, and it may be useful in the evaluation of a suspected malignancy [34]. It is the modality of choice for diagnosis of CD and CS [34–38]. Liver biopsy is rarely required to make the diagnosis of CD or CS.

The pathological findings of CD may show ectasia of the larger intrahepatic ducts with features of cholangitis. Intrahepatic bile duct ectasia and proliferation with severe periportal fibrosis are the pathological features of the CHF component of CS.

Differential Diagnosis

The differential diagnosis of CS and CD includes primary sclerosing cholangitis (PSC), recurrent pyogenic cholangitis, polycystic liver disease, choledochal cysts, and obstructive biliary dilatation. Some cases of choledochal cyst disease have been associated with mutations in the PKHD1 gene, usually with renal disease [39]. PSC and recurrent pyogenic cholangitis can be associated with duct dilatation, stenosis, intrahepatic calculi, and malignancy. The ductal dilatation in PSC is usually isolated and fusiform in opposition to the characteristic saccular dilatation of CS and CD. It may be difficult to differentiate between

polycystic liver disease and CS, as in both diseases the patients can have hepatic and renal cysts. However, the hepatic cysts of polycystic liver disease do not communicate with the usually normal bile ducts, and portal hypertension is rare in polycystic liver disease [18]. CS should be considered in the differential diagnosis for patients with intra- and extrahepatic biliary dilatation. Repeated bouts of cholangitis, stone formation (Fig. 14.4), and stone passage may explain extrahepatic duct dilatation in (Fig. 14.5) some patients with CD or CS [40].

Associated Conditions

CD has been associated with inherited and acquired disorders, especially with cystic diseases of the kidney. It has also been reported in association with Lawrence–Moon–Biedl syndrome [41], medullary sponge kidney [5], ADPKD, and rarely with ARPKD. CS is associated with renal involvement in up to 60% of patients. Kidney lesions in CS include ARPKD, renal tubular ectasia (medullary sponge kidney, cortical cyst), or rarely ADPKD.

Complications

Recurrent episodes of cholangitis and sepsis, liver abscesses, intra- and extrahepatic stones, and increased risk of cholangiocarcinoma are the main complications of CD and CS. Patients with CS can have complications of portal hypertension such as variceal bleeding [42–45].The incidence of cholangiocarcinoma was reported in patients with CD to be up to 100 times higher than in the average population [46]. It may occur in 5–10% of patients with CS [42]. The increased risk of cancer may be related to biliary stasis and chronic inflammation of the biliary epithelium [47, 48]. Systemic amyloidosis was described in patients with CD and it is probably related to chronic systemic inflammation from recurrent episodes of cholangitis [49]. Liver failure secondary to recurrent cholangitis or biliary cirrhosis, liver abscesses, or complications of portal hypertension increases the mortality rate of these patients.

Treatment

Medical Treatment

The treatment of CD and CS is largely supportive and directed toward treating the biliary tract infections and the complications of portal hypertension. Antibiotic therapy is used to treat cholangitis and sepsis. Gram-negative sepsis has a high mortality, especially if it is not

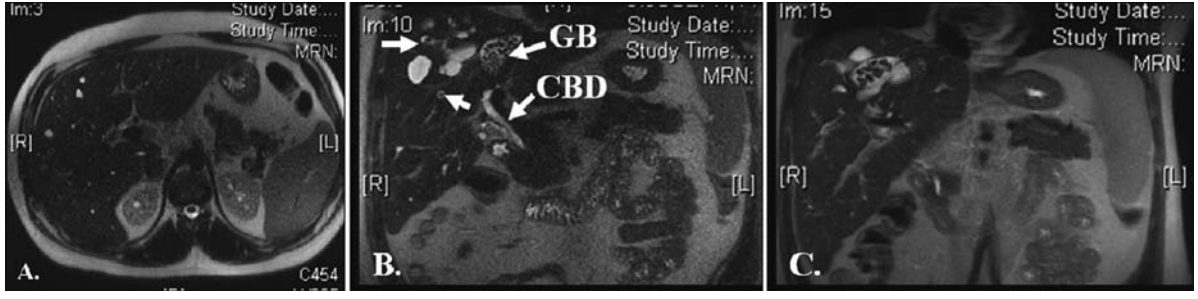

Fig. 14.4. (a–c) CD with choledocholithiasis. Axial coronal SS (HASTE) MRI image (**a**) of the abdomen shows a normal size liver with several dilated intrahepatic biliary ducts (*white dots*) and several small cysts within the normal size kidneys (*white dots*). Mild splenomegaly is noted. Coronal SS (HASTE) (**b**) demonstrates larger cysts within the liver representing dilatation of multiple bile ducts in addition to mild dilatation of the common bile duct (CBD). Each *arrow* points to a small defect, each of which is thought to be a stone with dilated intrahepatic ducts. Multiple stones are seen within the gallbladder (GB) lumen. The coronal HASTE sequence (**c**) shows multiple filling defects within the gallbladder consistent with stones, as well as cysts within the liver parenchyma and dilated biliary ducts.

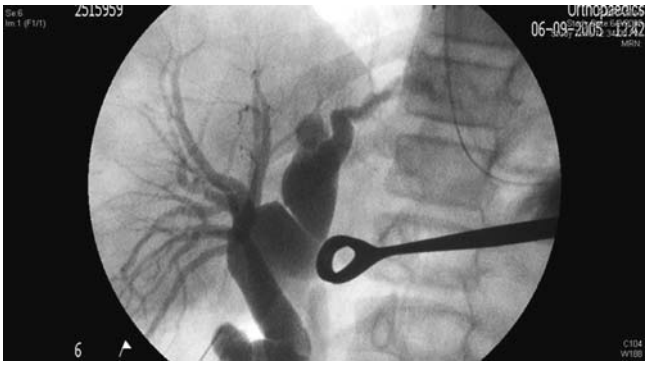

Fig. 14.5. Unilateral left-sided CD. A 9-year-old female S/P surgery for choledocholithiasis complicating choledochal cyst. Intraoperative cholangiogram demonstrating marked dilatation of the left intrahepatic and the entire common bile duct to the level of the ampulla of Vater, consistent with unilateral CD. The right intrahepatic ducts are of normal caliber.

diagnosed and treated early. It is not clear whether antibiotic prophylaxis for prevention of cholangitis is indicated. ERCP with sphincterotomy or PTC can be used for extraction of stones from the common bile duct, thus improving biliary drainage. Ursodeoxycholic acid (10–20 mg/kg/day) can be added to increase the bile flow, and fat-soluble vitamin supplementation is needed in cholestatic patients. In the case of a new biliary stricture or an unexplained clinical deterioration, the development of cholangiocarcinoma should be considered and evaluated. There are no proven or validated methods to screen for this complication.

Patients with CS may have portal hypertension. The presence of significant esophageal varices necessitates prophylactic treatment with non-selective beta-blockers. Therapy for acute variceal bleeding includes hemodynamic stabilization, use of intravenous somatostatin, early endoscopy with band ligation, or sclerotherapy (in small children). Shunting procedures such as splenorenal or portocaval shunts may be indicated for recurrent bleeding episodes and failure of endoscopic therapy since the liver function may be well preserved. TIPS (transjugular intrahepatic portosystemic shunt) is not a long-term solution for portal hypertension in this disease. Screening children with CHF for esophageal varices remains undefined. Fagundes et al. [50], however, reported that children with CHF with no previous history of digestive bleeding were more likely to have esophageal varices than cirrhotic patients. They recommend endoscopic screening in all patients with CHF.

Surgical Treatment

Surgical treatment consists of liver resection (segmental or lobar) or liver transplantation [30, 51]. If the biliary process is confined to a single lobe, lobectomy will relieve the symptoms. Surgical bypass procedures such as Roux-en-Y hepatojejunostomy may be helpful in diffuse forms of CD or CS. Liver transplantation is recommended for bilobar disease with progressive decompensation of liver function and complications of portal hypertension. Liver transplantation should also be offered in cases of refractory cholangitis. Special issues exist in the decision-making process regarding liver transplantation in children with ARPKD. Indications for combined liver and kidney transplantation in ARPKD include the combination of renal failure and either recurrent cholangitis or refractory complications of portal hypertension [52, 53]. Millwala et al. [54] published a study based on UNOS (United Network for Organ Sharing) data on 104 patients with CD/CS transplanted between 1987 and 2006 and showed excellent patient and graft survival similar to, or better than, that of patients transplanted for other causes. The overall 1-, 3-, and 5-year graft (79.9, 72.4, and 72.4%) and patient (86.3, 78.4, and 77%) survival rates were excellent. For combined liver/kidney transplantation ($n = 8$), the 1-year patient survival and graft survival were 100%. The authors proposed an algorithm for the evaluation and medical and surgical management of CD [54, 55].

Prognosis

The prognosis of CD and CS is variable depending on the severity of the disease, the presence of portal hypertension, and the presence of coexisting renal disease. Recurrent cholangitis, complications related to biliary stones, and cholangiocarcinoma effect patient morbidity and survival. Table 14.1 compares between CD and CS.

CONGENITAL HEPATIC FIBROSIS

Congenital hepatic fibrosis (CHF) is a hereditary developmental malformation that belongs to the family of hepatic ductal plate malformation, which results from persistence of excess embryonic bile duct structure in the portal tracts [56]. It is characterized by hepatic fibrosis, portal hypertension, and often associated with cystic diseases of the kidneys. It was first described by Kerr et al. [57]. The exact incidence and the prevalence of CHF is unknown. CHF is transmitted as an autosomal recessive trait. It may be associated with other liver malformations such as CS-bile duct dilatation with CHF. Although CHF

Table 14.1
Comparison between Caroli disease and Caroli syndrome

	Caroli disease (CD)	*Caroli syndrome (CS)*
Incidence	Estimated at about 1 in 1,000,000 births	Unknown higher than CD
Inheritance	• AR • Non-hereditary? • AD (rare report)	AR
Pathogenesis	Arrest in the ductal plate remodeling at the level of the large intrahepatic bile ducts	Arrest in the ductal plate remodeling at the level interlobular bile ducts
Pathology	Segmental, saccular dilatation of the large intrahepatic bile ducts (diffuse, localized)	Saccular dilatation of the large and small intrahepatic bile ducts associated with CHF
Genetic defect	Unknown	Mutations in PKHD1 gene that encode fibrocystin in cases that are associated with ARPKD
Clinical manifestations	Recurrent cholangitis	• Recurrent cholangitis • Manifestations of portal hypertension
Associated conditions	• ARPKD (rarely) • ADPKD • Medullary sponge kidney [5] • Lawrence–Moon–Biedl syndrome [40]	• ARPKD (common) • ADPKD
Complications	• Sepsis • Liver abscesses • Intra- and extrahepatic lithiasis • Cholangiocarcinoma	• Sepsis • Liver abscesses • Intra- and extrahepatic lithiasis • Cholangiocarcinoma • Complications of portal hypertension (e.g., variceal bleeding)

Table 14.1
(continued)

	Caroli disease (CD)	*Caroli syndrome (CS)*
Treatment	• Antibiotics • Ursodeoxycholic acid • Fat-soluble vitamins • Stone extraction • Liver resection • Liver transplantation	• Antibiotics • Ursodeoxycholic acid • Fat-soluble vitamins • Stone extraction • Liver resection • Liver transplantation • Treatment for portal hypertension (non-selective beta-blockers, band ligation, or sclerotherapy) • Shunting procedures
Prognosis	Variable, depending on the severity of the liver disease and the presence of coexisting renal disease	Variable, depending on the severity of the liver disease and the presence portal hypertension and coexisting renal disease

AR: autosomal recessive; AD: autosomal dominant; CHF: congenital hepatic fibrosis; PKHD: polycystic kidney and hepatic disease; ARPKD: autosomal recessive polycystic kidney disease; ADPKD: autosomal dominant polycystic kidney disease.

is usually associated with autosomal recessive polycystic kidney disease (ARPKD), it has been reported as an isolated entity [58] with autosomal dominant polycystic kidney disease (ADPKD) [59, 60] and nephronophthisis [61, 62]. ARPKD is a rare disease with an incidence of 1 in 20,000. It often presents as bilateral nephromegaly related to a generalized dilation of the collecting ducts [63, 64]. Until recently, it was not clear if CHF and ARPKD were a single disorder with wide spectrum of manifestations or two distinct entities that share phenotypically similar hepatic lesions. The identification of the gene for ARPKD, PKHD1 (polycystic kidney and hepatic disease 1), has shown that most cases of ARPKD with CHF are genetically homogeneous. PKHD1, as was discussed earlier, encodes fibrocystin/polyductin (FPC). Recent studies have demonstrated that FPC is localized to primary cilia and suggest a causative role for this organelle in cystic renal disease [65].

There is phenotypic variability among affected siblings with ARPKD with the same mutations that suggests the importance of modifier genes as well as possibly environmental influences. ARPKD may present with

a broad spectrum of clinical and histopathological phenotypes with two common characteristic features: fusiform non-obstructive dilatations of the collecting tubules and bile duct abnormalities of the DPM type [26]. The presentation of ARPKD ranges from severe renal impairment with a high mortality rate in infancy to more minimal renal disease and complications of CHF, cholangitis, and portal hypertension in older children and adolescents [66].

Blyth and Ockenden divided ARPKD into four clinical and pathological subgroups [67]. The first was termed "perinatal" and presented with severe renal disease, congestive heart failure, and respiratory distress from pulmonary hypoplasia. The second "neonatal" group also had progressive renal disease while the third "infantile" group was comprised of infants and young children who had less severe renal disease at presentation and gradual progressive hepatic fibrosis. The fourth group, termed "juvenile," had minimal renal disease, but complications of CHF were prominent. Approximately 30% of patients with ARPKD present perinatally with enlarged kidneys. Renal failure may be present at birth or develop later in life. Hypertension and growth failure are related to compromised renal function [1]. The kidney and liver disease are progressive in most patients with a variable rate of deterioration. More than half of the patients with ARPKD will require kidney transplantation before reaching 20 years of age [68].

Hepatic fibrosis was described in a variety of syndromes such as Jeune syndrome (asphyxiating thoracic dystrophy, with cystic renal tubular dysplasia and hepatic fibrosis) [69], Joubert's syndrome (oculoencephalo-hepato-renal) [70], COACH syndrome (cerebellar vermis hypoplasia, oligospermia, congenital ataxia, ocular coloboma, and hepatic fibrosis) [71], Bardet–Biedl syndrome (retinal degeneration, obesity, limb deformities, hypogonadism) [72], Ivemark syndrome (pancreatic fibrosis, situs inversus, polysplenia, cardiac, and CNS anomalies) [73], and congenital disorder of glycosylation-1b (chronic diarrhea, protein losing enteropathy, coagulopathy) [74]. When phenotypic features suggesting these disorders are excluded, a high rate of PKHD1 mutations can be detected even with isolated CHF. Yönem et al. [75] prospectively investigated the associated disorders and the clinical consequences of 19 patients (13 women, 6 men) with CHF. CHF-associated diseases included CS, polycystic kidney disease, cavernous transformation of the portal vein, Joubert's syndrome, von Meyenburg complex, polydactyly, medullary sponge kidney, and pancreatic duct atrophy. There was only one case with pure CHF. They concluded that CHF is not a pure liver disease but rather a multiorgan disorder involving the brain, portal vein, kidneys, and bile ducts.

Pathogenesis

The pathogenesis of CHF has not been fully elucidated, although studies done in rodent models of ARPKD have suggested that fibrogenesis and ongoing portal/septal fibrosis result from a combination of asymmetric and disproportionate overgrowth of biliary epithelial cells and their supporting connective tissue [76]. Proliferating bile duct epithelial cells are a major source of connective tissue growth factor (CTGF), a factor that modulates liver fibrogenesis [77]. CTGF stimulates fibroblast proliferation and extracellular matrix synthesis [78, 79]. Heparin sulfate proteoglycan (HSPG), a component of the extracellular matrix, regulates the presentation of various cytokines and growth factors, which may be transferred and bound to the receptors of stellate cells [80]. In CHF, profuse deposition of CTGF and HSPG has been colocalized in the fibrous portal tract/septa, and this suggests that CTGF retained in HSPG may be involved in ongoing and unresolving portal/septal fibrosis [81].

Pathology

The liver of patients with CHF is normal in size without macroscopically visible cysts but is speckled with irregular whitish areas of fibrosis. Microscopically, CHF is characterized by islands of normal liver parenchyma with unaltered vascular relationships that are separated by broad and narrow septa of dense, mature fibrous tissue. The fibrous tissue contains elongated or cystic spaces lined by regular biliary epithelium (Fig. 14.6). These represent a cross section of the hollow structures comprising the DPM. The lumen may contain inspissated bile [82]. There is a sharp demarcation between the portal fibrosis and the hepatic parenchyma. There is no necrosis, inflammation, hepatocellular regeneration, or dissection of the lobules by fibrosis. Portal vein branches often appear reduced in size and number, and the sparsity of venous channels may account in part for portal hypertension. The liver lesions of CHF may progress with time; however, in some patients they remain relatively unchanged [83]. The fibrosis may increase secondary to recurrent episodes of cholangitis. The hepatic lesions in patients with ARPKD are indistinguishable from those of isolated CHF. The fibrosis of CHF can be differentiated from cirrhosis, in which there is nodular regeneration, often inflammation and necrosis. The portal tracts in CHF are expanded by mature collagenous tissue which forms interportal bridges that initially do not disrupt the acinar architecture. The preserved lobular architecture explains why children with CHF do not exhibit hepatocellular dysfunction unless liver damage is superimposed by bouts of cholangitis and obstruction from biliary sludge.

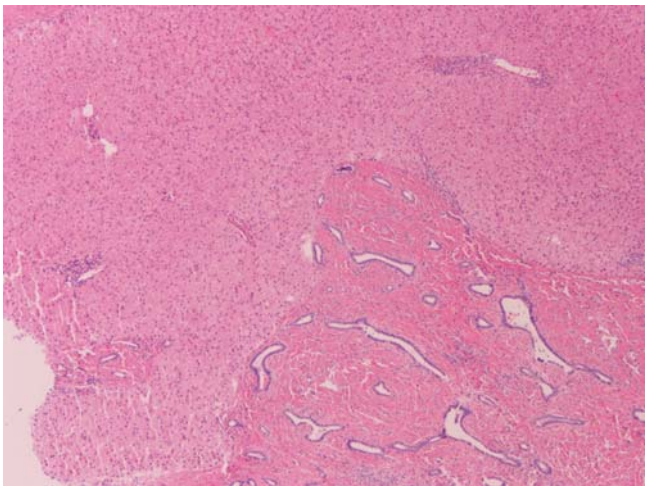

Fig. 14.6. Liver biopsy specimen from 12-year-old girl with congenital hepatic fibrosis. The picture shows portal tract expanded by fibrosis with numerous bile duct structures, some with curvilinear profiles of ductal plate malformation.

Clinical Manifestations

The onset of signs and symptoms is variable, ranging from early childhood to the fifth or sixth decade of life. Most cases are diagnosed during adolescence or young adulthood [84]. Clinical symptoms vary and different manifestations of CHF have been described such as portal hypertensive CHF, cholangitic CHF, combined portal hypertensive and cholangitic CHF, and latent forms of CHF [64, 85].

Portal Hypertensive CHF

The portal hypertensive type of CHF is the most common form. The most common manifestation is usually recurrent upper gastrointestinal hemorrhage from ruptured esophageal varices. These episodes have been described as early as 19 months of age [86], although they are more commonly seen in older children. These patients usually have other features of portal hypertension such as splenomegaly and hypersplenism with thrombocytopenia and leukopenia [53]. The precise pathogenesis of the development of portal hypertension is unknown, but it has been attributed to the compression of portal vein radicles in the fibrous bands and to an anomaly in the branching pattern of the portal vein, giving rise to hypoplastic and involutive branches [57]. Hypoplasia and a paucity of intrahepatic portal veins in patients with

CHF have also been described by Summerfield et al. [9]. The aminotransferases and growth of patients with CHF and portal hypertension are usually normal.

Cholangitic CHF

In this form the main manifestations are cholestasis and recurrent episodes of cholangitis that may lead to sepsis. Patients with cholangitic CHF have intra- and extrahepatic biliary ductal abnormalities and they may have liver dysfunction and poor growth.

Mixed Type

These patients have manifestations of portal hypertension and episodes of recurrent cholangitis. The cholangitic and mixed forms may be due to coexisting CS, although some authors have reported cases with no evident macroscopic dilatation of the bile ducts [56]. In some patients the disease is latent and manifests late in life or is noted incidentally. Alvarez et al. described their experience with 27 children with CHF that were followed for 3 months–12 years [87]. Hepatomegaly was present in 25 of the patients and splenomegaly was present in 24. The left lobe was predominantly enlarged in 24 patients and was palpable in the epigastrium. The serum bilirubin, prothrombin time (PT), aminotransferases, and alkaline phosphatase were normal in all the children. No impairment of growth was observed except in those with renal failure. Gastrointestinal bleeding (hematemesis) was observed in 11 patients and was the first sign of disease in 8. Esophageal endoscopy showed varices in 21 patients and cholangitis was present in only 3 children.

In patients with ARPKD, the liver disease can present as cystic dilatation of the intrahepatic biliary tree (CD), with fibrosis of the portal tracts without dilated bile ducts (CHF) or with both (CS). There are two major clinical manifestations of the liver disease in patients with ARPKD: biliary disease and portal hypertension. The prevalence of clinically relevant CHF in ARPKD ranges from 46 to 83% [88, 89]. The prevalence of portal hypertension from CHF in ARPKD ranges from 20% in infants to 60% in the larger studies in all age groups [90]. Progression of clinical signs of hepatic fibrosis occurs with increasing age [87].

Cholangitis and biliary disease is much less common in ARPKD than portal hypertension, occurring in 6–12% of cases [89]. The common manifestations of biliary disease are sepsis/cholangitis and/or the complications of cholelithiasis or biliary sludge. Cholangitis in infants and young children with ARPKD may be fatal [90]. A significant number of children have been described who have died of complications related to biliary sepsis after renal transplantation for ARPKD [63, 64, 66, 91].

Guay-Woodford and associates described findings of liver disease in 209 patients who had ARPKD followed at 34 centers in North America [64]. In patients born prior to 1990, portal hypertension was present in 32%, variceal hemorrhage in 11%, and bacterial cholangitis in 5%; 3% of patients required liver transplantation. In patients born after 1990, portal hypertension was present in 15%, a history of variceal bleeding in 4%, and bacterial cholangitis in 4%; 4% of patients required liver transplantation. Of patients from both groups who had liver imaging studies, 78 (53%) had normal images, 46 (31%) had echogenic livers, and 24 (16%) had dilated intrahepatic bile ducts consistent with CD. Bergmann et al. reported 164 patients with ARPKD and documented PKDH1 mutation [65]. Sequelae of CHF and portal hypertension developed in 44% of patients who survived the neonatal period and correlated with increasing age. A correlation between renal and hepatobiliary-related morbidity was also found.

Laboratory Findings

Laboratory evaluation is usually unremarkable in patients without portal hypertension or episodes of cholangitis. The serum aminotransferase and bilirubin levels are characteristically normal. Thrombocytopenia and neutropenia are seen in patients with portal hypertension and hypersplenism. The bilirubin, GGT, and alkaline phosphatase may be elevated during episodes of cholangitis. Blood urea nitrogen (BUN) and creatinine levels may be elevated in patients with renal involvement.

Physical Examination

On physical examination, abdominal distension may be present with hepatomegaly and splenomegaly in patients with portal hypertension. The liver is firm with a prominent left lobe.

Diagnosis

The diagnosis of CHF is suggested by abdominal US, CT, or MRI. Definitive diagnosis is determined on the basis of liver biopsy, but its routine use is rarely required and it may be reserved for the patients with equivocal findings. The diagnosis of CHF in patients with ARPKD/CHF can be confirmed on the basis of genetic mutation analysis. Mutations are distributed throughout the PKHD1 gene and polymorphisms are common. The current mutation detection rate is 80–85% [68]. There is marked allelic heterogeneity and most affected patients appear to be compound heterozygotes. Sequence analysis, combined with haplotype analysis in multiply affected families, provides the most

comprehensive results [92]. Prenatal diagnosis of ARPKD/CHF, on the basis of haplotype analysis and mutation analysis, has been performed [93].

Imaging

Cross-sectional imaging is indispensable for demonstration of the findings of CS, CD, and CHF. Duplex/color Doppler ultrasound (US) serves as a screening modality for assessment of the biliary system and may be used for follow-up once the diagnosis is established. US plays an important role in the evaluation of these patients as a cost-effective, portable, non-ionizing, non-invasive imaging modality that requires no intravenous contrast or sedation. The diagnosis of CS and CD is based on the demonstration of multiple dilated tubular structures typical of biliary radicals. They can be shown to extend to the periphery of the liver and at times to communicate with larger cystic areas which represent more focally ectatic portions of the biliary tree. Color Doppler is essential for the differentiation of these structures from vascular structures. The dilated ducts may contain intraluminal protrusions arising from the walls or echogenic bands or bridges. Sludge and stones are often visible within the dilated ducts. In addition, small branches of the portal vein are often seen within the dilated ducts, each of which appears as a small, echogenic dot in the nondependent part of the dilated duct ("central dot sign") [94]. Color Doppler examination can show flow in the central vascular portal venous radicles. In addition, US plays a role in the evaluation of the hepatic parenchyma. Although diffuse coarsening and increased echogenicity are non-specific findings in the presence of chronic inflammation and cholestasis, they are characteristic of deposition of dense material, including fibrous tissue. Periportal cuffing (thickening and increased echogenicity) along the portal tracts is highly suggestive of periportal fibrosis. Assessment for the complications of cholangitis may also be accomplished with US. Abscesses generally appear as cavities with walls thicker than those of the ducts and, when present, are another clue to the diagnosis. At times, the gallbladder and common bile duct may become enlarged and splenomegaly is easily visualized. Duplex/color Doppler also plays an important role in patients with portal hypertension by determining the patency and direction of flow within the biliary and abdominal vessels and for demonstrating the presence of collateral venous channels, portosystemic shunts, and ascites [84].

Renal findings include discrete cysts or markedly enlarged kidneys with brightly echogenic medullary pyramids. As the appearance of the kidneys may be confused with autosomal dominant polycystic kidney

disease, the demonstration of continuity of the intrahepatic cysts with the bile ducts is important to exclude ADPKD, polycystic liver disease, and simple liver cysts.

Computed tomography (CT) and/or magnetic resonance imaging (MRI) are used for clarification and confirmation of the US findings and for demonstration of the extent of the disease (Figs. 14.7, 14.8, and 14.9). CT and MRI provide a more complete assessment of blood flow and the entire biliary ductal system (magnetic resonance cholangiopancreatography – MRCP). They also provide better tissue characterization and thus improve differentiation between fibrocystic liver diseases and polycystic liver disease [53, 95]. Endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC) also provide additional information but are generally reserved for those patients in whom therapeutic intervention may be beneficial. Although they have high sensitivity in the diagnosis of CS and CD, they are invasive procedures with risk of complications such as bleeding, bile leaks, and/or infection.

Therapy

The therapy of CHF depends on the clinical manifestations of the disease. It is largely supportive and it is directed toward treating biliary tract infection and the complications of portal hypertension. Antibiotic therapy is indicated for cholangitis. The management of portal hypertension is complex in pediatric patients and lacks evidence-based approaches [96]. Some centers proceed with primary variceal prophylaxis with non-selective beta-blockers for large varices identified by surveillance endoscopy. Variceal bleeding can be treated endoscopically by sclerotherapy or band ligation. The liver function in CHF is usually well preserved for prolonged period, and a selective shunting procedure can provide relief from the complications of portal hypertension. Moon et al. reported the usefulness of distal splenorenal shunt (DSRS) in 15 children (5 with CHF) with portal hypertension for treatment of severe thrombocytopenia and leukopenia [97]. Nine patients underwent multiple sessions of endoscopic variceal ligation to control variceal bleeding prior to the DSRS. Alvarez et al. reported that 17/27 children with CHF and portal hypertension underwent successful portosystemic shunt (11/17 after episodes of variceal bleeding) [87].

Liver transplantation is indicated in patients with recurrent uncontrolled cholangitis and in patients with end-stage liver disease. Reversal of severe hepatopulmonary syndrome in a child with CHF after living-related liver transplantation was described by Nagral et al. [98]. The prognosis of CHF is good if the complications of portal hypertension

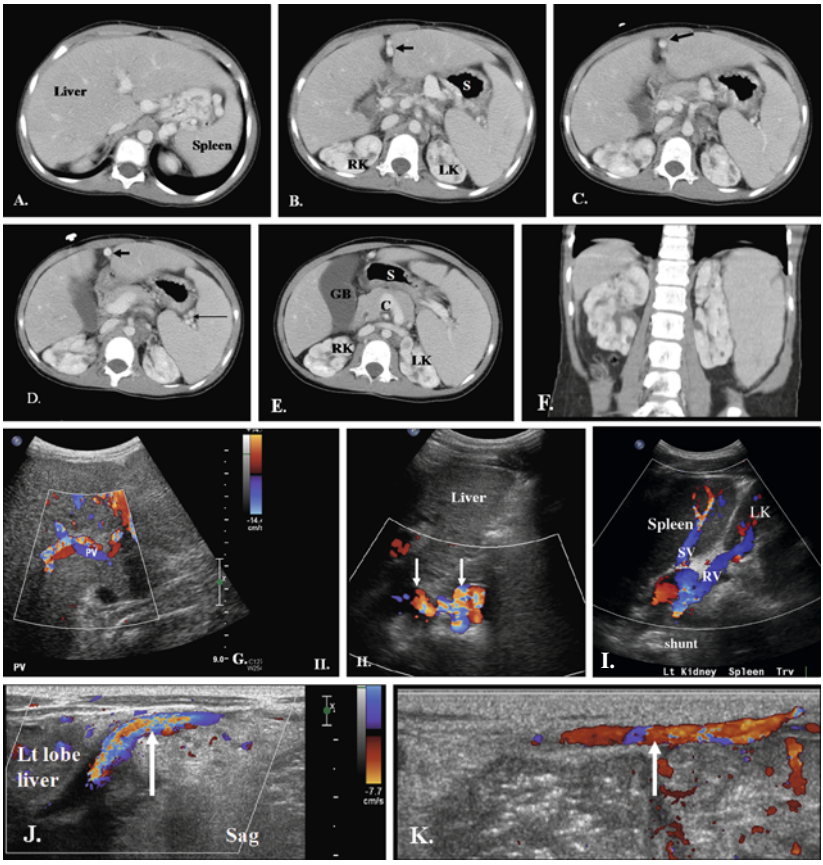

Fig. 14.7. (a–k). CHF with mild changes of ARPKD. A 14-year-old with COACH syndrome, S/P splenorenal shunt; 5 mm axial CT slices of the abdomen following intravenous injection of contrast material. Axial CT slices of the abdomen (**a–e**) demonstrate hepatosplenomegaly with no focal defects within either organ. Extensive varices are seen in the region of the splenic hilum and at the greater and lesser curvatures of the stomach. A recanalized paraumbilical vein (*small arrow*) is seen in (**b–d**). The portosplenic confluence is dilated as shown in (**d**). The kidneys are normal size but lobulated in contour with multiple low-density lesions of varying sizes are present in each kidney suggesting small cysts seen best on the coronal image (**f**). Note that hepatofugal flow (**g**) is recognized by reversal of color Doppler flow. Various collateral venous channels may be shown with US. In (**h**) gastroesophageal varices (*arrows*) are demonstrated, in (**i**) a splenorenal shunt (SV=splenic vein, RV=renal vein), and in (**j**) and (**k**), recanalization of the umbilical vein as evidenced by a vessel (*arrow* in each figure) coursing away from the liver and extending along the abdominal wall toward the umbilicus.

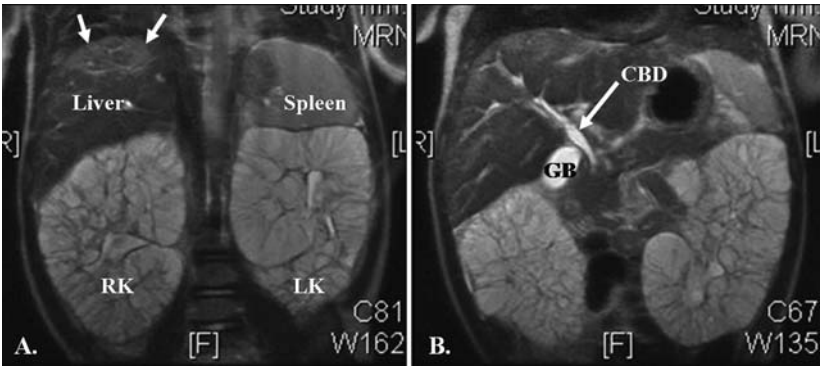

Fig. 14.8. (a, b). CHF and ARPKD: 4-year-old male with known CHF and ARPKD. T2-weighted MRI coronal images of the abdomen demonstrate (in **a**) a wedge-shaped area of bright signal intensity (*small arrows*) in the subcapsular area of the liver. The kidneys are markedly enlarged with multiple cysts throughout. Dilatation of the common bile duct (CBD) is noted in **b**. A normal appearing gallbladder (GB) is seen.

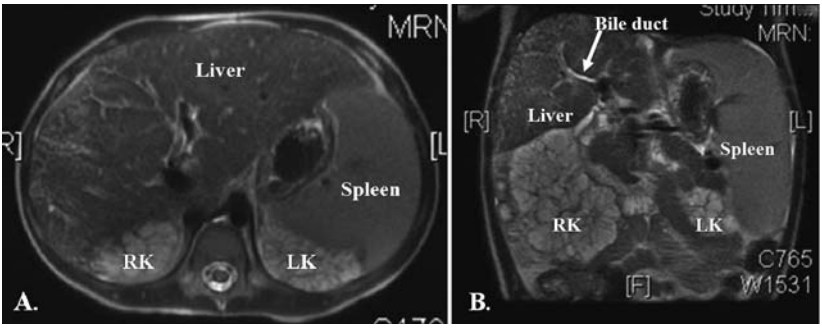

Fig. 14.9. (a, b). CHF and ARPKD: 6-year-old male with known ARPKD and portal hypertension. Axial (**a**) and coronal (**b**) T2-weighted MRI images of the abdomen demonstrate mild hepatomegaly with reticular areas of increased signal intensity in the subcapsular regions of the liver consistent with hepatic fibrosis, mild dilatation of the central intrahepatic bile duct, moderate splenomegaly, and marked renomegaly with high water content.

and cholangitis are controlled [99]. Shneider et al. discussed the special issues in the decision-making regarding liver transplantation in children with liver disease (CHF or CS) and ARPKD [53]. Clear-cut indications for combined liver and kidney transplantation in ARPKD included the combination of renal failure and either cholangitis or refractory

complications of portal hypertension (including significant hepatopulmonary syndrome). Khan et al. evaluated the morbidity and mortality from CHF in 14 patients with ARPKD post-renal transplantation [66]. Complications of CHF developed in 78% of children who received a renal transplant for ARPKD. Mortality related to CHF occurred in 29% and accounted to 80% (4/5) of the deaths. Causes of death were hepatic failure immediately post-transplant (one patient) and septicemia related to bile duct dilatation (three patients). Their study highlights the increased incidence of CHF-associated morbidity and mortality in patients with ARPKD after renal transplantation and may broaden the potential indications for earlier liver transplantation in these patients.

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15 Simple Hepatic Cysts/Choledochal Cysts

André A. S. Dick, MD and Patrick J. Healey, MD

CONTENTS

Summary

Cystic lesions of the liver can be malignant or benign, congenital or acquired. They represent a broad group of disorders that differ in presentation, etiology, and prevalence. Diagnosis requires a comprehensive history and physical as well as appropriate imaging studies. Simple hepatic cysts are symptomatic when they become large, and most do not require any intervention. Choledochal cysts are rare and most are diagnosed in childhood. Patients generally present with non-specific symptoms but once diagnosed should undergo curative resection. In this chapter we will discuss the diagnosis and management of patients with simple hepatic as well as choledochal cysts.

Key Words: Endoscopic retrograde cholangiopancreatography, MRCP, Choledochal cyst, Cholangiocarcinoma, Cholangitis

SIMPLE HEPATIC CYSTS

Liver cysts are generally classified into two broad categories: congenital and acquired. Simple hepatic cysts, the cysts of polycystic liver disease,

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and biliary cysts are considered congenital liver cysts. Congenital liver cysts are further subdivided into either true or false cysts. This designation is based on the presence of an epithelial lining [1]. Simple hepatic cysts are true hepatic cysts. Acquired liver cysts include neoplastic, traumatic, and infectious cysts. In this chapter we will focus on simple hepatic cysts. The other types of cysts will be discussed in more detail in other chapters of this book.

Epidemiology

Historically, simple hepatic cysts were considered to be rare and determining their precise incidence was difficult. These cysts were generally found incidentally at the time of laparotomy or at autopsy. The incidence has increased with the advent of increasing abdominal imaging studies. The incidence of simple hepatic cysts in the literature ranges from 0.14 to 4.75% [2–4]. Simple hepatic cysts occur more frequently in females and their incidence increases with age [3, 4].

Pathogenesis

The true pathogenesis of simple hepatic cysts is unknown. These cysts are congenital and are lined by cuboidal epithelium. They are thought to originate from abnormal development of intra-hepatic bile ducts in utero [5]. These malformed ducts have an indolent course and can enlarge overtime and present with symptoms later in life.

Clinical Features and Diagnosis

Most patients with simple hepatic cysts are asymptomatic and the cysts are found incidentally by diagnostic imaging such as ultrasound (US) or computed tomography (CT) (Fig. 15.1). They tend to have an indolent course but a minority may be associated with significant symptoms [6]. In contrast, larger cysts, neoplastic cysts, and traumatic cysts tend to be symptomatic. The most common presenting signs and symptoms in patients with simple hepatic cysts include right upper quadrant or mid-epigastric abdominal pain, abdominal mass, nausea, vomiting, or early satiety. Occasionally, patients may present with jaundice secondary to mass effect from an enlarging cyst. Biochemical evaluation and liver laboratory tests are usually normal. The majority of these signs, symptoms, and biochemical findings are non-specific and are not sufficient to make a conclusive diagnosis.

Most patients with simple hepatic cysts are diagnosed after referral for non-specific abdominal symptoms or are found incidentally after undergoing radiographic evaluation for an unrelated problem.

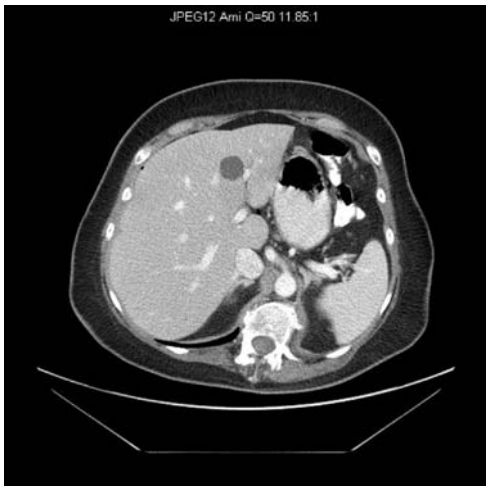

Fig. 15.1. Simple hepatic cyst (courtesy of Dr. Orpheus Kolokythas, University of Washington, Department of Radiology).

Ultrasound and CT are the two most common radiologic modalities used to diagnose simple hepatic cysts. Occasionally, magnetic resonance imaging (MRI) has been used to make the diagnosis. It is imperative to differentiate simple hepatic cysts from neoplastic or infectious cysts because the treatment algorithm is completely different (therapy for neoplastic and infectious cysts is discussed in another chapter). Ultrasound is a noninvasive and inexpensive way to diagnose simple hepatic cysts and is recommended as the initial diagnostic modality. Simple hepatic cysts appear on US as unilocular, well-circumscribed, anechoic cystic lesions without septations or debris [7]. The presence of septations or debris is suggestive of a neoplastic or an infectious process and warrants a more aggressive workup. CT offers superior spatial relationship between the cyst and surrounding structures such as the biliary system and the hepatic vasculature, and therefore offers a roadmap for patients who are symptomatic and may require surgical management. Like US, if septations or debris are seen on CT, a neoplastic process should be suspected. Simple hepatic cysts appear on MRI as well circumscribed with high signal intensity on T2-weighted images and low intensity on T1-weighted images.

Surgical Management

The degree of symptoms largely guides the decision for surgical management of simple hepatic cysts. Asymptomatic patients who are found to have simple hepatic cysts incidentally or at laparotomy are managed

nonoperatively. If found incidentally, they should be observed with clinical or radiographic follow-up for growth in size and or malignant degeneration [8, 9]. When discovered at laparotomy and the diagnosis is in question, it has been suggested that the cyst should be aspirated and its contents sent for cytologic and infectious evaluation. Tumor markers such as CA 19-9 may be sent as well [8, 10].

Surgical management is indicated when patients with simple hepatic cysts have symptoms that are attributable to the cyst. The goals of surgical management are twofold: to relieve symptoms via either complete excision or aspiration and to prevent recurrence. There are various surgical strategies for management of simple hepatic cysts depending on the size and the location of the cysts. The techniques employed include aspiration and observation, aspiration and sclerotherapy, local cyst excision, cyst fenestration, and hepatic lobectomy; these procedures may be performed using an open or a laparoscopic approach.

CT or US-guided percutaneous cyst aspiration and drainage is typically reserved for patients who are unfit for surgery; the diagnosis of simple hepatic cyst is uncertain or to ascertain whether the patients' symptoms are secondary to the cyst [8, 9]. The disadvantage of aspiration and drainage is the high recurrence rate [6]. Aspiration and sclerotherapy with various sclerosants such as ethanol and hypertonic saline has been advocated with improved outcomes compared to cyst aspiration alone [11, 12]. It is imperative to demonstrate that there is no communication to the biliary system prior to sclerotherapy so as to avoid sclerosant injury to the bile ducts. This can be achieved by a pre-operative endoscopic retrograde cholangiopancreatography (ERCP). Sclerosants cause destruction of the epithelial lining, thereby preventing the cyst's ability to secrete fluid and hence limiting their enlargement or recurrence.

Open surgical resection is generally reserved for patients where access to the cyst is not amenable to laparoscopic approach or the cyst is deep within the liver parenchyma. Patients undergoing open surgical resection should undergo intra-operative US to assess the relationship of the cyst to vascular and biliary structures in the liver. Liver resection for simple hepatic cysts has had good outcomes [13]. The benefits of liver resection, including absolute confirmation of the benign nature of the cyst and the negligible recurrence rate, must be weighed against the potential for procedure-related morbidity and mortality. Laparoscopy has changed the management of many surgical diseases including simple hepatic cysts. Managing simple hepatic cysts via the laparoscopic approach has been shown to be a viable option with excellent outcomes [6, 14, 15]. The advantages of the laparoscopic approach include

decreased postoperative pain, better cosmesis, and shorter hospital stay [16].

CHOLEDOCHAL CYSTS

Choledochal cysts are congenital cystic dilatations of part of the, or the entire, biliary system. Choledochal cysts were first described by Vater in 1723 [17]. Alonso-Lej and colleagues published the landmark paper in 1959, describing the first classification system for choledochal cysts [18]. This classification system has since been modified by others including Todani and associates [19]. The Todani modification of the Alonso-Lej classification system for choledochal cysts is the classification system that is currently most often used.

Epidemiology

Choledochal cysts are most frequently diagnosed in Asians, with an incidence of 1 in 1,000 live births in Japan [20]. In the Western world, the incidence is estimated at 1 in 13,000 to 1 in 15,000 live births overall [21]. Choledochal cysts occur more frequently in females, regardless of race (4:1), and the majority of patients diagnosed with choledochal cysts are children and young adults.

Classification

Choledochal cysts are classified into five different types: Type I (a, b, c), Type II, Type III, Type IV (A and B), and Type V. Type I cysts, the most common, represent approximately 85% of choledochal cysts. Type I choledochal cysts are cystic or fusiform dilatations of the common bile duct. Type II cysts, the most rare, are diverticular sacculations of the common bile duct and account for approximately 2% of cases. Type III cysts are referred to as choledochoceles and account for only 2% of all choledochal cysts. Choledochal Type III cysts affect the intraduodenal portion of the common bile duct. Type IV cysts, the second most common, account for the remaining 10% of choledochal cysts. These cysts are further subdivided into Type A and B. Type IV (A) cysts involve the intra and extra-hepatic biliary system and are multiple. Type IV (B) cysts are extra-hepatic only and can be single or multiple. Type V choledochal cysts affect only the intra-hepatic biliary system. Caroli's disease is characterized by Type V choledochal cysts without underlying hepatic abnormality [22]. Caroli's syndrome is characterized by Type V choledochal cysts in association with congenital hepatic fibrosis [23].

Pathogenesis

The etiology of choledochal cysts is unknown and several theories have been proposed for their development. Two predominant theories have emerged. One theory suggests that choledochal cysts exist as a result of abnormal recanalization of the primitive bile ducts, leading to obstruction and secondary cystic dilatation. The more widely accepted theory, however, was first described by Babbitt [24]. This pancreaticobiliary reflux theory suggests that there is an abnormal union between the distal common bile duct and the pancreatic duct. This malunion occurs outside the duodenal wall, proximal to the ampulla of Vater, and is referred to as pancreaticobiliary malunion [25–27]. It is thought that pancreatic enzymes reflux into the common bile duct because of the malunion, leading to chronic inflammation, localized weakness, and secondary cystic dilatation. Neither of these theories can fully explain the various types of choledochal cysts, however. Choledochal cyst formation may represent a spectrum of malformation of the pancreaticobiliary system during development. A genetic link has been suggested because of the preponderance of choledochal cysts in females and Asians, although proof has not yet been forthcoming.

Diagnosis and Clinical Features

The majority of choledochal cysts are diagnosed during infancy. Ultrasonography and ^{99m}Tc-HIDA [*N*-(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid] are the most common radiologic methods of diagnosing choledochal cysts in infants and small children [28]. Ultrasonography is noninvasive and is the diagnostic modality of choice for evaluating the entire intra- and extra-hepatic biliary system. Ultrasonography can also be used to diagnose choledochal cysts prenatally. ^{99m}Tc-HIDA characteristically demonstrates communication of the biliary system with the cystic mass and may also show delayed excretion from the liver into the intestines. In older children and adults, percutaneous transhepatic cholangiogram (PTC) (Fig. 15.2), ERCP, CT, or magnetic resonance cholangiopancreatography (MRCP) (Fig. 15.3) are highly sensitive and specific for confirming diagnosis. As stated earlier, the latter studies have the advantage of offering an anatomic road map prior to surgery.

The clinical presentation of patients with choledochal cysts varies according to age. The radiological differential diagnosis includes cystic biliary atresia, duodenal atresia, ovarian cysts, and duplication cyst. It is important to differentiate choledochal cysts from these other entities since the treatment urgency and recommendations vary. Infants and children rarely present with the classic triad of fever, right upper

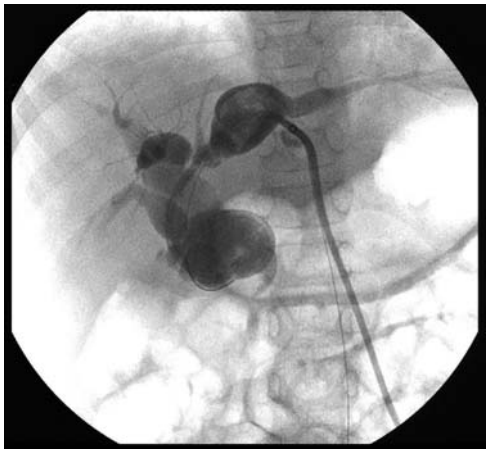

Fig. 15.2. Transhepatic cholangiogram demonstrating dilated intra- and extrahepatic bile ducts (courtesy of Dr. Patrick Javid, Seattle Children's Hospital, Division of Pediatric Surgery).

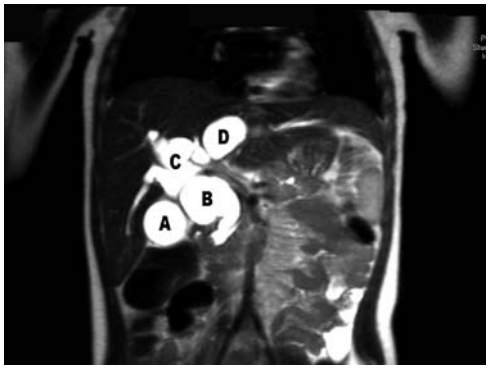

Fig. 15.3. MRCP illustrating Type IV(a) choledochal cyst (courtesy of Dr. Patrick Javid, Seattle Children's Hospital, Division of Pediatric Surgery). *A* (gallbladder), *B* (dilated common bile duct), *C* (right dilated intra-hepatic ducts), *D* (left dilated intra-hepatic ducts).

quadrant pain. and jaundice [29, 30]. The majority of infants present with a conjugated hyperbilirubinemia in conjunction with an abdominal mass. In older children, recurrent abdominal pain is the most common presenting sign, often with intermittent jaundice with or without elevated serum amylase. The cause of the abdominal pain is thought to be secondary to cholangitis or distension of the dilated bile duct.

Surgical Management

The definitive management for choledochal cysts is operative repair. Historically, cystenterostomy was the therapy of choice. This has been abandoned because of the long-term morbidity from retained cyst mucosa. Complications from cystenterostomy included inadequate drainage and consequently recurrent episodes of cholangitis, choledocholithiasis, anastomotic strictures, and secondary biliary cirrhosis [29]. The most important reason for complete cyst excision, however, is the increased risk of malignant degeneration of the retained cyst wall related to chronic inflammation [31, 32]. Adenocarcinoma is the most common cancer that develops, although squamous cell carcinoma has also been described, but occurs less frequently. The risk of malignancy increases with age and once it is present, it has a dismal prognosis [31, 34, 35]. Complete excision of the cyst is now the standard of care.

The surgical approach has to be tailored to the specific anatomic lesion. Type I choledochal cysts are optimally managed with complete cyst excision and roux-en-Y hepaticojejunostomy [33]. Patients who have previously undergone cystenterostomy should undergo surgical excision of the cyst remnant, with roux-en-Y hepaticojejunostomy if necessary, to prevent the development of cancer. Older children or adults may have dense pericystic inflammation which may make complete cyst excision from the surrounding portal structures difficult. In these cases, intramural dissection should follow the posterior wall of the cyst. This allows for the mucosal lining to be dissected free without injury to the other portal structures. Management of Type II choledochal cysts by diverticulectomy with ductoplasty has been described but roux-en-Y hepaticojejunostomy is the standard of care. Type III choledochal cysts are treated by transduodenal resection of the cyst or marsupialization to provide adequate drainage of the remnant pancreaticobiliary ducts.

Type IV (A) cysts are managed by complete excision of the extrahepatic segment of the cyst with roux-en-Y hepaticojejunostomy (Figs. 15.4 and 15.5). If intra-hepatic cysts are unilobar or focal, then hepatic resection may be indicated. Type (V) choledochal cysts are more difficult to manage. If segmental multifocal disease is limited to one lobe of the liver, then hepatic resection is indicated. If diffuse bilobar disease exists, this may be complicated by recurrent episodes of intra-hepatic lithiasis and cholangitis. In these circumstances, transhepatic drainage can provide temporary relief of symptoms. Ultimately these patients, especially in the setting of cirrhosis and portal hypertension, may need liver transplantation.

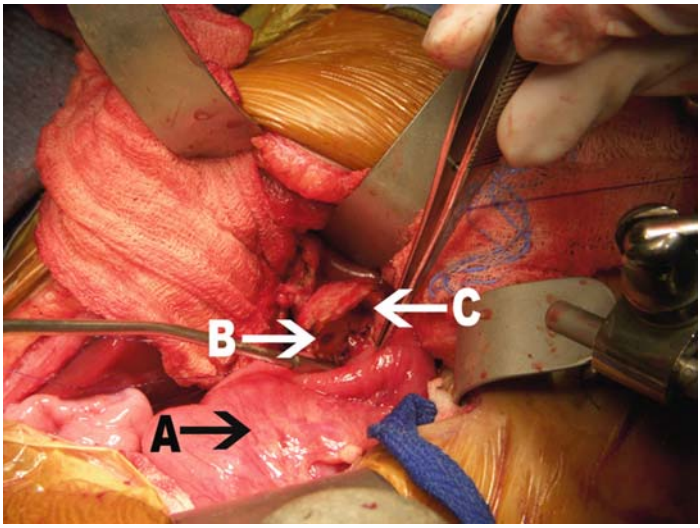

Fig. 15.4. Resection of extra-hepatic duct. (*A*) Roux-en-Y limb, (*B*) right hepatic duct, (*C*) left hepatic duct (courtesy of Dr. Patrick Javid, Seattle Children's Hospital, Division of Pediatric Surgery).

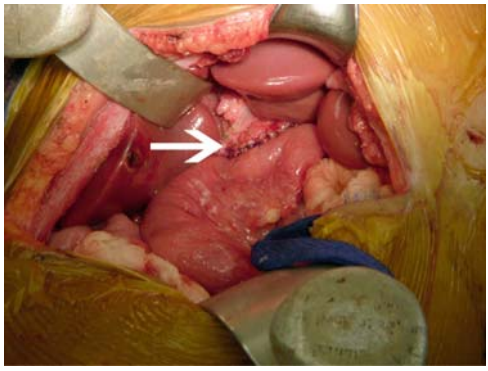

Fig. 15.5. Completed roux-en-Y choledochal jejunostomy (courtesy of Dr. Patrick Javid, Seattle Children's Hospital, Division of Pediatric Surgery). Arrow demonstrating completed roux-en-Y hepaticojejunostomy.

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16 Autosomal Dominant Polycystic Liver Disease

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CONTENTS

Summary

Autosomal dominant polycystic liver disease (ADPLD) can occur as an independent disease entity affecting primarily the liver with few extrahepatic manifestations or as part of the disease spectrum of autosomal dominant polycystic kidney disease (ADPKD). The clinical course of ADPLD is heterogeneous and dictated by the extent and severity of hepatic and, in the case of ADPKD, extrahepatic manifestations. Currently, the management of ADPLD is directed toward palliating symptoms and treating complications.

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DEFINITION

ADPLD is a disease characterized by the occurrence of multiple liver cysts inherited in an autosomal dominant manner. The extent of the liver involvement ranges from a few cysts to massive cystic enlargement of the liver. In cases where the disease is restricted to the liver with only a small number of cysts and a family history of ADPLD is absent, it may be impossible to distinguish ADPLD from acquired simple hepatic cysts.

GENETICS

Polycystic liver disease was first observed in autopsy studies [1–3]. Its autosomal dominant pattern of inheritance in association with ADPKD was first reported by Steiner in 1899 [4]. Polycystic liver disease also occurs as a genetically separate entity with autosomal dominant inheritance independent of ADPKD [5]. Offspring of either sex from an individual with ADPLD, with or without concurrent ADPKD, have a 50% risk of inheriting this disease. While the trait shows autosomal dominant inheritance, the mechanism of disease at the cellular level is thought to be recessive. Cyst initiation occurs after somatic second-hit mutations inactivate the normal copy of the respective polycystic disease gene resulting in functional loss of the gene product in the subset of cells that will give rise to cysts [6].

ADPLD with ADPKD

ADPLD may occur as a part of the extra-renal manifestations of ADPKD. ADPKD results from mutations in two genes, *PKD1* or *PKD2*. *PKD1* is located on the short arm of chromosome 16 (16p13.3) [7] and *PKD2* the long arm of chromosome 4 (4q13–q23) [8, 9].

The *PKD1* gene contains 46 exons. It encodes a 14.1-kb transcript that translates to a 4302-amino acid protein, polycystin-1 (PC1). PC1 is a receptor-like membrane protein with yet to be identified ligands [10]. The *PKD2* gene contains 15 exons and encodes a 5.3-kb transcript that translates to a 968-amino acid protein, polycystin-2 (PC2) [9]. PC2 is a member of the TRPP family of cation channel proteins and functions as a calcium channel [11].

PC1 and PC2 are enriched in primary cilia of renal and biliary epithelial cells, where they interact and exert functions in cellular

mechanochemical sensing. Mutations to PC1 or PC2 alter ciliary sensing. Although yet to be fully elucidated, the alteration in ciliary sensing is recognized to cause an array of aberrant downstream signaling that ultimately results in pathogenic cyst development.

The *PKD1* mutation accounts for approximately 85% of patients with ADPKD and *PKD2* the remaining 15%. Disease-causing mutations have been detected throughout the entire *PKD1* and *PKD2* genes without discernible hot spots or clustering. Studies have suggested that the position of mutations may predict the severity of kidney cystic phenotype and the risk of intracranial aneurysms [12–14].

ADPLD Without ADPKD

ADPLD can occur independently of ADPKD and is genetically heterogeneous [15]. Approximately 50% of the cases are due to mutations in either *PRKCSH* located on the short arm of chromosome 19 (19p13.2–13.1) [16] or *SEC63* on the long arm of chromosome 6 (6q21–q23) [17].

PRKCSH consists of 18 exons and encodes a 1.6-kb message that translates to a 527-amino acid protein, the β-subunit of glucosidase II also named hepatocystin [18]. It is an endoplasmic reticulum (ER) luminal protein that is the non-catalytic subunit of the glucosidase II holoenzyme that participates in the quality control and processing of oligosaccharide chains of integral membrane and secretory proteins [19], including cilia membrane proteins such as PC1 and PC2.

SEC63, a 21-exon gene, encodes a 3.4-kb message that translates into a 760-amino acid integral membrane protein of the ER [17, 20]. It is a part of a multicomponent translocon that comprises of the protein translocation machinery for integral membrane and secreted proteins. In mammalian systems, the translocon mediates cotranslational passage of nascent peptides into the ER and is functionally upstream to PRKCSH-dependent protein processing [21].

Mutations in either *PRKCSH* or *SEC63* are postulated to result in defects in the maturation of integral membrane proteins, including the polycystins, as well as secreted proteins. Defects in these processes in the bile duct are postulated to result in ADPLD. The relation between the phenotype of ADPLD and the mutation type or position in *PRKCSH* and *SEC63* genes has not been characterized.

PATHOGENESIS

Although several genes (*PKD1*, *PKD2*, *PRKCSH*, and *SEC63*), when mutated, are capable of causing the ADPLD phenotype, the cellular

mechanisms by which gene mutations ultimately lead to the development of liver cysts have not been fully elucidated. Morphological studies of individual cysts in ADPLD reveal that the liver cysts originate from biliary microhamartomas, also termed von Meyenburg's complexes [22], that arise by proliferation of biliary ductules, and from peribiliary glands [23]. Liver cysts are lined with epithelium of biliary origin [24, 25]. As the cysts enlarge, they become detached from their origins. It is generally accepted that the expansion of cysts detached from their origin in ADPLD is attributable to concerted effects of proliferation in cyst-lining epithelia, solute and fluid secretion into the cysts, remodeling of the extracellular matrix surrounding cysts, and neovascularization.

Proliferation

Excessive proliferation of biliary epithelial cells is required for the development and expansion of ADPLD liver cysts. Studies, primarily in kidney cyst epithelia, have shown that a number of signaling pathways are activated and likely contribute to the proliferative phenotype of cystic epithelia, including the biliary cystic epithelium. These include cAMP activation of the mitogen-activated protein kinase/extracellular regulated kinase (MAPK/ERK) pathway [26], cAMP, and the mammalian target of rapamycin (mTOR) signaling cascade [27, 28].

Secretion

Once disconnected from their origin, liver cysts require transepithelial transport of solutes and fluid for cyst expansion. Secretion by the cyst-lining epithelium is an integral part of force that supports cyst growth [29]. The epithelia lining the cyst in ADPLD retain their secretin responsiveness and secretory capacity [30]. Secretin activates cAMP-dependent signaling cascades, which promote cyst expansion by stimulating fluid secretion from cyst-lining epithelia.

Matrix Remodeling and Neovascularization

Remodeling of the matrix surrounding the cysts is necessary for liver cyst expansion. Such remodeling requires the secretion and activation of metalloproteases [31]. The activity of metalloproteases is highly elevated in the liver cyst epithelia [31], indicating ongoing remodeling of the extracellular matrix surrounding the cysts. Cyst expansion also requires a vascular supply for metabolic support. The density of the vascular beds surrounding the cysts is significantly increased [32, 33],

consistent with a process of adaptive angiogenesis and neovascularization. Neovascularization is promoted by a number of factors including cytokines IL-6 and IL-8 and growth factors such as VEGF [34, 35]. Inhibition of angiogenic factors such as VEGF antagonists may have a role for polycystic liver disease [36].

Estrogen

Estrogen has been shown to influence the development and progression of ADPLD [5, 37, 38]. Biliary epithelia (cholangiocytes) and cyst-lining cells in ADPLD, but not in normal liver, express estrogen receptors [37, 39]. Estrogen is able to act directly through estrogen receptors and indirectly by potentiating the effects of growth factors to promote cholangiocyte proliferation and secretion [40, 41]. Moreover, by potentiating the effects of vascular endothelial growth factor (VEGF), estrogen promotes adaptive angiogenesis [42], critical for sustained cyst growth. Thus, estrogen affects multiple aspects in ADPLD to promote cyst growth.

EPIDEMIOLOGY

ADPLD occurs worldwide with no known racial or gender preference. In autopsy series, the occurrence of polycystic liver disease defined as the presence of liver cysts consistent with ADPLD phenotype and in excess of what is expected in individuals of the same age with simple liver cysts varies from 0.05 to 0.53% [1–3]. The number and volume of liver cysts increase with age, and severe cystic liver enlargement occurs predominantly in female patients. ADPLD associated with ADPKD is relatively common. ADPKD occurs 1:400–1,000 live births. By age 30, up to 94% of ADPKD individuals have detectable hepatic cysts by magnetic resonance imaging (MRI) studies [43]. With age, almost all ADPKD patients have varying degrees of cystic liver disease.

ADPLD independent of ADPKD is rare, with an estimated incidence of <0.01%. However, in several autopsy studies, polycystic liver disease with a cyst burden out of proportion to that of age-related simple cysts and consistent with the definition of ADPLD has an occurrence only slightly less than that of ADPKD [1–3]. This discrepancy may be explained by the different clinical presentations in the two diseases; ADPLD without polycystic kidney manifestations is much less symptomatic than ADPKD and therefore would less frequently be detected. Hence, the incidence of isolated polycystic liver disease likely has been underestimated.

CLINICAL MANIFESTATIONS

The majority (~80%) of ADPLD patients are clinically asymptomatic [5]. Symptoms may occur in patients with a few large dominant cysts or in patients with severe cystic liver enlargement. ADPLD associated with ADPKD is further complicated by concurrent cystic kidney enlargement, kidney failure, and other organ manifestations detailed below. By contrast, ADPLD without ADPKD, when symptomatic, exhibits only liver cysts with liver enlargement-related manifestations [5]. Although cysts may be innumerable in ADPLD, liver synthetic function is typically well preserved in vast majority of the cases [44]. The only laboratory abnormalities seen are generally in severe cases and are usually mild elevations of γ-glutamyltransferase and alkaline phosphatase [5, 44].

Hepatic Manifestations

These manifestations are indistinguishable between ADPLD patients with or without ADPKD.

Pain. Acute pain may be caused by cyst rupture, hemorrhage, or infection. Chronic pain is usually caused by mass effect from liver enlargement.

Cyst Rupture and Hemorrhage. These complications can occur spontaneously or secondary to trauma. Spontaneous cyst rupture and/or hemorrhage is thought to result from overstretching of the cysts and the surrounding vessels. The precise frequency of their occurrence is unknown. The associated pain is usually local and acute. In majority of the cases, pain subsides spontaneously or with analgesia and supportive care. Rarely, a cyst can rupture into the peritoneal cavity and presents as an acute abdomen.

Cyst Infection. Cyst infection is a rare but serious complication with a reported mortality rate of 2% [45]. Affected patients typically present with fever, chills, and right-upper quadrant pain. However, not all patients exhibit the typical presentation; some may show a vague, nonspecific generalized malaise, diffuse abdominal discomfort, and intermittent low-grade fever and chills. Signs of cyst infection include leukocytosis, positive blood culture, and changes on imaging studies, i.e., thickened, irregular cyst walls and hyperdense cyst content with air–fluid levels [46, 47]. An indium-labeled white blood cell scan may be helpful in providing supportive information and localizing the infection. Cyst infection should be managed in a timely fashion to reduce the risk of life-threatening systemic spread. A combination of antibiotics and cyst drainage provides the best outcome [48].

Mass Effect. An enlarged cystic liver can cause abdominal distension and discomfort. In cases of massive cystic enlargement, the neighboring organs may be compressed resulting in chronic abdominal pain, bloating, early satiety, dyspnea, lower extremity edema from compression of the inferior vena cava, and, rarely, jaundice from compression of the biliary system [49].

Extrahepatic Manifestations

Extrahepatic manifestations in ADPLD can be grouped by the presence or absence of ADPKD.

ADPLD with ADPKD. In addition to cystic liver disease, patients with ADPKD may develop cystic changes in multiple organ systems. Most prominently, bilateral kidney cysts with kidney enlargement which occurs in 100% ADPKD patients are associated with a urine concentration defect, hypertension, nephrolithiasis, and a decline in glomerular filtration rate (GFR). More than 50% ADPKD patients develop end-stage kidney failure. Cysts also develop in the pancreas (5–10%) and arachnoid space in the central nervous system (5–10%). The cystic changes in these organs are generally clinically silent. Noncystic manifestations include intracranial aneurysms and aneurysmal rupture, vasospasm, pericardial effusion, mitral valve prolapse (MVP), intestinal diverticular disease, and abdominal/inguinal hernias. These manifestations can occur individually or concurrently.

ADPLD Without ADPKD. ADPLD in patients without ADPKD has a more restricted manifestation. The major extrahepatic abnormality reported is an increased incidence of heart valve abnormalities, including MVP and incompetence, occurring in up to 30% affected individuals [5]. Additionally, an increase in the occurrence of intracranial aneurysms has also been suggested [5, 50].

PATHOLOGY

The hepatic pathology of the cysts seen in ADPLD with or without ADPKD is indistinguishable [5, 50].

Gross Examination

The liver is enlarged with multiple, variable-sized cysts. The cysts can be diffusely distributed or, more typically, confined to a single area, leaving part of the parenchyma relatively cyst free. Quantitative analysis has shown that the intact hepatic parenchymal volume is preserved in the majority of ADPLD patients, even in those with massive cystic enlargement [44].

Microscopic Examination

The liver cysts are lined by a layer of cuboidal and/or flattened epithelia, originating from the biliary epithelia. Biliary microhamartomas (von Meyenburg's complexes), collections of proliferating bile ductules encased in a fibrous, hyalinized stroma, are common.

NATURAL HISTORY

Liver cysts usually begin to appear after patients reach puberty. The rate of cyst growth and liver enlargement is highly heterogeneous. Significant variations may occur even among affected individuals from the same family. However, for each patient, the liver cysts grow steadily with age in both number and size. Although men and women have an equal frequency of inheriting ADPLD, women present more frequently with massive and symptomatic cystic liver enlargement [44, 51], largely attributable to the effects of estrogen on the polycystic liver growth. Accordingly, multiparous women or women who have used oral contraceptives or estrogen replacement therapy for many years tend to exhibit a higher degree of cystic liver enlargement and to be symptomatic [38].

With an increase in the average life expectancy, more patients with ADPLD, with or without ADPKD, are expected to develop significant cystic liver enlargement and become symptomatic. However, the natural course may differ for ADPLD depending on whether or not it is associated with ADPKD. Due to the absence of kidney dysfunction, ADPLD patients without ADPKD often have a milder course [52]. By contrast, ADPLD patients with ADPKD, with the concurrent enlargement of cystic liver and kidneys and kidney dysfunction, tend to be more symptomatic. Studies have shown that the severity of symptomatic cystic liver disease in this group of patients appears to parallel the severity of cystic kidney enlargement and kidney dysfunction [51, 53]. One report shows that ADPLD-related complications account for 10.5% mortality in ADPKD patients on dialysis [54].

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

ADPLD is diagnosed by imaging studies, including ultrasound, computed tomography (CT), and MRI. Ultrasound is generally preferred because of its low cost and lack of radiation exposure. However, CT and MRI are more sensitive and accurate in detecting the presence and size of liver cysts.

ADPLD should be differentiated from simple liver cysts, which often occur with age in normal individuals. An arbitrary set of criteria have

been devised to differentiate simple liver cysts from ADPLD [5]. For individuals with a family history of ADPLD (at-risk individuals) and who are <40 years of age, the presence of any liver cysts is considered to be diagnostic of ADPLD; for at-risk individuals >40 years of age, four or more cysts are considered indicative of disease. Individuals >40 years of age who have one to four cysts are considered indeterminate. The adequacy of such criteria has been validated from gene linkage analysis of the two largest ADPLD families [55].

ADPLD should also be differentiated from occasional cysts associated with autosomal recessive polycystic kidney disease (ARPKD). ARPKD is a rare (1:20,000) disease with congenital hepatic fibrosis as its major hepatic manifestation. Occasionally, liver cysts in ADPLD may be confused with cystadenomas [56], especially when cysts contain hemorrhagic fluids. In such cases, further investigation and close follow-up are necessary.

TREATMENT

To date, no specific medical regimen has been established to prevent ADPLD development or retard its progression. The mainstay of management is interventional and directed at minimizing and treating the complications, with a goal to palliate the symptoms without compromising liver function.

Interventional Managements

Interventions for symptomatic ADPLD include percutaneous cyst aspiration followed by sclerotherapy, cyst fenestration, hepatic resection with fenestration, selective hepatic artery embolization, stenting of obstructed bile ducts and large abdominal veins, and, rarely, liver transplantation. Each intervention has its own indications. ADPLD patients with specific characteristics and cyst anatomy may be better suited for one of them than the others [57].

Percutaneous Cyst Aspiration Followed by Sclerotherapy. This modality is feasible when treating one or several large dominant cysts (Fig. 16.1A). Sclerotherapy with 95–99% alcohol or acidic solutions of tetracycline or minocycline ablates the fluid-producing cyst epithelia to attenuate the regrowth of the cysts. With this method, ~70–90% treated cysts can be successfully obliterated. However, for larger-sized (diameter >10 cm) cysts, repeat treatments may be necessary to achieve a sustained cyst obliteration [58–60].

Cyst Fenestration. Cyst fenestration [61], either laparoscopically or by open laparotomy, involves unroofing and excising the cystic area

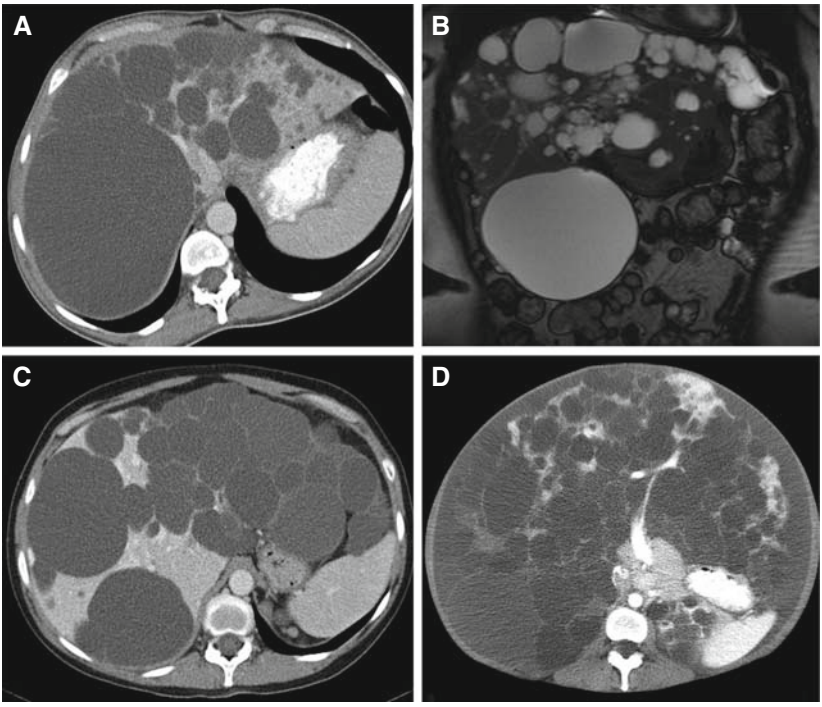

Fig. 16.1. (**a**) Large symptomatic cyst in the right hepatic lobe treatable by cyst aspiration and alcohol sclerosis. (**b**) Superficial large cyst arising from the right lobe of the liver suitable for laparoscopic fenestration. (**c**) Polycystic liver with nearly complete replacement of the left lobe of the liver by cysts and relative sparing of the right lobe of the liver with few large cysts that can be fenestrated at the time of a combined liver resection/cyst fenestration. (**d**) Severe polycystic liver disease without sparing of any liver segment in a patient listed for liver transplantation.

down to the interface of the liver parenchyma. The unroofed cyst-lining epithelium is then treated by argon laser beam coagulation or electrocoagulation or both to reduce cyst recurrence. This technique requires careful selection of the cystic area to avoid postoperative bleeding or leakage of bile. Patients with a small number of large cysts, particularly with superficial cysts, are the most suitable candidates (Fig. 16.1B). The success rate in properly selected patients is over 80%. The morbidity and mortality rates are 30 and 1%, respectively, and are similar in patients with laparoscopic or open fenestration [62].

Hepatic Resection with Fenestration. Hepatic resection is effective in reducing liver mass. Additionally, large, superficial, and deep-seated

cysts in the remaining liver segments can be fenestrated during the operation. Suitable candidates are those with massive hepatomegaly due to many small- to medium-sized cysts with relative preservation of at least two contiguous liver segments with adequate hepatic venous drainage (Fig. 16.1C). The mortality and morbidity rates are highly dependent on the experience and degree of expertise in the team of caregivers. In the largest series, rates of morbidity and mortality were 58 and 3%, respectively [63]. The most common morbidities are development of ascites that may require stenting of the inferior vena cava or hepatic veins or more rarely placement of a LeVeen shunt, and bile leak. The long-term outcome, a mean follow-up of 28 months, was excellent with only 1 of the 31 patients having recurrence of the symptoms.

Selective Hepatic Artery Embolization. Selective hepatic artery embolization has recently been reported from a single center [64, 65]. This method targets hepatic regions without discernible intact parenchyma. It appears to be safe with an average hepatic mass reduction by 23%. The major side effects are post-procedural pain and fever, which typically resolve within a week. This method is best suited for patients who have diffuse cystic liver without cyst-free parenchyma and are poor candidates for surgery.

Stenting of a Compressed Hepatic Vein or Inferior Vena Cava. Reestablishing patency of the compressed bile ducts and veins can relieve obstruction-associated symptoms. Stenting of a compressed hepatic vein or inferior vena cava may correct intractable ascites and lower extremity edema and significantly improve symptoms [66, 67].

Liver Transplantation. Patients with ADPLD typically have normal liver function, and liver transplantation is limited by organ shortage, perioperative risks, and lifelong immunosuppression. Liver transplantation for ADPLD patients is therefore reserved for severely symptomatic patients with massive hepatomegaly without spared liver segments who are not suitable for other treatment modalities (Fig. 16.1D). In patients who underwent liver or combined liver–kidney transplantation, the reported 5-year survival rate is ∼85%. Survivors of the transplantation enjoy a sustained improvement in quality of life [68, 69].

Non-interventional Management

Avoidance of Estrogen. Higher levels of estrogen exposure tend to be associated with rapid cyst growth and larger liver size that often lead to clinical complications that more frequently require palliative interventions. Estrogens should be avoided in patients with progressive polycystic liver enlargement.

Limiting Caffeine Intake. Caffeine stimulates cAMP accumulation, which promotes fluid secretion from cyst-lining epithelia. Patients should be instructed to avoid excessive caffeine consumption.

Investigational Treatments: Ongoing Clinical Trials

Octreotide. Long-acting synthetic somatostatin analogs inhibit secretin-induced, cAMP-mediated secretion in biliary and cyst-lining epithelium. Octreotide, one of these analogs, has been shown to inhibit hepatic cyst growth in a rat model of PKD [70]. Prospective pilot trials of octreotide and lanreotide are currently in progress.

Sirolimus. Enhanced proliferation of the cyst-lining epithelium is a prominent feature of ADPLD. Sirolimus, an immunosuppressive agent, exerts an antiproliferative effect by inhibiting mTOR. A retrospective study in ADPKD patients after renal transplantation showed that a sirolimus-containing immunosuppressive regimen was associated with a significant reduction in polycystic liver volume, suggesting a role for sirolimus in the treatment of severe ADPLD. Prospective clinical trials of sirolimus and everolimus are currently in progress.

SUMMARY

ADPLD is a group of genetically and phenotypically heterogeneous diseases. Although the majority of these patients are asymptomatic, a small fraction do suffer from polycystic liver-related complications that lead to significant morbidity and mortality. To date, management of ADPLD rests primarily on modifying the known risk factors and correcting complications by interventions. A number of candidate medications are currently undergoing clinical trials that will likely offer useful insights.

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17 Echinococcal/Hydatid Cysts of the Liver

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CONTENTS

Summary

Human hydatid disease, also known as echinococcosis, is caused by infection with dog tapeworms of the genus *Echinococcus*. Although the parasite's life cycle is understood, considerable obstacles remain in the quest to prevent further disease transmission, and the incidence of this infection remains high among many populations. Diagnostic and therapeutic tools for echinococcosis have improved in recent decades, but challenges persist in both arenas. This chapter focuses on the epidemiology, microbiology, prevention, diagnosis, and therapy for hydatid cysts of the liver – the most common organ site of clinical disease.

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HISTORY

Although it is impossible to know when humans began to sustain hydatid disease, the historical record indicates that this is an ancient condition. Mummies from ancient Egypt have been found to contain hydatid cysts [1]. In light of what we now know of the pathogen's life cycle and mode of transmission, this is not surprising: the intimate relationship between canids, herbivores, and humans has probably always been responsible for this condition. Indeed, it is possible that the incidence of this disease was considerably higher in ancient times, although the paleontological record is insufficient to prove this hypothesis.

MICROBIOLOGY AND LIFE CYCLE

Echinococcal disease in humans is caused principally by two species of metazoan (multicellular) eukaryotic parasites: *Echinococcus granulosus* and the less prevalent *Echinococcus multilocularis*. Infection with other species has also been documented, including *Echinococcus oligarthrus* and *Echinococcus vogeli*, although they are far less frequent. In all cases, humans serve as "incidental" and "dead-end" hosts, meaning that they are neither required for persistence of the parasite in nature nor do they allow transmission to other hosts.

The life cycle for *E. granulosus* begins with mature cestodes, each 3–6 mm in length, attached to the small intestinal mucosa of a canine (e.g., dog) (Fig. 17.1). These adult tapeworms adhere to the intestines via their scolex, or "head," which contains two rows of tiny hooklets as well as four suckers. Below the scolex are attached three segments called proglottids, which contain male and female reproductive organs. The eggs are fertilized within the last proglottid. These embryonated eggs, each approximately 30 μm in diameter, are passed from the proglottid into the fecal stream. By definition, the passage of fertilized eggs within canines makes them the "definitive hosts" for this parasite.

Once defecated into the environment, the eggs are then consumed by grazers (e.g., sheep or cattle) when they eat contaminated grass. Upon contacting the herbivore's small intestines, each egg releases an embryo called an "oncosphere" that burrows through the intestinal mucosa and into the parenchyma. In many cases, the oncosphere then enters the portal venous drainage, and enters the liver, where it lodges in the hepatic parenchyma. However, in approximately 30% of cases, the oncosphere penetrates neighboring organs or enters the systemic venous drainage system, and from there is swept to distant sites (end organs) such

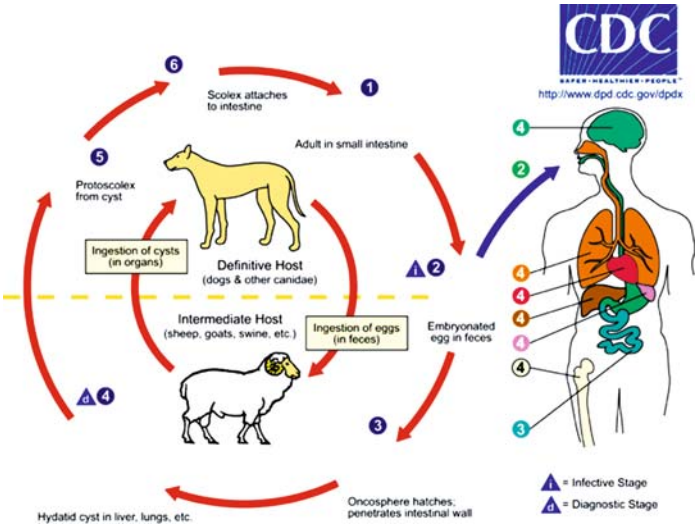

Fig. 17.1. Life cycle of *Echinococcus* species. The adult *Echinococcus granulosus* (3–6 mm long) ❶ resides in the small bowel of the definitive hosts, dogs or other canids. Gravid proglottids release eggs ❷ that are passed in the feces. After ingestion by a suitable intermediate host (under natural conditions: sheep, goat, swine, cattle, horses, and camel), the egg hatches in the small bowel and releases an oncosphere ❸ that penetrates the intestinal wall and migrates through the circulatory system into various organs, especially the liver and lungs. In these organs, the oncosphere develops into a cyst ❹ that enlarges gradually, producing protoscolices and daughter cysts that fill the cyst interior. The definitive host becomes infected by ingesting the cyst-containing organs of the infected intermediate host. After ingestion, the protoscolices ❺ evaginate, attach to the intestinal mucosa ❻, and develop into adult stages ❶ in 32–80 days. The same life cycle occurs with *E. multilocularis* (1.2–3.7 mm), with the following differences: the definitive hosts are foxes, and to a lesser extent dogs, cats, coyotes, and wolves; the intermediate host are small rodents; and larval growth (in the liver) remains indefinitely in the proliferative stage, resulting in invasion of the surrounding tissues. With *E. vogeli* (up to 5.6 mm long), the definitive hosts are bush dogs and dogs; the intermediate hosts are rodents; and the larval stage (in the liver, lungs and other organs) develops both externally and internally, resulting in multiple vesicles. *E. oligarthrus* (up to 2.9 mm long) has a life cycle that involves wild felids as definitive hosts and rodents as intermediate hosts. Humans become infected by ingesting eggs ❷, with resulting release of oncospheres ❸ in the intestine and the development of cysts ❹, ❹, ❹, ❹, ❹, ❹ in various organs. *Image and Legend Credit: Center for Disease Control and Prevention, Division of Parasitic Diseases (CDC-DPDx).*

as the spleen, kidneys, adrenal glands, lungs, brain, bone marrow, or peritoneal cavity. Once they arrive in an end organ, the oncospheres develop gradually into fluid-filled cysts – hence, the terms "hydatid (water-filled) cysts" and "cystic echinococcosis (CE)." The cysts are bound by a dense "false capsule" of fibrotic tissue of host origin, as a well as a luminated membrane of parasite origin. The innermost cyst layer contains germinal membrane from which offspring will bud. These offspring are called "protoscolices," because they contain the hooklets and suckers found in the scolex of the adult – however, the protoscolices are folded in upon themselves ("invaginated"), which presumably protects them during their journey back into the canine host.

Over time, as the hydatid cyst grows, these protoscolices will accumulate and settle by gravity to the bottom of the cyst, where they mix with cast-off cells shed by the germinal layer, forming a sludge called "hydatid sand." Hydatid cysts may also contain smaller "brood capsules" that bud from the germinal layer, which may eventually grow into full "daughter" cysts, each with its own inner germinal layer, protoscolices, and sand. By definition, herbivores are "intermediate hosts" for this parasite, because they enable the worms to develop, but not to pass infectious eggs. Thus, completion of the life cycle depends upon consumption of invaginated protoscolices by canines when they feed upon cysts in raw organ meat ("offal"). Once the protoscolices reach the canine's small intestines, they unfold ("evaginate"), adhere to the mucosa via hooklets and suckers, and develop into sexually mature, infectious adults over the next 1 to 2.5 months, thereby completing the life cycle.

As described below, humans act as incidental hosts when they unknowingly ingest tapeworm eggs via unwashed fingers or food contaminated with canid feces, thus allowing oncospheres to hatch, invade, migrate, and grow into cysts. Thus, humans are dead-end hosts for the parasite – unless canids consume their infected entrails. Because this disease is spread from other vertebrates to humans, it qualifies as a zoonotic infection.

The life cycle of *E. multilocularis* is very similar to that of *E. granulosus*, except that foxes and wolves frequently serve as definitive hosts, and small rodents such as voles often serve as intermediate hosts. Furthermore, unlike the germinal epithelium of *E. granulosus* which creates daughter cysts within the parent cyst, the germinal epithelium of *E. multilocularis* cysts often buds *outward* into the surrounding parenchyma, producing multilocular cysts that invade target organ parenchyma in a fashion very reminiscent of a cancerous tumor. For this reason, disease caused by *E. multilocularis* is referred to as "alveolar echinococcosis (AE)."

EPIDEMIOLOGY

Hydatid cyst disease of humans has been reported in all continents except for Antarctica. The global distribution of this illness, however, varies significantly by region, probably due to differences in animal husbandry practices rather than climate or ecology. *E. granulosus* is found in the northern and southern hemispheres, with heaviest burdens reported in Latin America, Africa, coastal Mediterranean regions, states of the former USSR, the northern Middle East, and Southeast Asia. Prevalence of *E. granulosus* infection is reported from 1 to 220 per 100,000 population [2]. Certain regions, however, are hyper-endemic for this illness. In Sichuan Province, China, up to 12.1% of the ethnic Tibetan population, in certain villages, were detected to have cystic echinococcosis by ultrasound [3]. The number of cysts in this cohort increased with age, and also with duration and intensity of exposure to dogs. Both men and women may become infected. Infection is rare in the United States, but has been demonstrated in Minnesota, Alaska, California, Arizona, and New Mexico [4].

In contrast, disease due to *E. multilocularis* is found only in the northern hemisphere. Although less common than cystic echinococcosis overall, alveolar disease may be similar in prevalence in certain populations. In the survey described above, the prevalence of AE detected by ultrasound among the same cohort peaked at 14.1% [3]. Presumably, the most common mechanism of transmission in this population was via exposure to domestic dogs rather than to foxes. A separate survey of Gansu province, China, detected seropositivity to *E. multilocularis* in 8.8% of the population studied [5]. In Europe and North America, AE is of particular concern to those exposed to foxes, where 75–100% of red foxes have been found to be infected [5].

Hydatid disease is rarely reported among travelers from industrialized regions to underdeveloped areas. However, this is possible, and the diagnosis should be considered among travelers with cystic liver disease of unknown etiology – in particular those who have pursued "adventure travel" or who have lived with local families in underdeveloped regions. Because hydatid cysts may not present for years following infection, a careful and complete travel history may be required to elicit relevant exposures.

PREVENTION

Because of its mode of transmission from dogs to humans, *Echinococcus* infection can – in theory – be prevented using a tantalizingly simple strategy. Humans who avoid the ingestion of dog

feces will not acquire this infection. Thus, careful hand washing is recommended after contact with dogs which may harbor tapeworms, particularly working dogs on ranches or feral dogs. Similarly, washing fruits and vegetables that may have been contaminated by canid feces should be routine practice. Indeed, the food safety strategy advised for patients traveling to underdeveloped nations – "peel it, boil it, cook it, or forget it" – should be effective in preventing this infection, along with a great many others that are transmitted by fecal–oral route. However, the great majority of hydatid disease does not affect travelers, but rather people living with infected dogs in endemic areas. Individuals who are exposed to contaminated food on a regular basis are unlikely to benefit from such simple advice, unless it is accompanied by broader efforts to curtail the transmission of infection.

Stopping the practice of feeding uncooked organ meat to dogs would break the life cycle of *E. granulosus*. It is a practice engrained in many cultures, however, and for good reason. Dogs may protect families and their livestock from predators and intruders; in return, they require nutrition. Organ meat is usually discarded by herders, and thus allowing dogs to feed upon that meat makes for a tidy, sustainable arrangement. Because hydatid cysts may not be apparent to ranchers handling the organ meat, and because human illness is removed in time from that activity, ranchers and villagers may not appreciate the impact of this practice upon their own health. And, because cooking meat requires resources in time and fuel, they may not be willing or able to render the organs non-infectious before feeding them to their dogs. Nevertheless, educating affected populations must remain a cornerstone of prevention.

Enticing work is underway to develop and deploy a safe and efficacious Echinococcus vaccine for livestock [6, 7]. However, no vaccine is currently commercially available for herbivore or human use. A complementary approach focuses on vaccinating dogs to prevent them from acquiring adult tapeworm infestations [8]. However, there are significant hurdles to overcome in order to generate effective immunity to the intraluminal, non-invasive life form found in dogs, and evidence to date is not convincing that this can be accomplished [9]. Finally, the "deworming" of infested dogs using the antihelminthic drug praziquantel has been shown to dramatically decrease transmission to intermediate hosts [10], although it generates no host immunity in the dog and thus requires repeat administration.

The concerted efforts of public health officials and ranchers in a variety of nations have dramatically decreased the incidence of *E. granulosus* infection among sheep and humans [11]. These experiences demonstrate the fact that a multi-pronged approach to this

problem can succeed – and the fact that the governments of many nations remain unable or unwilling to commit the time, effort, and money required to break the chain of transmission. The challenge of controlling *E. multilocularis* infection is even greater, based on the sylvatic nature of its life cycle, and the fact that neither the definitive hosts (foxes) nor the intermediate hosts (rodents) are under direct human control.

CLINICAL MANIFESTATIONS

Cystic Echinococcosis (CE) may present in protean fashion, with manifestations that run the gamut from asymptomatic carriage to sequelae of pressure-induced end organ necrosis to anaphylaxis following cyst rupture.

Invasion of the intestines by infectious oncospheres produces no symptoms, nor does the initial phase of cyst growth. Prevalence studies using serology and ultrasound imaging support the hypothesis that many patients with hydatid cysts of the liver remain asymptomatic forever; in these cases, infection might be detected incidentally during imaging for other indications, or at autopsy. On the other hand, some patients with CE of the liver *will* develop symptoms at some point in their lifetime. The rate of cyst growth is difficult to predict, regardless of the end organ affected, and has been documented at a maximum of between 1 and 5 cm in diameter per year [12]. Because symptoms are typically directly related to cyst size, as well as location, a delay of years or even decades may occur from infection to symptom onset. Onset of symptoms may be insidious, and these frequently include aching pain in the upper abdomen, early satiety, nausea, emesis, and a sensation of abdominal bloating [13].

Hydatid cysts of the liver are multiple in approximately one-third of cases, and the size, number, and location of cysts may influence the speed and severity of symptoms [14]. Any portion of the liver parenchyma may be involved, although most cysts involve the right lobe of the liver. If they grow large enough, cysts may impair biliary flow, leading to cholestatic obstructive jaundice or ascending bacterial cholangitis. Compression of the hepatic and portal veins may lead to portal hypertension, and even thrombosis of the hepatic veins and/or inferior vena cava (the Budd–Chiari syndrome).

Large cysts may erode into the biliary system, leading to spillage of cyst contents into the bile ducts and causing intermittent obstruction, local irritation, inflammation, cholangitis, and pancreatitis [15]. If liver cysts erode into the diaphragm, they may lead to direct communication with the pulmonary parenchyma, causing bronchorrhea with

cyst contents. Similarly, erosion into the abdominal cavity may allow drainage into the peritoneal space. Cysts which spontaneously drain are at risk of developing bacterial superinfection. Regardless of the site of drainage, contact of hydatid sand with other visceral surfaces also raises concern for implantation of viable brood capsules or germinal tissue, leading to spread of infection. The incidence of this occurrence is unclear, and it is likely rare, perhaps in part due to the fact that large cysts are sometimes "burned out" and contain non-viable sand. However, spread of infection during spillage of cyst contents has been described and should be taken seriously, both for this reason and for its association with anaphylaxis to the antigens in hydatid sand. This may happen either during chronic drainage of cyst contents into the body or more classically during an acute rupture, as might happen in blunt trauma to the abdomen.

Thus, the symptoms, signs, and physical examination findings associated with hydatid cysts of the liver are non-specific and vary from case to case. Although the majority of hydatid cysts are detected within the liver parenchyma, extrahepatic sites of implantation are also common (Table 17.1.). Hydatid cysts in each of these extrahepatic sites may present with unique symptoms and signs, depending on anatomic location and size. For example, cysts in the long bones may weaken them, leading to pathological fractures. Intracranial cysts may present with seizures, reminiscent of neurocysticercosis due to infection with *Taenia solium*, the pork tapeworm. Pulmonary cysts may present with pleuritic pain, bronchorrhea when cysts erode into bronchi, or massive hemoptysis when they erode into the ascending aorta [16]. Thus, it may be wise

Table 17.1
Approximate frequency of hydatid cysts by anatomic location

Location	*Frequency (%)*
Liver	66
Lung	10
Abdominal (including spleen and pancreas)	8
Kidney	7
Intracranial	7
Bone	2
Other (e.g., ocular, cardiac, muscle, breast)	<1

Adapted from Chiodini et al. [49].

to think of this condition as a space-occupying mass that can grow, slowly, in virtually any end organ; it may be well tolerated there, it may cause the insidious onset of non-specific complaints, or it may lead to sudden or even catastrophic complications – all depending on the location involved. Finally, the history and physical examination may be further obscured when cysts develop in multiple organs simultaneously, which has been reported to happen in approximately 10% of cases [12].

In the case of AE due to *E. multilocularis*, clinical presentation may be indistinguishable from that of CE described above. However, because of the infiltrative and invasive nature of this infection, patients are more likely to present with severe, advanced, inoperable disease than those with CE – although, as described below, the introduction of anti-parasitic medications may still offer these patients a chance for prolonged survival, or even a cure.

DIAGNOSIS

History. Symptoms described above should prompt consideration of possible hydatid disease. Because of the potential for long delay between infection and clinical presentation, a history should be elicited which includes the suspected patient's lifetime risk profile, including work on sheep or cattle ranches, exposure to herding dogs, feral dogs, or foxes, regardless of how long ago these exposures may have happened. Anaphylaxis following blunt abdominal trauma should raise the possibility of cyst rupture.

Physical Examination. Large liver cysts may manifest upon inspection as a visible bulge in the right upper quadrant, but this is unusual. Fullness and tenderness of the liver may be elicited during palpation and percussion. Because of the fluid-filled nature of the cyst, it may produce a thrill when fluid waves are generated during percussion [26]. However, this finding is neither sufficiently sensitive nor specific to make a diagnosis. In advanced cases of hydatid liver cysts, patients may present with scleral icterus or jaundice, but this, too, is non-specific and is poorly sensitive.

Imaging. A significant proportion of patients with hydatid liver cysts will come to the attention of hepatologists and hepatobiliary surgeons because of imaging findings. Ultrasonography typically demonstrates a hypoechoic lesion with smooth contours and roughly spherical shape. It carries a negative predictive value greater than 90% [17]. Several ultrasonographic features should be considered. The presence of diffuse echogenic signals from the cyst may represent hydatid sand that is disturbed during the examination. The presence of cyst surface calcifications implies advanced cyst age, and thus a decreased chance of

viability, as do asymmetry of the cyst and the presence of germinal tissue that has separated from the cyst wall [18]. Predictive criteria for the viability of hydatid liver cysts on ultrasonography have been developed by various authors, including the World Health Organization, but treatment schemes based on these have yet to undergo rigorous validation (see Management below) [19]. Finally, ultrasound's positive predictive value is adversely impacted by the fact that, in the absence of these findings, benign cysts may have an identical appearance.

For patients with ultrasonographic findings concerning for hydatid liver cysts, computed tomography (CT) is a reasonable next step in the evaluation when it is available. CT provides unsurpassed sensitivity for detecting hydatid cysts both in the liver and in the extrahepatic sites (Fig. 17.2) [17]. An aggressive protocol for assessing presumptive hydatid disease of the liver includes scanning the head to rule out the presence of intracranial cysts; this practice is rational, but is of unproven utility in terms of a population-based, cost/benefit analysis. CT also provides radiologists with more reliable information regarding possible superinfection of cysts, as implied by contrast enhancement of the surrounding tissue or presence of gas within the cyst [20]. Magnetic resonance imaging (MRI) offers no advantages over CT in terms of sensitivity or specificity, although magnetic resonance angiography (MRA)

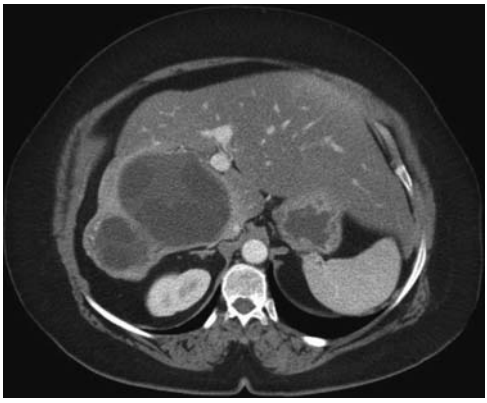

Fig. 17.2. CT Image of a complex, long-standing hepatic hydatid cyst. The patient had been infected approximately four decades earlier as a child on a sheep ranch in Uzbekistan. Note the heterogenous nature of cyst contents, which were confirmed to represent daughter cysts during surgery. This patient's cysts were in significant contact with the liver capsule (thus making her a poor candidate for PAIR), and there was significant peri-cystic fibrosis leading to inferior vena cava adherence (thus making her a poor candidate for hemi-hepatectomy). (*Image credit: Orpheus Kolokythas, MD.*)

may allow a more complete evaluation of suspected hepatic vein thrombosis [21]. In cases of biliary communication and obstructive jaundice, endoscopic retrograde cholangiopancreatography (ERCP) may be required to bypass biliary obstructions (see Management below), thus simultaneously offering radiographic data, fluid for microscopic analysis, and providing at least temporizing therapy until definitive treatment for the cyst can be implemented [22].

The diagnostic challenge for radiologists increases substantially when extrahepatic cysts are detected, as these may mimic other benign or even malignant etiologies [23] (see Chapter 5)

Routine Biochemical Testing. Screening blood work is typically normal in hepatic echinococcosis. Hepatocellular function is usually preserved, except in rare instances of advanced infection with large liver cyst involvement, leading to widespread pressure necrosis, portal hypertension, and biliary cirrhosis. Thus, except in severe cases, clinicians should consider alternative explanations for clinical hepatitis manifesting with elevations of serum aminotransferase levels or prothrombin time. Peripheral blood eosinophilia is usually absent in hydatid disease, regardless of the organ(s) involved; the finding of peripheral eosinophilia implies leakage of cyst contents and presentation of antigens to surrounding lymphatic tissue [12]. If solid organ malignancy remains on the differential diagnosis, serum tumor markers such as CA 19-9 and CA 125 can also be sent as part of the evaluation. However, results should be interpreted with caution, as experience with this modality is limited, and hydatid cysts may "falsely" elevate these markers [24].

Serology. A variety of serological assays have been developed for hydatid disease [25]. Some have been used both for diagnosis and to monitor response to therapy. Many are not currently available on a commercial basis, either in developing nations or in industrialized settings. Perhaps the first clinically available immunological assay for echinococcal infection is the skin test, "Casoni's test," in which 0.2 mL of hydatid fluid is injected intradermally followed in positive cases by a local wheal and flare within 20 min [26]. Problems with this test include the lack of access to standardized antigen, and the concern for falsely elevating subsequent serologic results. However, this test is still used in some highly endemic areas, such as Turkey, where antigens are readily available, and where it has compared favorably with indirect hemagglutination assays (IHA) [27].

For most practitioners in industrialized settings, blood testing – rather than skin testing – will be available. The diversity of assays, and their different operating characteristics, can be a source of frustration. For example, IHA has been described as having a sensitivity of 87% for

CE by one group of investigators [28], whereas others found it to be significantly less sensitive at only 56% [27]. Variability on this scale has also been described with the negative and positive predictive values for CE of IgG enzyme-linked immunosorbent assays (ELISA), latex agglutination, radioimmunoassay, and other modalities [12]. These differences may have a variety of causes, including cyst number, location (e.g., bone cysts may be more immunogenic than eye cysts), condition (e.g., draining cysts may be more immunogenic than intact ones), host immune status (e.g., pregnant women may demonstrate lower immune response to antigens than non-pregnant women), infection with other parasites that generate cross-reactive immunological response (e.g., neurocysticercosis), and assay reagents used (e.g., freshness, mixture, and source of material). Because of the chronic nature of this condition, comparing acute with convalescent antibody titers is unlikely to be useful. One bright spot in this confusion comes from the use of dot-ELISA, an affordable, hardy, rapid test that has tested favorably in the field in Kenya, with sensitivity and specificity greater than 90% [29].

In summary, the serological tests currently available for CE do not carry a negative predictive value high enough to exclude the diagnosis. Likewise, their positive predictive values are not perfect and should be interpreted in light of the pre-test probability of hydatid disease. Laboratories should furnish health care providers with references specific to the operating characteristics of tests they perform; however, no test will exclude or confirm the diagnosis with infallible certainty.

AE due to *E. multilocularis*, in contrast, is generally believed to be easier to diagnose by serology, based perhaps on its greater antigen presentation when the germinal membrane penetrates into the surrounding host tissue. A sensitivity of 95% and specificity of 100% have been reported [30].

Pathology. As described below, therapeutic drainage of possible hydatid cysts may simultaneously offer a diagnosis as well as a cure (see Management, Percutaneous Drainage below). However, a small-volume bedside diagnostic tap of suspected hydatid cysts is *not* generally recommended, because of concern for spillage of cyst contents into adjacent liver tissue or the peritoneal cavity, which has been associated on rare occasions with anaphylaxis and/or spread of infection due to escaped germinal tissue.

Neither *E. granulosus* nor *E. multilocularis* can be cultured outside of highly specialized research settings [31], and thus cultivation of the organisms from cyst fluid is not a diagnostic option. Instead, fluid from putative hydatid cysts should be examined rapidly by light microscopy. Viable cysts are said to contain clear-appearing fluid, whereas older, less infectious cysts may contain turbid yellow-colored

fluid. Direct, unfixed, unstained wet-mount preparations of the fluid may reveal intact, viable protoscolices (Fig. 17.3) or detached hooklets (Fig. 17.4). Because of their tough, proteinaceous composition, hooklets may remain intact for months or years after all protoscolices have died, and thus their presence merely confirms the diagnosis of hydatid disease, not viability of the cyst in question. On the other hand, the negative predictive value associated with the absence of healthy-appearing protoscolices remains undefined, and thus hooklets by themselves *may* still be associated with a viable cyst. If direct microscopic examination

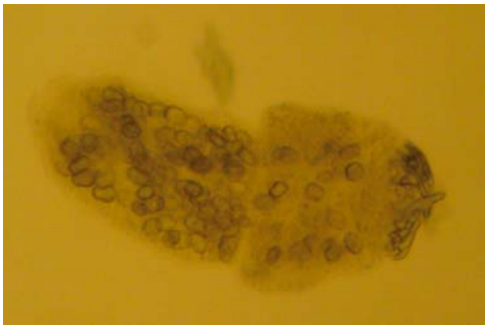

Fig. 17.3. Protoscolex visualized by light microscopy (high power) of fluid aspirated from a solitary hydatid liver cyst during PAIR procedure. The patient had been infected approximately 12 years earlier when caring for a feral canine-wolf hybrid. This protoscolex has already evaginated, but may be viable, based on its intact anatomy and retained hooklets. (*Image credit: Paul Pottinger, MD, DTM&H.*)

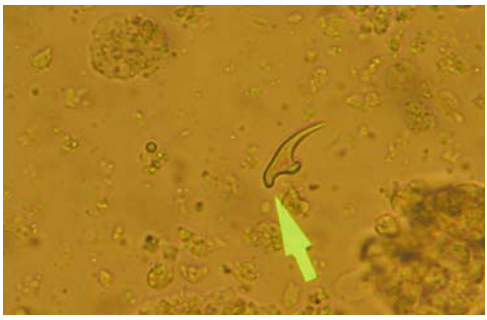

Fig. 17.4. Hooklet visualized by light microscopy (high power) in cyst fluid aspirated during open surgical drainage from the patient in Fig. 17.2. Note the heterogenous, amorphous debris typical of non-viable cystic fluid. (*Image credit: Paul Pottinger, MD, DTM&H.*)

of cyst fluid contents reveals no parasites or hooklets, it may be profitable to gently centrifuge the fluid, stain it using iodine, and examine the concentrated specimen. Although the absence of parasites or hooklets in cyst fluid speaks against the diagnosis, the negative predictive value of this maneuver remains undefined and should be considered in the context of history, physical examination, imaging, blood work, and cyst wall pathology (if available).

If a putative hydatid cyst has been surgically removed and is delivered for pathological examination, it can be analyzed in two ways. First, cyst contents can be examined by light microscopy as described immediately above, as well as macroscopically for the presence of brood capsules and daughter cysts. Second, the cyst wall may be sampled and examined microscopically. Hydatid cysts should demonstrate an outer layer of host adventitia (comprised of endothelial cells, giant cells, eosinophils and other leukocytes, fibroblasts, and fibrin strands), a middle layer of parasite origin consisting of nonnucleated opaque tissue, and an inner germinal layer of parasite origin, which may rarely demonstrate budding brood capsules.

MANAGEMENT

Management options for CE of the liver include observation, medical therapy, percutaneous drainage, and surgical removal. The choice of approach may depend on individual signs and symptoms, comorbidities, certainty of diagnosis, radiographic features, available resources, and patient preference. The World Health Organization has published guidelines for the treatment of CE and AE [32].

Observation. Asymptomatic patients are sometimes found to have incidental imaging changes consistent with possible CE. These patients may be appropriate candidates for watchful waiting, provided that a careful evaluation is performed as described above, and that there is a low suspicion for a dangerous alternative diagnosis, such as malignancy. In that setting, patients should be reassured regarding the nature of this infection and counseled regarding the risk of traumatic cyst rupture – a risk which is influenced by patient activities as well as cyst size and location. In particular, if asymptomatic patients are not engaged in occupations or hobbies that place them at risk for blunt trauma, and if they have simple, solitary hepatic cysts, cysts which are smaller than 10 cm in diameter, cysts with heavily calcified walls, or separation of germinal membrane from the cyst wall on ultrasound, then it may be reasonable to repeat imaging perhaps every 6–12 months for 1–2 years. If the cyst is stable, and if no new cysts are detected, then patients can be counseled to return for onset of unexpected symptoms.

Medical Therapy. Symptomatic patients should be offered treatment for their infection. Antihelmintic drugs are typically administered in combination with definitive cyst removal or drainage (see below). However, medical therapy alone may have a role to play in a small number of patients with CE, in particular those who are felt to be poor candidates for surgery or PAIR (*p*uncture of cyst wall, *a*spiration of cyst contents, *i*njection of protoscolicidal agents, and *r*e-aspiration of cyst contents) (see below). Medical therapy is also recommended when two or more cysts are found in two or more organs or the peritoneum, when multiple small cysts are found in the liver, or when cysts spontaneously rupture [32, 33].

The antihelminthic medications mebendazole and albendazole are benzimidazoles which impair microtubular assembly in the cyst and protoscolices, thus interfering with glycogen processing, resulting in slow parasite death. These medications are taken orally and are generally well tolerated, although a minority of patients will develop nausea, vomiting, rash, hepatitis, or myelosuppression. Furthermore, the cyst walls seem to act as barriers to drug penetration into the center, where drug levels may reach only 30% of those found in the serum. For this reason, therapeutic drug monitoring is recommended by the WHO – peak serum levels should be obtained 2 weeks after initiating albendazole, or 4 weeks after initiating mebendazole, and repeated every 3 months thereafter while on therapy; peak levels should be checked 4 hours following oral administration (target: 250 nmol/L for mebendazole and 650–3,000 nmol/L for albendazole). Many practitioners also check serum liver damage indices periodically while patients are on therapy, although this practice is not strictly evidence-based. A rational approach to safety monitoring includes checking serum aminotransferases, alkaline phosphatase, bilirubin, and prothrombin time 1 week into treatment with either of these medications and then monthly thereafter among patients who have underlying liver disease. However, all patients should be counseled to contact their providers immediately with unexplained abdominal pain, nausea, or jaundice.

Among those unable to tolerate either of these medications, praziquantel can be used as an alternative drug. This isoquinoline medication's mechanism of action is unknown, but may relate to interference with purine uptake by the parasite. It, too, may cause gastrointestinal upset and reversible hepatocellular damage. Preliminary data suggests that nitazoxanide may be useful for this condition [34], but clinical experience with it is lacking, and thus it cannot be recommended except on an experimental basis among patients who cannot tolerate benzimidazoles or praziquantel.

Clinical improvement in a variable proportion of patients with CE has been reported with albendazole alone. However, results are often discouraging. A concerning report from Turkey reported radiographic improvement in only 12 of 65 patients treated with 6 months of albendazole monotherapy after 2 years of follow-up [35]. Reports on the combined use of benzimidazoles plus praziquantel assert superior outcomes versus monotherapy with albendazole for either perioperative use, post-spillage prophylaxis, or medical-only approaches [36, 37]. Many practitioners have adopted the use of combination treatment among patients who tolerate it. However, a lack of strict control cohorts in these trials, and concern for potential toxicity with multiple medications, continue to generate debate regarding combination therapy.

The optimum duration of drug treatment using either class is unclear, but courses usually last for months or longer, especially in the absence of adequate cyst drainage or removal. Most symptomatic patients should be offered definitive drainage, but if those procedures are not feasible, then patients treated with medication only should undergo regular assessments for toxicity and efficacy. Repeat CT scans may be performed every 3–6 months in this cohort, and consideration of percutaneous or surgical intervention might be entertained if cysts continue to enlarge and cause increased symptoms.

Surgical Removal. The conventional treatment of hydatid cysts in all organs has been surgical, as this has the potential for complete cyst removal and cure. Successful eradication requires the entire elimination of the parasite without contamination or compromise of affected organ system(s) during extirpation. Surgery remains the most efficient primary and salvage therapy, and the evolution of hepatic surgery has fortunately facilitated less morbid interventions. The WHO considers superficial or large liver cysts with multiple daughter cysts, evidence of infection, or violation of the biliary or other organ systems as indications for surgery [13]. Although the procedure of choice is still debated, given the lack of randomized controlled trials, the accepted objective remains the entire elimination of the cyst. Surgical techniques are divided into either radical or conservative methods based on complete cyst removal versus evacuation and inactivation of the protoscolices, respectively.

Prior to any form of diagnostic or therapeutic intervention, a course of antihelminthic therapy, preferably albendazole, is empirically initiated – for a minimum of 4 days pre-operatively [32]. Given that spilled cyst fluid may contain viable protoscolices which could contaminate the peritoneal cavity, protection of the operative field is imperative. The peritoneal (and pleural if appropriate) cavities should be isolated

with gauze soaked in 20% saline or parasiticidal solution. Unless radical en bloc resection is being entertained with confidence that there will be no cyst violation, it is prudent to evacuate the cyst. After verifying control of the cyst wall, it is punctured and evacuated with a large caliber suction device, followed by instillation of protoscolicidal agents such as 20% saline, 70–95% ethanol, or 0.5% cetrimide, for approximately 15–20 min. The use of hypertonic saline minimizes the risk of chemical cholangitis in cases where biliary communications are present [14].

Radical surgery ideally results in complete removal of the cyst along with the pericyst, including exocysts when present, and adjacent hepatic parenchyma. Anatomic and nonanatomic liver resections, including formal hepatectomy, may be used to eradicate the cyst(s), but true anatomic resection for large cysts is rarely feasible. Pericystectomy is the resection of the cyst and essentially the surrounding rim of hepatic parenchyma. Similar to formal anatomic resection, this strategy likely sacrifices normal healthy liver tissue and may be compromised in scope by cyst adherence to adjacent vessels and bile ducts. Although more aggressive resection is associated with higher complication rates, the reported rate of disease recurrence is lower [2]. However, many endemic areas are not equipped with the technical expertise or equipment for these procedures.

Given that the level of evidence is currently inadequate to recommend the correct level of aggressiveness, many surgeons utilize more conservative methods for open and/or laparoscopic resections. Conservative measures include removal of the endocyst and subtotal resection of the pericyst without spillage of cyst contents. After isolation and evacuation of the cyst(s) as previously described, fenestration and/or unroofing of the residual cavity is performed. The cavity may then remain open with or without drainage [38]. Although previously utilized, marsupialization with externalization has been abandoned secondary to high morbidity rates. In all cases, vigilance for cyst-biliary communication(s) should be maintained, and there should be a low threshold for cholangiography and potential repair and/or drainage. Omentoplasty following either radical or conservative treatment is efficient in preventing post-operative complications such as abscess formation [39].

There is growing support for the safety of laparoscopic approaches, and, indeed, recent reports demonstrate lower rates of post-operative morbidity and mortality versus open procedures. The major disadvantage to laparoscopic procedures is the inherent difficulty in preventing spillage and controlling contamination during cyst penetration. Although promising, the laparoscopic data are limited in that

clinical studies were not randomized and high-risk patients were likely ultimately handled with open procedures. If there is any question of possible spillage of cyst contents during any of the aforementioned procedures, patients are offered post-surgical treatment with albendazole, usually for 8–12 weeks.

Retrospective studies report recurrences following open surgery in 10–30% of individuals compared with approximately 10% for laparoscopic series [40]. However, the laparoscopic experience is much more limited and is subjected to significant patient selection bias. Further prospective studies are needed to better define the role of open versus laparoscopic approaches in the primary versus salvage therapy of hydatid cyst disease.

PAIR (see below) has emerged as a less invasive and more attractive option in patients with increased surgical risk, underlying comorbidities, or impaired access to surgical expertise. In this alternative to surgical intervention, the cyst is punctured transcutaneously, ideally under ultrasound guidance, and the parasite is killed through repeated aspiration and injection of protoscolicidal agents such as 20% saline. For simple hydatid liver cysts that do not abut the liver capsule, this appears to be a safe and advantageous option, especially in endemic areas where more aggressive intervention is unavailable [41, 42].

The WHO guidelines recommend a minimum of 4 days of pre-operative antihelminthic therapy, preferably using albendazole [32]. As described above, many practitioners prescribe a combination of albendazole plus praziquantel for a month pre-operatively, in hopes of achieving greater sterilization of the cyst than with either medication alone. This practice is based in part on a study that demonstrated the superiority of combination therapy over albendazole monotherapy when cyst contents were examined immediately following removal [43]. However, these results are not convincing enough to obviate either meticulous handling of cysts intra-operatively, or consideration of post-surgical medical prophylaxis – which is generally given for 60 days in cases where spillage is not suspected, or considerably longer in cases where gross spillage is detected.

Percutaneous Drainage. In select cases, percutaneous drainage of cysts may yield excellent clinical results with minimal risk of either treatment failure or the peri-procedural complications associated with open surgery. This technique is called PAIR: *p*uncture of cyst wall, *a*spiration of cyst contents, *i*njection of protoscolicidal agents, and *r*e-aspiration of cyst contents. The procedure is guided by direct imaging, usually using ultrasound with or without fluoroscopy, or using CT, depending on cyst location, resources available, and operator comfort and preference. It provides both an opportunity to confirm the diagnosis

(see Diagnosis, Pathology above) as well as to attempt to kill existing protoscolices and the germinal tissue from which they have budded. Even patients with "non-viable" cysts may benefit symptomatically from this technique, although many patients with heavily calcified cysts that appear "deflated" may have no associated symptoms, and these cysts are not recommended for PAIR.

Patients may be considered for PAIR if they have "simple" liver cysts that do not communicate with the biliary tree, are not in direct contact with the liver capsule, are located in areas associated with high surgical risk, and are considered accessible by a radiologist. Patients who cannot tolerate open surgical excision due to medical comorbidities may tolerate this procedure comparatively well. Risks include pain, local bleeding, visceral injury, secondary infection, chemical cholangitis, spread of new cysts, and anaphylaxis. The largest published series to date analyzed 795 patients: 14% of patients sustained minor complications such as urticaria or biliary rupture and cholangitis, 1% of patients sustained major complications including anaphylaxis or gross spillage of cyst contents, and one patient died as a result of the procedure [12, 44].

A number of agents have been investigated for their protoscolicidal activity and suitability for use in the "Injection" portion of the procedure [45]. Concern regarding certain agents, including 100% ethanol and cetrimide, centers upon the risk of chemical cholangitis and pancreatitis if it comes into direct contact with the biliary drainage. An approach that ameliorates this risk includes ERCP pre-PAIR, in order to exclude wholesale biliary communication. However, this procedure itself entails added cost and risk. Hypertonic (20%) sodium chloride (NaCl) solution has been used safely in many cases, although concerns for hypernatremia have been raised, prompting investigation of other agents, including hypertonic 50% glucose [46] and 20% mannitol.

Regardless of the scolicidal agent used, the volume of scolicidal agent recommended is approximately 30% of the volume removed during aspiration. Dwell time has not been carefully standardized, and based on in vitro studies, the optimum dwell time may vary with the agent chosen. Many centers allow a dwell time of 20 min, although up to 45 min may be required to achieve maximum killing power with 20% NaCl [45]. And, regardless of agent selected or dwell time, fluid obtained from the cyst on re-aspiration should be examined immediately via light microscopy, and the presence of viable protoscolices should prompt repeat injection.

The WHO recommends a minimum of 4 days of antihelminthic oral drug therapy pre-PAIR, followed by continuation of treatment

post-PAIR for 1–3 months. When combined with pre-procedural and post-procedural oral antihelminthic therapy, this technique has compared favorably with traditional open cyst removal [42]. The risk of recurrent hydatid liver cyst enlargement following PAIR varies by case series, but at 1–5 years of follow-up is approximately 0–4% [33].

Extrahepatic cysts present unique challenges in terms of management. The fundamental treatment options remain the same – observation versus medical therapy combined with either PAIR or surgery. However, the consequences of surgery versus medical therapy may differ from uncomplicated CE, depending upon cyst location.

Experimental Therapies. High-intensity focused ultrasound energy has been investigated as a method to sterilize hydatid cysts, but it remains unproven and experimental [47].

AE due to *E. multilocularis* mandates immediate and aggressive therapy, regardless of the degree of symptoms at presentation, because of its very high mortality without treatment. These patients may present with advanced hepatic disease, making surgical resection or PAIR impossible. In all suspected cases of AE, medical therapy using albendazole with or without praziquantel should be initiated immediately. Based on the small number of cases in the literature in this circumstance, this approach has resulted in clinical improvement and survival ranging from 53 to 80% at up to 15 years of follow-up [12].

Follow-Up. Monitoring of patients post-PAIR or surgical resection involves periodic clinical assessment, with combined history, physical examination, and imaging to exclude the finding of new cysts, or complications of existing treated cysts, including enlargement or superinfection. Radiographic changes will be evident post-PAIR and post-resection, but imaging obtained every 6–12 months may be useful in order to exclude the presence of new cysts, or the unexpected enlargement of cyst remnants. Among patients treated with PAIR, in particular, the expected radiographic evolution includes a "sloughing" of the germinal layer into the cyst cavity.

Serological assays such as IgG titers available in most settings are expected to remain positive indefinitely and thus cannot be used as a gauge of disease activity [48]. However, complement fixation assays have been demonstrated to wax and wane in response to disease activity, and, if validated assays of this type are available, they may prove helpful in following clinical response. For most health care providers, assessment of outcomes following therapy will have to happen without the benefit of this information.

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18 Miscellaneous Cystic Lesions of the Liver

Iris W. Liou, MD

CONTENTS

Summary

Cystic diseases of the liver and biliary tract are comprised of a heterogeneous group of disorders. The pathogenesis, clinical presentation, histological description, and radiographic appearance of several less common conditions, including biliary hamartomas, ciliated hepatic foregut cysts, cavernous hemangiomas, peliosis hepatis, and peribiliary cysts, will be discussed here.

Key Words: Liver cyst, Biliary hamartoma, Ciliated hepatic foregut cyst, Cavernous hemangioma, Peliosis hepatis, Peribiliary cyst

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INTRODUCTION

Cystic diseases of the liver and biliary tract comprise a large heterogeneous group of disorders, the majority of which are discussed elsewhere in this text. Both clinical and radiographic features (Table 18.1) may help distinguish between these entities. Here we will discuss situations which are not generally associated with any of the known cystic syndromes – biliary hamartoma, ciliated hepatic foregut cyst, cavernous hemangioma, peliosis hepatis, and peribiliary cyst.

BILIARY HAMARTOMA (VON MEYENBURG COMPLEX)

Biliary hamartomas (also called bile duct hamartomas, microhamartomas, or von Meyenburg complexes) were first described by von Meyenburg in 1918 and are considered to be part of the spectrum of fibrocystic liver diseases occurring secondary to ductal plate malformation [1]. They are thought to originate from embryonic bile ducts which fail to involute [2–7], essentially a ductal plate malformation of the small peripheral interlobular bile ducts [8]. Biliary hamartomas (BH) are usually found incidentally at imaging or at autopsy without pre-existing symptoms. They can expand within a portal tract or to adjacent portal tracts and can lead to the development of non-cirrhotic portal hypertension. The prevalence in the general population has been estimated to be 0.69–5.6%, based on incidental discovery at autopsy, and they tend to be more common in women [9–11]. Patients may present with a mild elevation in serum gamma-glutamyltransferase (GGT) level. The hamartomas may occur in otherwise normal liver but are also found in patients with Caroli's syndrome, autosomal dominant polycystic kidney disease, and in almost all patients with congenital hepatic fibrosis.

Grossly, BH appear as grayish-white, green, or black nodular lesions from 0.1 to 1.5 cm in diameter and are well defined without encapsulation. They can be solid with narrow bile duct channels, intermediate with both solid and cystic components, or predominantly cystic lesions with very dilated bile channels [12]. BH are found throughout the hepatic parenchyma, most commonly in the subcapsular region [7]. Generally in the interlobular region, these lesions lie within dense fibrous stroma and are lined by a single layer of low columnar or cuboidal epithelium with irregular spaces which typically contain proteinaceous fluid and sometimes bile (Fig. 18.1) [9]. Histologically, BH have a thicker zone of fibrous tissue compared to simple hepatic cysts,

Table 18.1
Cystic lesions of the liver

Type	*Condition*	*Clinical features*	*Complications*	*Radiographic findings*
Congenital/genetic	Simple hepatic cyst	Asymptomatic		Homogeneous, round, no enhancement
	Autosomal dominant polycystic liver disease	Asymptomatic or abdominal fullness, pain	Cyst infection, hemorrhage	Homogeneous, round, no enhancement
	Caroli's syndrome	Asymptomatic or pruritus	Cholangitis, cholangiocarcinoma	Saccular dilatation of bile ducts
	Biliary hamartomas	Asymptomatic	Peripheral cholangiocarcinoma	Homogeneous, irregular borders, endocystic enhancement
	Choledochal cyst	Intermittent pain, jaundice	Cholangitis, cholangiocarcinoma	Dilated bile ducts
	Ciliated hepatic foregut cyst	Asymptomatic	Obstructive jaundice, portal hypertension, squamous cell carcinoma	Solitary, subcapsular, anterior medial segments of liver
	Cavernous hemangioma	Asymptomatic, upper abdominal pain, or early satiety	Hemorrhage, torsion, rupture, Kasabach–Merritt syndrome	Peripheral nodular enhancement with centripetal fill-in

Table 18.1
(continued)

Type	*Condition*	*Clinical features*	*Complications*	*Radiographic findings*
Neoplastic	Biliary cystadenoma	Asymptomatic, upper abdominal pain	Biliary cystadenocarcinoma	Thick and irregular walls, mural nodules, multilocular
	Hepatocellular carcinoma	Asymptomatic, symptoms of cirrhosis	Hemorrhage, rupture	Arterial phase enhancement with wash-out on delayed images
	Cystic metastases	Symptoms from primary cancer		Peripheral enhancement
	Ovarian carcinoma metastases	Symptoms from ovarian cancer		Implants on capsule
	Undifferentiated (embryonal) sarcoma	Abdominal pain		Solitary, pseudocapsule
	Primary squamous cell carcinoma	Abdominal pain		Hypodense
Infectious	Hepatic abscess	Fever, abdominal pain	Sepsis	Enhancing wall, loculations
	Echinococcal cyst	Asymptomatic, biliary colic, jaundice	Portal hypertension, peritonitis	Daughter cysts, hydatid sand, calcifications

Table 18.1
(continued)

Type	*Condition*	*Clinical features*	*Complications*	*Radiographic findings*
Miscellaneous	Peliosis hepatis	HIV (*Bartonella*), oral contraceptive, anabolic steroids	Hepatomegaly, portal hypertension, liver failure, cirrhosis, rupture	Hypodense with slow centripetal enhancement, target sign
	Peribiliary cysts	Asymptomatic, cirrhosis	Hepatolithiasis, cholangitis, cholangiocarcinoma	Cysts along large portal tracts
	Extrapancreatic (subcapsular) pseudocyst	Pancreatitis	Hemorrhage	Enhancing capsule
	Intrahepatic hematoma	Trauma or procedure	Rupture	High attenuation or fluid attenuation
	Intrahepatic biloma	Trauma or surgery	Infection	Pseudocapsule
	Endometriosis	Abdominal pain	Rare malignant transformation	Smooth or irregular wall
	Post-trauma hepatic cyst	Trauma	Infection, rupture	Hypodense

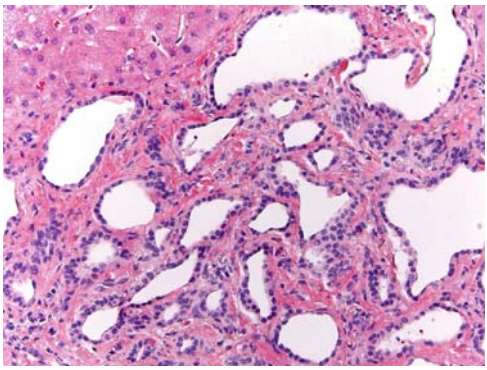

Fig. 18.1. Biliary hamartoma (von Meyenburg complex). Photomicrograph shows aggregates of bile ductal structures of variable sizes in a fibrous stroma. Some are dilated and may contain bile. They are lined by a single layer of cuboidal to flat epithelium (×200 magnification). Courtesy of Matthew M. Yeh, MD, PhD.

which are also lined with bile duct epithelium. The lumen of multiple microhamartomas within one complex may be interconnecting [10, 13].

BH lesions appear on ultrasound as multiple, small, discrete, irregular hypoechoic or hyperechoic lesions scattered throughout both lobes of the liver [3, 14, 15]. Multiple small comet-tail echoes have been reported in several cases [16, 17]. On non-contrast computed tomography (CT), BH appear as multiple uniform, hypoattenuating, cyst-like hepatic nodules, up to 1.5 cm in diameter, with irregular borders. They typically do not enhance after administration of intravenous iodinated contrast [4–7, 12], although enhancement has been described in a few cases [3, 18]. Magnetic resonance (MR) imaging reveals these lesions to be hypointense on T1-weighted images compared to the surrounding liver parenchyma and are hyperintense on T2-weighted images. In fact, the lesions are even more intense on heavily T2-weighted images [3, 7, 19, 20]. One series described mural nodules which were isointense to the liver parenchyma on T1-weighted images and of intermediate intensity on T2-weighted images. Most studies have demonstrated endocystic enhancement after administration of gadolinium, which has been hypothesized to represent the endocystic polypoid projections of connective tissue into the cyst as seen on histological review [13]. Enhancement following administration of gadolinium contrast may be present [4] or absent [5], but a thin rim of enhancement persisting into the late post-gadolinium phase correlates with compressed liver parenchyma surrounding these lesions, as can be seen histologically

[7]. Angiographically, the lesions appear as multiple areas of abnormal vascularity approximately 1 cm in diameter, persisting into the venous phase [21]. MR cholangiography has demonstrated the absence of communication with the biliary tree, but the presence of bile casts within the lumina of these lesions suggests that there actually is some connection to the biliary system [22]. Detailed investigations of isolated BH and those associated with polycystic liver disease have demonstrated a plexus of connecting cavities that are contiguous with the biliary system [23–27].

Since these lesions are generally asymptomatic, long-term prognosis may depend on any associated conditions. There are case reports that BH can become complicated by the development of peripheral cholangiocarcinoma [13, 28–33]. There are no guidelines established for long-term screening, but suspicious lesions should be investigated.

CILIATED HEPATIC FOREGUT CYST

Ciliated hepatic foregut cysts (CHFC) were characterized by Wheeler and Edmondson in 1984 [34], although there are descriptive reports dating back to 1857 [35]. Almost 100 cases have been reported to date. CHFC are rare benign solitary hepatic cysts, which occur slightly more frequently in men (male to female ratio 1.2:1) [36]. The average age at presentation is 50 years but they have been identified in neonates and children as well [37, 38]. Most cases are found incidentally; however, there are case reports of obstructive jaundice and of portal vein compression leading to portal hypertension and splenomegaly. Some cases are brought to attention during the evaluation of right upper abdominal pain.

CHFC are thought to be similar to esophageal and bronchial cysts. The esophagus, tracheobronchial tree, and the liver are all derived from the primitive foregut. These ciliated cysts may develop in the liver either as a detached outpouching of the hepatic diverticulum or adjacent enteric or bronchial foregut which migrated to the liver [34]. Foregut cysts have also been found in the neck, mouth, mediastinum, lung, pancreas, esophagus, stomach, and retroperitoneum.

CHFC are composed of four layers: ciliated pseudostratified columnar epithelium associated with hepatocytes and goblet cells, subepithelial loose connective tissue, smooth muscle (often an incomplete layer), and a surrounding fibrous capsule [39, 40] (Fig. 18.2). The ciliated epithelium has immunohistochemical and ultrastructural features which resemble bronchial epithelium but does not contain cartilage [41]. Endocrine cells (Clara cells) similar to those found in respiratory epithelium have been identified by immunohistochemistry [42].

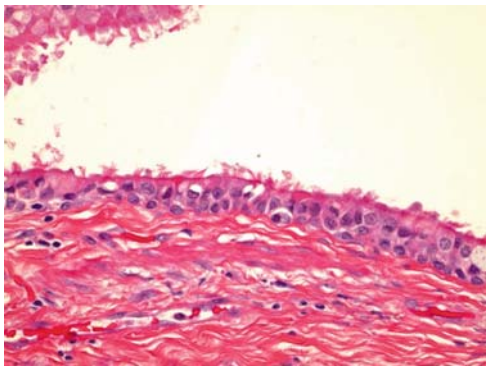

Fig. 18.2. Ciliated hepatic foregut cyst. The ciliated hepatic foregut cysts are lined by a ciliated pseudostratified columnar epithelium. Bundles of smooth muscle are usually present, surrounding these cysts (×400 magnification). Courtesy of Matthew M. Yeh, MD, PhD.

CHFC are usually solitary (90%), subcapsular, and are less than 3 cm in diameter, but can range in size from 0.4 to 9.0 cm. They can be located in either hepatic lobe but predominate in the anterior medial subcapsular segments (typically segments 4, 5, or 8). Rarely, they are multiloculated or septated. The cystic fluid is viscous and mucinous in about 78% of cases; however, clear serous or bilious fluid has also been described, despite the absence of communication with the biliary system. Specific testing has revealed that these cysts contain neutral, acidic, carboxylated, and sulfated mucins [41, 43].

These lesions appear anechoic or slightly hypoechoic on ultrasound. The variable properties of the cyst content (protein, lipid, and calcium) account for differences in CT attenuation characteristics and in signal intensity on MR imaging. There is no enhancement following administration of contrast material [39, 44]. All lesions demonstrate high signal intensity on T2-weighted MR imaging but can have variable signal intensity on T1-weighted imaging, depending on the content of the cysts [45]. Although radiographic features may suggest the diagnosis in 70% of cases, fine needle aspiration may confirm the diagnosis, with a positive predictive value of 76% [46–48].

High serum CA19-9 levels have been observed in benign CHFC. There have been reports of squamous cell carcinoma (4.4% of all reported cases) arising from these lesions via squamous metaplasia [40, 49–51], suggesting that large lesions(>5 cm) require close radiologic monitoring and could be considered for resection or laparoscopic excision [46, 52–54].

CAVERNOUS HEMANGIOMA

Hemangiomas are the most common benign hepatic tumor, occurring in 0.4–20% of individuals in autopsy series. They are typically discovered incidentally on imaging, laparotomy, or at autopsy, most often in women, with a female to male ratio of 2:1 to 6:1 [55–57]. Multiple hemangiomas occur in 10–50% of cases. While they are typically less than 5 cm in size, they can grow to more than 20 cm in diameter. Hemangiomas may increase in size with pregnancy or with estrogen use, as some cavernous hemangiomas have estrogen receptors; estrogen use is not contraindicated in women with uncomplicated hemangiomas.

Most hemangiomas grow very slowly with the blood supply arising from the hepatic artery. They may involute with thrombosis, necrosis, calcification, fibrosis, or sclerosis. Following involution, the vascular spaces may be replaced by hyalinized fibrotic tissue, creating a hyalinized or sclerosed hemangioma, without the classic vascular features seen on contrast-enhanced imaging studies [58]. They are usually asymptomatic, although nonspecific abdominal symptoms, such as upper abdominal pain, early satiety, or nausea, may occur following necrosis, infarction, or thrombosis of lesions. Serum liver studies are usually normal.

Hemangiomas are thought to represent congenital malformations or hamartomas that initially grow within the liver and then increase in size thereafter by ectasia. They arise from the endothelial cells of hepatic sinusoids and consist of multiple, large vascular channels lined by a single layer of endothelial cells supported by fibrous septa [56, 59]. On gross examination, they appear as well-defined compressible tumors with a dark purple or blue color; larger lesions may be pedunculated. They typically have round or lobulated margins, but larger lesions may have irregular borders without distinct fibrous interface and can be surrounded by multiple hemangioma-like vessels [60].

Hemangiomas are commonly located in the posterior right hepatic lobe in the subcapsular or peripheral region. They appear as well-defined, lobulated, homogeneous, hyperechoic masses on ultrasonography, but the center may be hypoechoic (15–20%) due to fibrosis [61]. Calcifications typically are phleboliths and can be found centrally or peripherally. Contrast-enhanced harmonic ultrasonography can demonstrate peripheral nodular enhancement in the portal phase and absence of intratumoral vessels in the arterial phases, as can be seen in hepatocellular carcinomas [62, 63]. On multiphasic CT imaging, there is peripheral nodular enhancement on early enhanced images, then contrast enhancement progresses in a centripetal fashion, such that the lesion appears uniformly enhanced in the delayed phase [64, 65]

(Fig. 18.3). This pattern of blood flow can be explained by the slow flow of blood within the vascular channels of the hemangioma. The lesions show low signal intensity on T1-weighted MR images and high signal intensity on T2-weighted MR images, and a similar enhancement pattern is seen after the administration of gadolinium; the diagnostic sensitivity and specificity for T2-weighted MR imaging in distinguishing cavernous hemangiomas from malignant lesions and focal hepatic lesions are both greater than 90% [66, 67]. Large lesions, however, may display a central scar that does not enhance on the later phases; on histologic evaluation, the scar results from thrombosis, myxomatous degeneration, fibrosis, or necrosis [68]. Perilesional enhancement is seen in 19–25% of cases, typically as transient hepatic attenuation differences, probably due to arteriovenous shunting [69, 70]. Single-photon emission computed tomography (SPECT) with

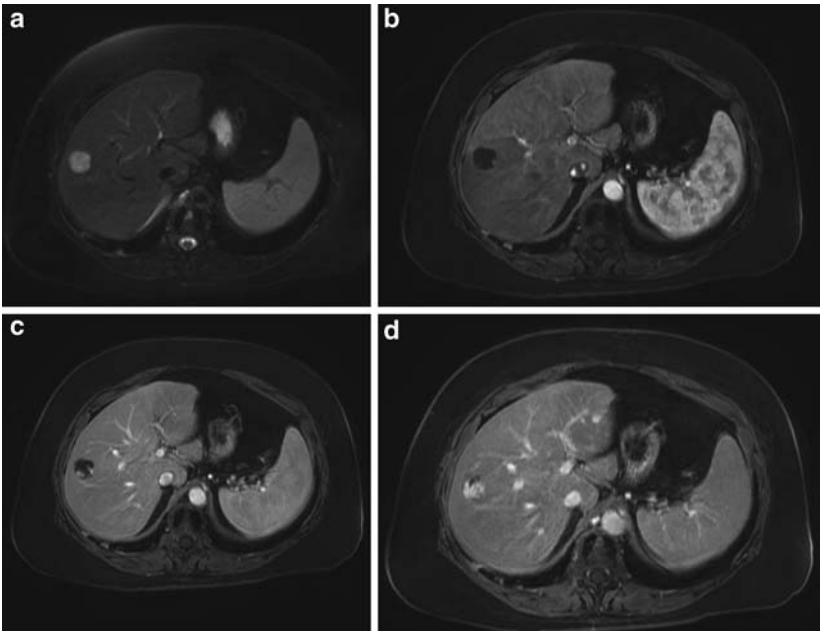

Fig. 18.3. Cavernous hemangioma (3 cm) in right hepatic lobe. (**a**) Fat-suppressed T2-weighted axial image of the liver without contrast demonstrates hyperintense lesion (indistinguishable from simple cyst). T1-weighted axial images after intravenous administration of gadolinium in the (**b**) arterial contrast phase shows initial puddle-like peripheral enhancement that becomes confluent in the (**c**) portal venous and (**d**) 5-minute delayed contrast phases, matching the enhancement behavior of the portal veins. Courtesy of Orpheus Kolokythas, MD.

technetium-labeled red blood cells has similar sensitivity and specificity to MRI only for lesions >3 cm and close to the surface; therefore, MR imaging is still the diagnostic study of choice [55].

Giant cavernous hemangiomas (>4 or >10 cm depending on the reference) may outgrow their blood supply, leading to central cystic degeneration, which does not enhance on CT and MR imaging [57]. These lesions are still identified by the peripheral nodular enhancement on contrast CT or MRI, which is typical for hemangiomas [71]. Rarely, there have been cases of multicystic cavernous hemangiomas, with the cystic change thought to be secondary to necrotic changes from infarction due to thrombosis or hemorrhage, presence of lymphangioma, or apoptosis [72–76]. For lesions that do not demonstrate classic enhancement features of hemangioma, a percutaneous biopsy of the lesion may be considered using a 20-gauge needle; the safety of this procedure, however, is still debated as there is a risk of severe bleeding.

Asymptomatic adult patients do not require any therapy, and stability of the lesion may be established on a 6-month follow-up imaging study. Patients with giant hemangiomas may need to be monitored more routinely but do not necessarily need treatment if they are asymptomatic. Lesions seen in infants and young children, however, demand greater vigilance as malignant tumors, such as hepatoblastomas, may mimic hepatic hemangiomas, so measurement of serum alpha-fetoprotein levels and serial imaging may be warranted [77, 78]. Spontaneous hemorrhage (intraparenchymal or intraperitoneal) is rare, even in large hemangiomas, but rupture has been known to occur. Complications are reported in 4.5–19.7% of large hemangiomas, including bleeding, compression of adjacent structures (e.g., biliary ducts and bowel), and torsion (pedunculated form) [79]. Infantile hepatic hemangiomas can be symptomatic with cardiac failure due to shunting, hypothyroidism, fulminant hepatic failure, and/or abdominal compartment syndrome [80]. Kasabach–Merritt syndrome occurs rarely in association with giant hemangiomas and presents with thrombocytopenia (sequestration and destruction of platelets in large hemangiomas), hypofibrinogenemia, and disseminated intravascular coagulopathy. It is occasionally seen in children but rarely in adults and may be treated by liver transplantation [81, 82]. Concurrent focal nodular hyperplasia has been reported in 20% of cases of hemangiomas, perhaps due to the disturbance of the vascular supply to a focal region [83, 84]. Treatment is indicated in symptomatic patients, those with complications, and in those in whom malignancy cannot be excluded. Treatment options for these large symptomatic cavernous hemangiomas include surgical enucleation, surgical resection, transarterial chemoembolization, radiation therapy, radiofrequency ablation, and liver transplantation [85].

Diffuse liver hemangiomatosis is rare in adults, but may be treated by radiofrequency ablation or liver transplantation [86].

PELIOSIS HEPATIS

Peliosis hepatis is defined as the presence of numerous blood-filled cysts in continuity with the hepatic sinusoids. These cysts may vary in size up to 5 mm in diameter and may occur in the liver, spleen, lungs, and bone marrow [87–89]. The condition was first recognized in association with wasting disorders, such as disseminated tuberculosis, but it is now recognized in association with HIV infection complicated by *Bartonella henselae* or *Bartonella quintana* infection [90], drugs (e.g., androgens [91], estrogen, tamoxifen, immunoglobulin, corticosteroids, danazol, azathioprine, and methotrexate), toxins (e.g., cadmium and arsenic), cystic fibrosis, bacterial sepsis, malignancies (e.g., hepatocellular carcinoma), hematologic disease (e.g., Hodgkin disease and multiple myeloma), and renal and cardiac transplantation. This condition may occur at any age and both genders are equally afflicted. Liver tests, including serum aminotransferases, alkaline phosphatase, bilirubin, and GGT levels, are usually normal or slightly elevated, so the lesions are often found incidentally or at autopsy. Symptomatic patients may present with jaundice, hepatomegaly, and/or fever. Thrombocytopenia can be present in cases attributed to *Bartonella* [92, 93].

The exact pathogenesis of peliosis hepatis is unknown. One hypothesis suggests that hepatocellular necrosis leads to disruption of the reticulin network and dilation of the sinusoids [94]. Another theory is that blockage of hepatic blood flow at the sinusoid–hepatic venule junction leads to sinusoidal congestion and dilation [95]. Others have proposed that there is a lesion at the sinusoidal barrier [96, 97]. For example, endotoxin may injure the sinusoidal lining, disrupting hepatic microcirculation, leading to hepatocellular injury and consumptive coagulopathy [98]. Infections and malignancies are thought to be associated with peliosis hepatis via the induction of angiogenic factors, including vascular endothelial growth factor [99–101].

There are two types of peliosis hepatis [102]. The “phlebatic” type is characterized by blood lakes lined by endothelium and communication with the central veins. They are typically found in the centrilobular region. In the “parenchymal” type, the blood lakes are not lined by endothelium and are found throughout the hepatic lobule. They can be associated with focal hepatocellular necrosis and calcifications. Liver biopsy by laparoscopic or transjugular approach may be helpful in establishing the diagnosis, but percutaneous liver biopsies should be avoided due to the risk of fatal intraperitoneal hemorrhage

[103]. In cases associated with *Bartonella* organisms, these bacteria are demonstrated upon staining of the liver tissue using the Warthin–Starry technique.

On ultrasonography, the lesions are hyperechoic in a normal liver and hypoechoic in a background of hepatic steatosis [104]. They appear as small hypodense lesions <1 cm on CT imaging, which remain hypodense after administration of contrast [105] or they may show slow contrast enhancement during the portal venous phase; hyperattenuating lesions, however, may be visible in the setting of hemorrhage. There is lack of mass effect on the hepatic vessels [88, 106]. The lesions have low T1 signal intensity and high T2 signal intensity on MR imaging, with delayed and slow centripetal enhancement after contrast administration. There is a case report of the lesions appearing with a "target sign" – central enhancement during the arterial phase and slow centrifugal enhancement during the later phases [88]. Notably, peliosis hepatis can also be found in a focal fashion within the liver, mimicking malignancy [107].

The condition may regress if the predisposing factor is withdrawn, or, if due to an infection, with appropriate antibiotic therapy (e.g., erythromycin or fluoroquinolone for *Bartonella*). It is typically harmless, but, rarely, liver failure, portal hypertension, cirrhosis, hemorrhage, and hepatic rupture have been reported to occur. Selective embolization of the hepatic artery or emergent laparotomy can be performed to treat intrahepatic or intraperitoneal bleeding [108, 109], and liver transplantation has been reported as treatment for cases complicated by cirrhosis and progressive liver failure [110]. Liver resection is contraindicated due to the risk of hemorrhage and the diffuse nature of the condition.

PERIBILIARY CYSTS

Peribiliary cysts are multiple small cystic dilations of the intrahepatic extramural peribiliary glands, which are found near the first to fourth branches of the intrahepatic ducts [111, 112]. They are located in the connective tissue of hepatic hilum and within the larger portal tracts. The cysts are typically unilocular and contain serous fluid. They are lined with a single layer of cuboidal/columnar epithelium, similar to bile duct epithelium. Cystography has shown that these cysts are isolated without communication with the bile ducts [113]. Necrosis and inflammation of the peribiliary glands can be seen in 23% of cases, suggesting intrahepatic circulatory disturbance. Rarely, they can cause obstructive jaundice and cholangitis [114–116], but, for the most part, they are found incidentally at surgery or autopsy. In fact, 50% of cirrhotic livers and 73% of polycystic livers may demonstrate cystic

dilation of the peribiliary glands, whereas the incidence is only 3% in normal livers [117, 118]. There is a potential genetic predisposition given the association with autosomal dominant polycystic kidney disease with liver involvement. They can also be seen following liver transplantation and may be accompanied by hepatolithiasis [119, 120].

On ultrasound examination, they appear as round or tubular anechoic areas or clustered small cysts around the large portal tracts. On CT imaging, there can be multiple small cysts <1 cm in diameter or larger discrete cysts >1 cm in diameter, scattered along the portal vein. On MR imaging, they appear as hypointense areas surrounding the portal vein on T1-weighted images and hyperintense areas on T2-weighted images. They also can be identified using MR cholangiography, appearing as a string of beads along the hepatic hilum or larger bile ducts [121]. Extrinsic compression of the bile ducts can be seen on ERCP or percutaneous transhepatic cholangiography.

These cysts may enlarge over time and increase in number [112, 122]. There has been one case in which these peribiliary cysts were associated with bile duct carcinoma [123]. Therefore, long-term observation seems warranted.

OTHER CYSTIC CONDITIONS OF THE LIVER AND BILIARY TRACT

Cystic Subtypes of Hepatic Neoplasms. Hepatic tumors of any type, including hepatocellular carcinoma, may compress small branches of the portal vein, leading to areas of infarction and the appearance of small cystic regions, which are called pseudoinfarcts of Zahn.

Cystic Metastases. Most hepatic metastases are solid but some may have partial or complete cystic characteristics. Rapidly growing hypervascular tumors, such as neuroendocrine tumors, sarcomas, melanomas, lung carcinomas, and breast carcinomas, may develop necrosis and cystic degeneration [124]. These lesions typically demonstrate enhancement of the peripheral viable tissue on CT or MR imaging with contrast. Cystic metastases also occur with mucinous cystadenomas, such as colorectal carcinomas and ovarian carcinomas [125, 126].

Primary Squamous Cell Carcinoma. There are several case reports of primary squamous cell carcinoma arising in the liver [127, 128]. These lesions are lined by stratified squamous epithelium. These patients tend to have a very poor prognosis.

Undifferentiated Embryonal Sarcoma. Undifferentiated embryonal sarcoma is a rare hepatic tumor which occurs in older children, adolescents, and young adults, with a mean age of 12 years. Although

over 80% are solid on gross examination, CT and MR imaging typically demonstrate a cystic appearance due to the high water content of the myxoid stroma [129]. They are typically large solitary lesions (10–25 cm in diameter), with well-defined borders and pseudocapsules [130, 131]. Some even have internal calcifications. Large areas of the lesion are hypointense on T1-weighted MR imaging and hyperintense on T2-weighted MR imaging. After administration of contrast, there is heterogeneous enhancement of the solid peripheral portions of the mass, especially on delayed imaging [129, 132].

Hepatic Abscess. Pyogenic, amebic, and fungal abscesses may develop within the liver [133]. Enteric bacteria may enter the liver via the portal venous system or the biliary tree, leading to the development of pyogenic abscesses. *Entamoeba histolytica* is the cause of amebic abscesses, which are the primary type of hepatic abscess worldwide. Fungal abscesses are less common, typically occurring in immunocompromised patients. Air inside the lesion would be indicative of gas-forming organisms. In the more acute stage, the abscesses may cluster as small low-attenuation or high signal intensity lesions. During the subacute phase of abscess development, the abscess may appear unilocular and cystic due to necrosis and liquefaction. They are typically thick walled with homogeneous low attenuation on CT, homogeneous low signal intensity on T1-weighted MR imaging, and high signal intensity on T2-weighted MR imaging, with an enhancing abscess wall and peripheral rim enhancement (peri-lesion edema) [134]. Appropriate drainage and antibiotic therapy is essential for treatment of these lesions.

Endometriosis. Endometriosis has been reported to be a rare cause of hepatic cysts, which have been treated by surgical resection [135, 136].

Post-traumatic hepatic cyst. Post-traumatic hepatic cysts have been recognized and a similar phenomenon occurs after hepatic artery embolization [137, 138].

Hepatic Extrapancreatic Pseudocyst. Rarely extrapancreatic pseudocysts may occur in the liver, predominantly in the left lobe of the liver due to the extension of fluid from the lesser sac [139]. On CT imaging, the lesion appears as a well-defined, subcapsular mass that is homogeneous and hypoattenuating and has a thin fibrous capsule. They may contain necrotic debris and even hemorrhage. On MR imaging, the pseudocyst lesion has low signal intensity on T1-weighted imaging and high signal intensity on T2-weighted imaging. The capsule enhances after administration of gadolinium in mature cysts [140].

Hematoma. Hemorrhage may occur in the liver due to trauma, surgery, or rupture of a neoplasm within the liver. In the acute phase, the fluid has high attenuation; however, in chronic cases, the

attenuation of the fluid may be identical to that of pure fluid [141]. On MR imaging, the hematoma appears as a heterogeneous mass with high signal intensity on T1-weighted images and intermediate signal intensity on T2-weighted images [142].

Biloma. Bilomas may occur due to spontaneous, traumatic, or iatrogenic disruption of the biliary tree. Pseudocapsular formation occurs due to the intense inflammatory response. It typically appears well defined on imaging.

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IV Complications

19 Vascular Complications of Fibrocystic Liver Disease

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CONTENTS

Summary

Vascular complications of fibrocystic liver disease can involve the hepatic or the extrahepatic vasculature. The hepatic vascular complications of fibrocystic liver disease involve the venous structures of the liver. Portal hypertension is common in congenital hepatic fibrosis, often manifesting as variceal bleeding. In autosomal dominant polycystic liver disease, enlarging hepatic cysts can obstruct the hepatic veins or the inferior vena cava, or both, resulting in the corresponding venous obstruction syndrome. Extrahepatic vascular complications of fibrocystic liver disease are related to abnormalities at the level of the arterial elastic lamina associated with aneurysm formation, most commonly at the level of the intracranial vessels.

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Rupture of intracranial aneurysms results in the devastating complication of subarachnoid hemorrhage. Autosomal dominant polycystic liver disease is also associated with cardiac valvular anomalies.

Key Words: Varices, Gastrointestinal bleeding, Biliary obstruction, Aneurysm

INTRODUCTION

Fibrocystic diseases of the liver are conditions related to ductal plate malformation which occur during embryonic development. In addition to biliary abnormalities, pathological features of fibrocystic liver diseases include the formation of multiple hepatic cysts of biliary origin; portal and periportal hepatic fibrosis; and dilatation of the large intrahepatic bile ducts in the absence of biliary obstruction (Caroli's disease). The term "Caroli's syndrome" is used when congenital hepatic fibrosis (CHF) is present along with biliary dilatation, that is, the combination of CHF and Caroli's disease. There is a considerable degree of overlap in the histological and clinical features of the different entities. For example, Caroli's disease may be associated with autosomal recessive polycystic kidney disease (ARPKD), CHF, both ARPKD and CHF, and, infrequently, autosomal dominant polycystic kidney disease (ADPKD) or autosomal dominant polycystic liver disease (ADPLD). Although ADPKD and ADPLD are genetically distinct diseases, the hepatic and extra-hepatic vascular abnormalities of polycystic disease associated with ADPKD and ADPKLD are very similar [1].

There are several hepatic and extrahepatic vascular abnormalities in the fibrocystic liver diseases (Table 19.1) [1, 2]. In fact, vascular

Table 19.1
Vascular complications associated with fibrocystic liver diseases

Portal hypertension:	CHF Caroli's syndrome Rarely with polycystic liver disease
Portal vein thrombosis:	CHF
Inferior vena caval obstruction:	Polycystic liver
Intracranial aneurysms:	ADPKLD ADPLD
Thoracic aortic aneurysms	ADPKLD

CHF: Congenital hepatic fibrosis; ADPKLD: Autosomal dominant polycystic kidney and liver disease; ADPLD: Autosomal dominant polycystic liver disease.

complications such as portal hypertension-related bleeding may be the major manifestation of CHF. The hepatic vascular complications are a direct consequence of the liver disease. For example, portal hypertension in CHF is secondary to portal fibrosis. Large cysts in ADPKD/ADPLD may compress the hepatic vein or inferior vena cava and cause hepatic venous outflow obstruction. Rarely, portal hypertension may be seen in polycystic liver disease secondary to pressure on portal venous radicles, but is more likely due to associated portal vein thrombosis. Extrahepatic vascular complications, on the other hand, may be due to altered expression or function of the PKD gene in abnormal smooth muscle cells and myofibroblasts [3]. These abnormalities result in disruption of the elastic lamina. Thus, cerebral aneurysms, aneurysms of the thoracic aorta, and cardiac valvular abnormalities may be associated with ADPKD/ADPLD.

HEPATIC VASCULAR COMPLICATIONS

The hepatic vascular complications of the fibrocystic liver diseases include portal hypertension and hepatic venous outflow tract obstruction.

Portal Hypertension

CHF may present with variceal bleeding, typically in the second decade of life or later. Other manifestations of CHF include cholangitis or a combination of variceal bleeding and cholangitis in Caroli's syndrome. In some patients, CHF may be completely asymptomatic.

The pathogenesis of portal hypertension in CHF has not been clearly defined. Potential mechanisms contributing to portal hypertension in CHF include portal fibrosis, portal vein thrombosis or, rarely, compression of the portal venous system by large cysts. Though periportal fibrotic bands compressing the intrahepatic portal vein branches are the major cause of the portal hypertension, portal vein anomalies and portal vein thrombosis (Fig. 19.1) may also be seen in association with CHF [4, 5]. Thus, the portal hypertension is largely presinusoidal.

In cohorts with ARPKD, portal hypertension secondary to CHF may be seen in 15–50% of patients and is more frequent as children get older [6–8]. Of note, CHF has been reported to present with complications of portal hypertension as late as the seventh decade of life [9].

The clinical manifestations of portal hypertension in CHF include asymptomatic splenomegaly, hypersplenism, and variceal bleeding. Because hepatic synthetic function is usually excellent in patients with

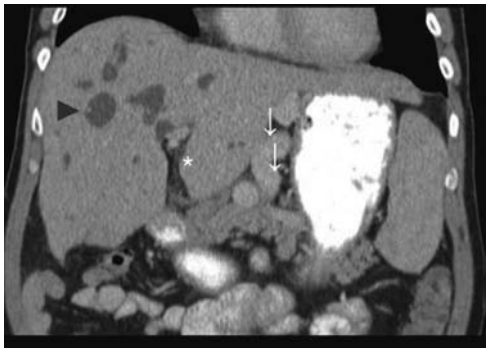

Fig. 19.1. CT scan coronal section, portal venous phase in patient with Caroli's syndrome with portal vein thrombosis. Note the portal vein radicle in association with dilated bile ducts (*black arrow head*); abdominal varices (*white arrows*); and replacement of portal vein by collaterals (*asterisk*).

CHF, and the portal hypertension is presinusoidal, ascites is uncommon. In fact, among 44% of children in a cohort of 164 children with ARPKD who developed portal hypertension, only 2% had ascites [6]. Hepatopulmonary syndrome may also be a vascular complication of CHF and is reversed by liver transplantation [10].

There are no data on the best management strategies for prevention of variceal bleeding, control of variceal bleeding, and prevention of variceal re-bleeding in patients with CHF due to the rarity of this condition. Unfortunately, there are also very limited data on management of variceal bleeding in children, in general. Therefore, recommendations regarding treatment of variceal bleeding in CHF have to be based on recommendations for management of variceal bleeding in adults with cirrhosis, except that the threshold for recommending surgical portosystemic shunts is lower in patients with CHF.

Primary prophylaxis to prevent the first variceal hemorrhage may be carried out using either endoscopic variceal ligation or non-selective beta-blockers, such as nadolol or propranolol. In adults, the use of prophylactic endoscopic variceal ligation is associated with a lower risk of variceal bleeding and bleed-related mortality than the use of beta-blockers, but overall survival is probably equivalent between the two groups. Complications of treatment are more frequent in patients on beta-blockers, but more severe (ligation-related ulceration and esophageal strictures) in patients who undergo endoscopic variceal ligation. The use of beta-blockers is probably more cost effective. However, the choice of treatment in the individual patient is best decided upon after discussion with the patient and the family [11, 12].

As in adults, the control of variceal bleeding should be carried out using a combination of a safe vasoactive agent (octreotide in the United States, even though data supporting the efficacy of octreotide are limited), endoscopic variceal ligation, and antibiotic treatment. The use of antibiotics is associated with fewer infections, a lower rate of re-bleeding and, most important of all, decreased mortality [13, 14]. Norfloxacin by mouth, or ciprofloxacin intravenously, is used in adults. Parenteral ceftriaxone is also an excellent choice [15]. In patients in whom variceal bleeding cannot be controlled in spite of two sessions of endoscopic treatment, a portosystemic shunt is recommended. If the procedure can be carried out semi-electively, a portosystemic shunt created surgically is preferable in this setting in view of the longevity of the shunt. There are limited long-term data on the use of TIPS (transjugular intrahepatic portosystemic shunts) in children with CHF.

To prevent the recurrence of variceal bleeding, a combination of endoscopic variceal ligation and non-selective beta-blockers is recommended [16]. Nadolol has theoretical benefits over propranolol in that it is longer acting, is excreted predominantly via the kidney, and is less lipid soluble and, therefore, associated with fewer central nervous system side effects. If variceal bleeding recurs in spite of a combination of endoscopic and pharmacological treatment, then a surgical portosystemic shunt would be recommended. A selective portosystemic shunt such as a distal splenorenal shunt is likely to be associated with a lower risk of hepatic encephalopathy and learning disability than is a less selective shunt such as a mesocaval shunt or a portocaval shunt [17].

Hepatic Venous Outflow Obstruction

The hepatic venous outflow tract comprises of the right, middle, and left hepatic vein which drain into the inferior vena cava. The right and middle hepatic vein usually join together and drain into the inferior vena cava adjacent to but separate from the left hepatic vein. Obstruction of the hepatic venous outflow tract, either at the level of the hepatic veins or the suprahepatic inferior vena cava, results in elevation in hepatic sinusoidal pressure. This results in ascites, as well as some worsening of hepatic parenchymal function. Hepatic venous outflow tract obstruction should be suspected in any patient with polycystic liver disease who has worsening liver function associated with ascites. Ultrasound examination is often not helpful in making the diagnosis because of the difficulty in visualizing the hepatic veins secondary to the large number of hepatic cysts. Magnetic resonance imaging is the non-invasive imaging modality of choice in patients without advanced renal insufficiency, but hepatic venography with pressure measurements across the hepatic

veins and inferior vena cava is essential in confirming the diagnosis of hepatic venous outflow tract obstruction. Treatment of the ascites may be carried out with sodium restriction and a combination of diuretics such as spironolactone and furosemide. However, mobilization of ascitic fluid is usually difficult as a result of the associated renal insufficiency. Primary treatment, therefore, is aimed at relieving the hepatic venous outflow obstruction. The simplest way of achieving this is by cyst aspiration and alcohol sclerosis, or surgically by fenestration of the cysts with or without hepatic resection, assuming occluding vascular thrombosis has not occurred [18]. An inferior vena caval stent is feasible in these patients, but TIPS is technically not possible because of extensive involvement of the liver by hepatic cysts. Liver transplantation is often the treatment of choice for hepatic venous outflow obstruction occurring in the presence of polycystic liver disease.

Inferior vena caval obstruction may occur as a consequence of its compression by hepatic cysts. A portion of the inferior vena cava runs through the liver (intrahepatic IVC) and is circumferentially surrounded by the liver except on its posterior aspect. Compression of the inferior vena cava by large hepatic cysts can result in inferior vena caval syndrome with lower extremity edema, and abdominal wall edema typically below the diaphragm. When the suprahepatic inferior vena cava is obstructed, the clinical features are of both inferior vena caval obstruction and hepatic venous outflow tract obstruction, including ascites. Treatment of inferior vena caval obstruction can again be carried out by fenestration of the hepatic cysts or hepatic resection. Alcohol ablation of cysts in close proximity of the inferior vena cava should be avoided because of the risk of alcohol leakage into the tissues surrounding the inferior vena cava which can result in back pain and inferior vena caval fibrosis. An endovascular stent can also be placed across the inferior vena caval obstruction [19].

EXTRAHEPATIC VASCULAR COMPLICATIONS OF FIBROCYSTIC LIVER DISEASE

The extrahepatic vascular complications of fibrous polycystic disease of the liver are related to abnormalities of the elastic lamina in the vessels or in the mitral valve. These associations are noted both in isolated ADPLD, as well as in autosomal dominant polycystic kidney and liver disease (ADPKLD). On the other hand, such abnormalities are not common in patients with ARPKD and CHF.

The majority of patients with ADPKD have a mutation of either the PKD1 (chromosome 16) or PDK2 (chromosome 4) genes. The gene products, polycystin 1 and polycystin 2, are involved in regulation of

vascular development in the kidney and other organs, including the heart. Both PKD1 and PKD2 are also expressed in human vascular smooth muscle in normal arteries and in vascular smooth muscle in intracranial aneurysms [2, 20].

Intracranial Aneurysms

The most common vascular abnormality of ADPKD is the formation of intracranial saccular aneurysms. Brain aneurysms occur in approximately 8% of patients [21]. Rupture of these aneurysms may be associated with a mortality rate of up to 50% at 30 days and a 25% rate of substantial long-term disability in those who survive. Asymptomatic intracranial aneurysms in patients with ADPKD are four to five times more frequent than in the general population, which has a prevalence of intracranial aneurysms of only about 2%. Saccular aneurysms are more frequent in the anterior circulation. Intracranial aneurysms from ADPKD differ from those which occur sporadically in the general population in that they are larger in size, occur at a younger age, have a familial tendency, may be multiple, and tend to involve vessels of the middle cerebral artery territory rather than the internal carotid artery [22]. The risk factors for intracranial aneurysm rupture include location in the posterior cerebral circulation (relative risk 4.1–13.6), the presence of a prior history of subarachnoid hemorrhage (relative risk 1.7–8.3), and aneurysm size <7 mm (<0.7% risk of rupture per year) or >7 mm (0.7–4.0% risk of rupture per year) diameter. Smoking, hypertension, older age, and female gender are also risk factors for rupture of the intracranial aneurysms [23, 24].

Screening of patients with polycystic kidney and liver disease for intracranial aneurysms has been proposed. Unfortunately, such screening is expensive and, even when aneurysms are identified, treatment of these aneurysms may be associated with significant mortality and morbidity. Treatment options for the intracranial aneurysms include surgical clipping or angiographic coil embolization. Surgical clipping of aneurysms is associated with a mortality of 2.3% and an approximately 10% risk of significant disability with risk of cognitive dysfunction. The risk of mortality and morbidity is higher in patients over the age of 45 years. Coil embolization of aneurysms in the posterior circulation also carries a similar risk of mortality and permanent complications, and the long-term outcome is not as satisfactory as with surgery. Therefore, it is difficult to conclude with a high degree of confidence that screening for intracranial aneurysm should be carried out in all patients with ADPKLD. The benefit of screening may be best

restricted to patients younger than 45 years of age; screening is usually started at age 20 years and repeated every 5 years [25].

Nonetheless, screening for intracranial aneurysm would be recommended in a patient with a history of subarachnoid hemorrhage or a family history of intracranial aneurysm or subarachnoid hemorrhage or stroke; in patients who request screening to allay their anxiety and in patients whose occupation is such that subarachnoid hemorrhage would pose a great deal of risk to them and those around them, such as airline pilot or truck drivers; and those with headache or neurological symptoms. If aneurysms are detected, treatment would be recommended for aneurysms >7 mm in diameter. Other aneurysms may be followed with serial imaging every 6–12 months. When screening is carried out, magnetic resonance imaging is preferred, except in patients who have renal insufficiency because of the concern for gadolinium-related fibrosing dermopathy.

Other Aneurysms

Whereas the formation of intracranial saccular aneurysms is the most common extrahepatic vascular complication of fibrocystic disease of the liver, other types of aneurysms may occur. Intracranial arterial dolichoectasia (IAD) is an elongated fusiform aneurysm of the cerebral circulation. This type of aneurysmal dilation is rare in the general population and is associated with connective tissue diseases and ADPKD; the prevalence of IAD in ADPKD is around 2%. Unlike with saccular intracranial aneurysms, rupture is rare in IAD, but an ischemic stroke may result from dissection or due to its mass effect. Prophylactic intervention is not indicated in patients with asymptomatic IAD [26].

Coronary aneurysms and aortic aneurysms, both thoracic and abdominal, with the fatal complications of dissection and rupture, have also been described in association with ADPKD [27, 28]. However, the degree to which the vasculopathy of ADPKD contributes to the pathogenesis of extracranial aneurysms is not very clear, because of the frequent association with hypertension and atherosclerosis, which are confounding risk factors often present as a complication of the chronic renal disease of ADPKD.

Cardiac Abnormalities

Polycystin-1 and polycystin-2 are both expressed in cardiac myocytes and cardiac valvular myofibroblasts [29]. Echocardiographic abnormalities, including left ventricular hypertrophy, aortic regurgitation, mitral regurgitation, and mitral valve prolapse, are frequent in patients with polycystic liver disease [30]. Since many of these findings are also

common in hypertension, whether these changes represent a primary association of ADPKLD or findings secondary to hypertension has been subject to debate. It appears that diastolic dysfunction, associated with left ventricular hypertrophy, is present even in young normotensive patients, which argues for a primary association as opposed to a secondary finding [31]. Similarly, mitral valve prolapse, but not valvular regurgitation, appears to be a primary association of ADPKLD [30].

CONCLUSION

The vascular complications of the fibrocystic diseases of the liver occur either as a direct consequence of the liver disease or because of its extrahepatic associations. These complications contribute substantially to the morbidity and mortality from fibrocystic liver diseases and warrant special attention.

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20 Biliary Cystadenoma and Cystadenocarcinoma

Paolo Del Poggio, MD, Ezio Pezzica, MD, and Daniela Corti, MD

CONTENTS

Summary

Biliary cystadenoma (BCA) and its malignant counterpart biliary cystadenocarcinoma (BCAC) are rare cystic tumors originating from the biliary system. BCA appears as a multilocular intrahepatic cyst lined by benign cuboidal or columnar non-ciliated epithelium. If the epithelium shows neoplastic degeneration it is defined as BCAC. According to the presence or absence of ovarian-like mesenchymal stroma, BCA is classified into two subtypes with different prognosis. BCA is a tumor with a propensity for recurrence or malignant degeneration and should always be completely resected. Partial interventions like marsupialization or cyst drainage are not considered adequate. The diagnosis is based on radiologic imaging and the role

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of cytology and histology is limited to doubtful cases, the most frequent being the differentiation from hemorrhagic cysts. In these cases fine needle aspiration (FNA) may be used to confirm BCA by the identification of atypical biliary cells and elevated intracystic CA19-9 levels. FNA, however, lacks adequate sensitivity and specificity and should always be used in conjunction with imaging. Due to the slow growth of these tumors, the prognosis after adequate resection is good and prolonged survival has been unanimously reported in the literature. Even in the case of BCAC, long-term survival can be achieved, provided that the tumor is discovered and resected without distant foci or metastasis.

Key Words: Cystadenoma, Cystadenocarcinoma, Biliary cysts

INTRODUCTION

Biliary cystadenoma (BCA) and its malignant counterpart biliary cystadenocarcinoma (BCAC) are rare cystic tumors originating from the biliary system and accounting for less than 5% of all cystic neoplasms of the liver [1–3].

Willes first reported on a case of adenocarcinoma arising in a seemingly benign liver cyst in 1943 [4], but it was Ishak in 1977 who first identified BCA and BCAC as specific entities. He described eight patients with BCA and six with BCAC and discussed the main characteristics of these lesions that were later confirmed in other series in the literature [1, 3, 5]. These characteristics include the prevalence of BCA in middle-aged women, the likely derivation of BCAC from BCA, and its good prognosis following resection. The largest series to date of this rare tumor is that of Devaney who reported on 52 BCA and 18 BCAC [1].

It is not possible to determine the true incidence of BCA because of its rarity, although it is estimated to be in the range of 1 in 20,000–100,000 individuals [6]. BCAC is much rarer with an estimated incidence of 1 in 10 million [6]. The low incidence of this tumor is in contrast with the more common benign liver cysts, which can be demonstrated in about 3% of the general population. BCA thus represents a rare subtype of hepatic cystic disease, being 1,000 times less prevalent than simple liver cysts, which are by far the most frequent cystic lesions observed in the liver [7]. BCA can be classified by its location: 85% of these lesions are intrahepatic [8, 9]. The remainder (15%) arise in the extrahepatic biliary ducts or more rarely from the gallbladder wall [10], causing confusion with intrahepatic biliary papilloma [11]. There is no consensus in the literature regarding the location

of intrahepatic BCA: some authors describing a predominantly right lobe location [2–6] and others reporting a more frequent involvement of the left lobe [3, 12].

BCA shows a strong female predominance – approximately 90% of the patients are women [13, 14], while BCAC is equally distributed between genders [2–12]. These tumors have been described at any age, but presentation during middle age is the most common [2, 13]. Their occurrence in the younger age group is, however, exceptional and only one case has been described in a 20-year-old patient [15].

PATHOLOGY

Biliary cystadenoma (BCA) was originally defined by Wheeler and Edmondson as a multilocular intrahepatic cyst lined by benign cuboidal or columnar non-ciliated epithelium [3]. The World Health Organization more recently classified biliary cyst tumors as BCA if the biliary epithelium lining the cyst was benign and as biliary cystadenocarcinoma (BCAC) if neoplastic changes were present [16, 17].

Macroscopic Appearance

Grossly, the tumor is globular with a bosselated surface through which stretched vessels course in different directions (Fig. 20.1). The tumors usually are large and almost always multilocular, with a mean diameter of 15 cm, but they occasionally can attain size up to 28 cm. Macroscopic sections reveal multiple communicating locules with a smooth or trabeculated internal surface. Biliary cyst tumors are only rarely unilocular and multilocularity is the key feature distinguishing cystadenoma from benign biliary cysts [18, 19]. The locules contain a clear to turbid fluid which may be mucinous or serous. Purulent material is rarely present and only seen if the tumor becomes infected. Bloody fluid is more often observed in cysts undergoing malignant change. Large polypoid or dense masses in the wall or papillary excrescences usually indicate zones of malignant transformation [1, 6, 20].

Microscopic Appearance

These tumors are histologically heterogeneous with regard to tumor cell types and stroma. Microscopically, cystadenomas are usually mucinous but a serous variety has been recognized. The tumor wall consists of three layers: (1) a cystic layer of biliary-type epithelium, (2) a moderately to densely cellular stroma, and (3) a dense outer rim of collagenous connective tissue [6, 21]. In men, the underlying fibrous

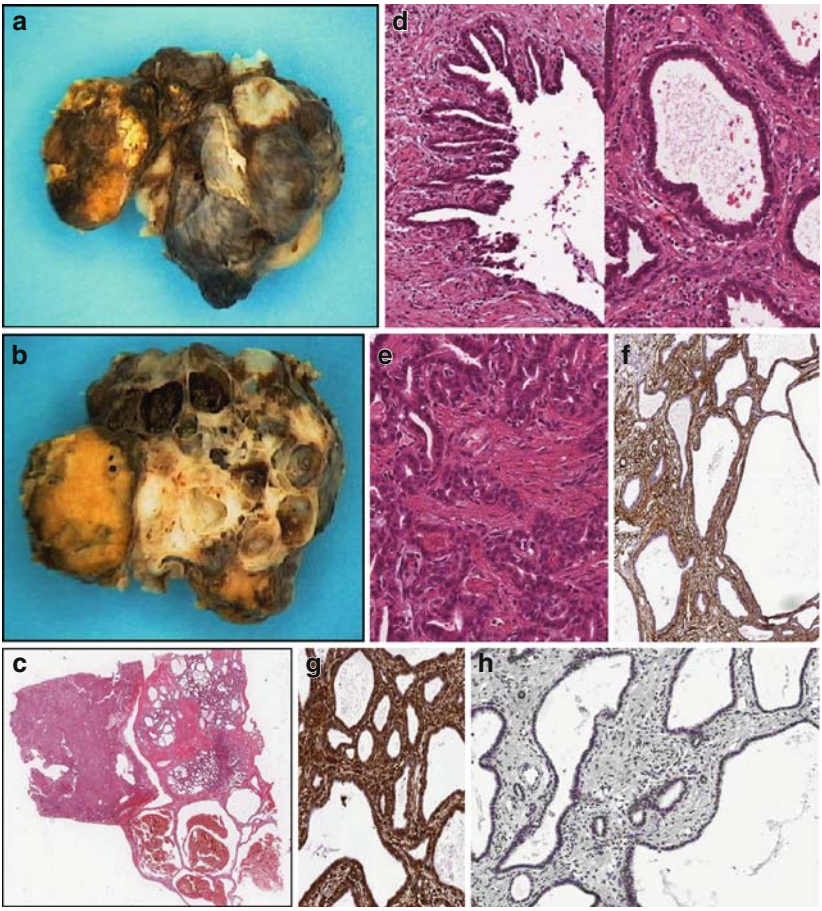

Fig. 20.1. (**a**) Liver resection specimen of segments II and III showing a multiloculated cystadenoma with globular shape and smooth surface. (**b**) The tumor is characterized by multiple locules of varying dimension containing coagulated hemorrhagic fluid. The presence of solid areas on macroscopic examination is the best clue of malignant change. (**c**) The histologic examination of the same tumor (Haematoxylin–eosin stain, low magnification) shows a typical biliary cystadenoma with hemorrhagic areas. (**d**) The columnar epithelium is supported by dense fibrous tissue, without mesenchymal stroma (**e**) The epithelial lining shows a focal invasion of the underlying stroma by atypical proliferating glandular cells. This finding is consistent with biliary cystadenoma undergoing initial malignant degeneration. Immunohistochemistry shows a strong cytoplasmic staining of the stromal cells by SMA (smooth muscle actin) (**f**) and Vimentin (**g**). No staining for estrogen receptors is identifiable (**h**), confirming the absence of ovarian-like mesenchymal stroma.

stroma is often hyalinized whereas in women it is densely cellular with spindle-shaped cells resembling the ovarian stroma: a variant called cystadenoma with ovarian-like mesenchymal stroma [2]. On light microscopy BCA is lined by a single layer of flattened or cuboidal non-ciliated mucin-producing cells, similar to those of the intrahepatic bile ducts. The cells contain mucin, which is easily demonstrable by special stain [22]. The nuclei are poorly stained and basally located, the cytoplasm is pale and eosinophilic. Occasionally, small papillary structures or invaginations of the epithelial layer can be observed within the lesion, while in other portions of the cyst the epithelium may be focally flattened or even absent, exposing the underlying stroma [1, 16]. An endocrine cell component can be demonstrated in about one-third of the cases [23] and oncocytic differentiation of the epithelial layer has been described [24, 25]. Inflammatory infiltration by both acute and chronic inflammatory cells may be noted within the cyst wall. In some cases the wall can be thickened by xanthogranulomatous inflammation and focal calcium deposition may occur [1]. However, none of these features has prognostic significance regarding malignant transformation or propensity for recurrence after surgery.

Cystadenoma with mesenchymal ovarian-like stroma has been exclusively described in women, while the variant with hyaline stroma is equally distributed between genders and more frequently observed in the elderly [1, 6, 16]. Both lesions are associated with neoplastic transformation and are truly precancerous, but carcinomas arising in BCA with mesenchymal stroma seem to have a more indolent course. On the contrary carcinomas arising in BCA with hyaline stroma are more aggressive and they are more likely to progress to cholangiocarcinoma. The mesenchymal stroma is very cellular and similar to the ovarian stroma, but it has also some resemblance with the developing mesenchymal tissues of the fetal gallbladder [6, 26].

Malignant transformation is a recognized complication of mucinous cystadenoma. In the largest series around 10% of cases showed signs of dysplasia or borderline malignancy. On the contrary malignant transformation has never been reported in the rare variant of serous cystadenoma [1]. When malignant transformation occurs it is usually of papillary or tubulo-papillary type. Invasion of the underlying stroma must be clearly documented to establish a definite diagnosis of malignancy. This is sometimes difficult to demonstrate, because benign and malignant areas often coexist. The need for careful pathologic evaluation is here emphasized since malignant transformation may be focal and can be detected only after thorough sampling of the surgical specimen. Foci of epithelial atypia, loss of polarity, and mitotic activity indicate dysplastic premalignant changes and are not by

themselves a sign of malignancy. The demonstration of intestinal metaplasia with goblet and Paneth cells may precede dysplasia, although the full intestinal metaplasia–dysplasia–carcinoma sequence has been documented only in few cases [26]. Severe architectural atypia, exophytic papilla, and grossly anaplastic changes with cellular pleomorphism are additional features of frank malignant transformation [1, 27–29]. The malignant epithelial cells have the typical features of adenocarcinoma and only in very rare cases acquire a spindle-shaped pseudosarcomatous appearance [30].

Immunohistochemistry

The epithelial lining of the cystadenoma shows strong and diffuse cytoplasmic staining with antibodies to cytokeratins CK7, CK8, CK18, and CK19, typical of biliary epithelium. The spindle cells in the stroma show strong diffuse cytoplasmic staining with Vimentin and alfa-smooth muscle actin (SMA), which is specific for actin present in smooth muscle cells, myofibroblasts, and myoepithelial cells [31]. The ovarian-like stroma usually has positive immunostaining for estrogen receptors (ER), progesterone receptors (PgR), and inhibin-alfa receptors. The presence of these receptors suggests that the growth of BCA with ovarian stroma may be hormonally regulated and also explains its exclusive occurrence in women [32, 33].

New Proposed Classification

Recent advances in imaging modalities have revealed a direct luminal communication with the bile duct in some cases of these tumors [34] similarly to what occurs in cystic tumors of the pancreas, which may communicate with the pancreatic duct. In the pancreas two types of cystic neoplasms have been described [35]. The first is mucinous cystic neoplasm with ovarian-like stroma that usually occurs in women and does not have a luminal communication with the pancreatic duct and the second is intraductal papillary mucinous neoplasm (IPMN) which has equal gender distribution and communicates with the pancreatic ductal system. Recently Zen and colleagues [17] proposed a new classification of BCA and BCAC, speculating that biliary cystic tumor with ductal communication and biliary cystic tumors with ovarian-like stroma might correspond to IPMN and mucinous cystic neoplasms of the pancreas, respectively. According to these authors the terms BCA and BCAC should be restricted to cystic neoplasms with ovarian-like stroma, not communicating with the bile ducts. If the tumor communicates with the bile ducts it should be defined as IPMN, as in the case

of the pancreas and classified into four types according to the histological characteristics of the neoplastic cells: (1) the pancreatic biliary type, (2) the intestinal type, (3) the gastric type, and (4) the oncocytic type [17]. MUCs (mucins) are used to distinguish these four subtypes of IPMN: MUC1 is usually detected in pancreaticobiliary type and in oncocytic type (albeit focally). MUC2 is considered to be a marker of intestinal differentiation and is strongly expressed in the intestinal type. MUC5AC is, on the contrary, less useful, being detected in all types of IPMN [17]. In support of this new classification it is interesting to note that in the series of Devaney [1] and Ishak [2] most cases of benign BCA had an ovarian-like stroma and no communication with the biliary system, while most cases of BCA undergoing malignant transformation were actually devoid of ovarian-like stroma and communicated with the bile ducts. It seems therefore plausible that at least some of the cystadenocarcinomas without ovarian stroma could be a hepatic variant of IPMN [35].

ETIOLOGY

Two hypotheses have been proposed to explain the histogenesis of BCA: either a congenital or an acquired mechanism. According to the former, the presence of mesenchymal stroma similar to the primitive mesenchyme of the embryonic gallbladder and large bile ducts suggests that this tumor may arise from ectopic embryonal tissue destined to form the gallbladder or main ducts [36]. Alternatively, it may originate from a primitive hepatobiliary endodermal stem cell [37] or from intrahepatic peribiliary glands. This is supported by the finding that about 50% of BCAs harbor endocrine cells which are located within the epithelial lining of these glands [38]. Moreover, hamartomatous nests can be found in some BCA, and this adds credibility to the hypothesis of a congenital origin [2, 39]. Despite the presence of ovarian-like mesenchymal stroma, a derivation from ectopic ovarian tissue is highly unlikely since heterotopia of the ovary is restricted to the pelvis and has never been observed within the liver [3].

The second hypothesis, that tumor formation is an acquired reactive process secondary to some type of injury, has been proposed by Ishak [2], but is supported only by a single and outdated experimental study with aflatoxin [40]. If external stimuli are an unlikely cause of this tumor, they may possibly act as promoters, at least in the case of BCA with ovarian stroma. Immunohistochemical studies have in fact revealed the strong expression of progesterone and estrogen receptors in the mesenchymal stromal cells [41], and there are also reports of BCA arising in oral contraceptive users. This suggests that sex hormones may

act as tumor promoters [42] through stimulation of the mesenchymal stromal cells.

BCAC is commonly thought to be derived form BCA, since focal areas of severe dysplasia and even frank cystadenocarcinoma have been demonstrated in apparently benign BCA [3, 43, 44]. Malignant degeneration of BCA with mesenchymal stroma has also been reported after several years of follow-up, further strengthening the theory of a derivation of BCAC from BCA [45]. There are however sporadic cases of de novo BCAC, not originating form pre-existent BCA: these cases seem to be more frequent in men and have never been associated with mesenchymal stroma [1, 46].

CLINICAL PRESENTATION

The clinical presentations of BCA and BCAC vary widely. Some patients are asymptomatic with the tumor incidentally discovered during radiographic imaging or at surgical exploration for concomitant disease [26, 47]. The majority of the patients however present with right upper quadrant pain or discomfort, due to the large dimension that these tumors may achieve [26]. In one series about 60% of patients complained of right upper quadrant or epigastric pain and many of them noted increasing abdominal girth [48] while in another series 74% had abdominal pain, 26% abdominal distension, and 11% nausea or vomiting [49]. On physical examination it is sometimes possible to palpate an upper abdominal mass with variable stiffness and consistency [26].

Unusual presentations are less frequent and are related to complications. They include jaundice, cholangitis, intracystic hemorrhage, intraperitoneal rupture, and caval or portal compression with ascites or peripheral edema [6, 26]. Jaundice may be caused by the enlarging tumor itself [48] or by the secretion of mucin into the biliary tract through a communication between the biliary system and BCA [50]. Obstructive symptoms are more frequent in cases of extrahepatic BCA and are rarely observed with intrahepatic BCA, unless the tumor reaches very large dimensions. Extrahepatic BCA is more difficult to diagnose on imaging: at times intermittent jaundice and recurrent episodes of cholangitis may be the presenting symptoms of an extrahepatic BCA and occur a long time before the correct diagnosis is made [51].

Laboratory tests are usually normal in uncomplicated BCA, but mild elevation of alkaline phosphatase and bilirubin may be noted. If alkaline phosphatase and bilirubin are high or progressively increasing, a complication should be suspected. Serum carbohydrate antigen CA19-9 may be normal or elevated and does not aid in the diagnosis, while serum carbohydrate embryonic antigen (CEA) and alfa-fetoprotein

(AFP) are always within the normal range [52]. Although useless as a diagnostic tool, an elevated preoperative serum CA19-9 may be a valuable prognostic marker after resection, since it has been reported to completely normalize after radical surgery [53, 54]. It is also interesting to note that histological immunoreactivity for CA19-9 is lost when BCA transforms into BCAC; therefore, serum CA19-9 has no role in monitoring BCA for malignant transformation [55].

DIAGNOSIS

The diagnosis of BCA and BCAC relies heavily on radiologic imaging, and the role of cytology and histology is limited to doubtful cases. The typical appearance of BCA on ultrasound (US), computerized tomography (CT), and magnetic resonance imaging (MRI) is that of a septated multilocular cyst [5]. US and CT scan disclose a well-demarcated, thick-walled, anechoic, or hypodense round cystic mass with internal septa (Fig. 20.2A), which are better demonstrated by US than by CT

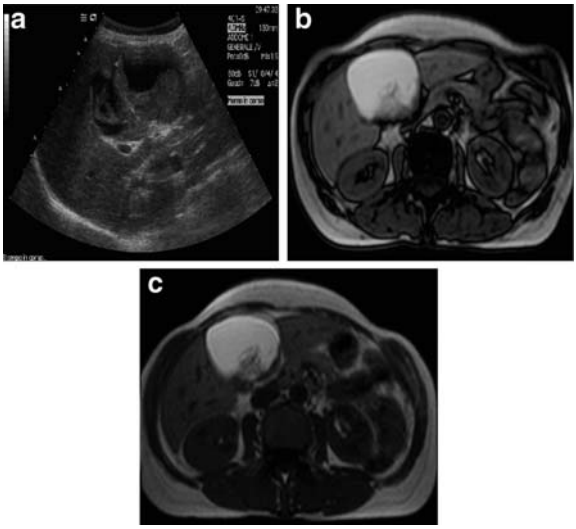

Fig. 20.2. (**a**) Ultrasound examination showing a 4-cm cystic lesion of segment 5 of the liver with a broad thick septum in the middle. Out-of-phase T1-weighted (**b**) and T2-weighted resonance imaging (**c**) confirmed the cystic nature of the lesion with the thick septum appearing hypointense in both acquisitions. Ultrasound-guided aspiration of the cyst was performed giving yield to thick mucinous fluid with scanty well-differentiated epithelial cells and elevated intracystic CA19-9 levels (5,739 U/ml). The lesion was resected, and in the surgical specimen a biliary cystadenoma was found with a focus of cystadenocarcinoma within the septum.

[5, 56]. The role of US and CT scan is considered complementary with no clear advantage of CT over US [56]. MRI confirms the multilocularity of the cystic tumor (Fig. 20.2B,C). It shows variable intensity on T1-weighted images and strong hyperintensity on T2-weighted images, due to the fluid contained in the cyst, irrespective whether it is mucinous or serous [57]. MRI does not give any specific information on the presence of pseudovarian mesenchymal stroma, but has unique value in detecting intracystic bleeding. In this case a fluid–fluid level can be observed within the cyst with the typical T1 hyperintensity in its lower portion, characteristic for the presence of hemoglobin [57]. Another advantage of MRI is the possibility to perform at the same time magnetic resonance cholangio-pancreatography (MRCP) in order to exclude a communication of the tumor with the bile ducts. For these reasons, when a suspected BCA or a complex cyst is evidenced on US, MRI is now supplanting CT scan as the second diagnostic step.

Imaging cannot reliably differentiate BCA from BCAC, although some characteristics are more peculiar to the latter. The presence of papillary infoldings, mural nodules, and pedunculated projections are more frequent in BCAC, and among these signs the identification of mural nodules seems to be the most predictive of malignant degeneration [57]. Intravenous contrast on CT and MRI enhances the cyst wall and internal septa in virtually all cases of BCAC, but also in a proportion of BCA cases varying from 15 to 100% [16, 57], which does not help much in the diagnosis. However, the identification of strong Doppler signals within the septations of BCA can strongly raise the suspicion of malignancy [44]. Fine punctuate calcifications and internal bleeding may be present in both tumors, but thick and coarse calcifications are more suggestive of BCAC [1, 57]. Local dilatation of intrahepatic bile ducts may also be detected around the tumor due to compression of the peripheral bile ducts [58] but it is not by itself a sign of malignant degeneration or of communication with the biliary system. As mentioned, it is advisable to perform an MRCP in all cases of suspected BCA, in order to exclude such a communication, which may change the therapeutic approach. It should be noted, however, that connections with the biliary ducts cannot be demonstrated in the majority of BCA, either at imaging or at the time of surgery [53]. Moreover, according to the new classification, the communicating variant of BCA probably represents an intraductal papillary neoplasm with cystic dilatation rather than a true BCA [17].

If it is practically impossible to rule out a malignant transformation of BCA before surgery, every effort should be made to differentiate it from biliary or echinococcal cysts and other benign tumors with similar radiographic appearance. In these cases fine needle aspiration (FNA)

may be useful and reasonably safe, the risk of pleural or peritoneal seeding being very low [60]. Tumor sampling may be problematic with FNA, however, because of poor cellularity of the smears or because of the predominant fluid component of the mass. Additionally, FNA is unreliable to rule out BCAC, since small neoplastic foci may go undetected [44]. For this reason, FNA is not considered part of routine practice in managing cystic liver lesions and should be considered on a case by case basis [16]. If atypical biliary cells are not easily demonstrated in FNA smears, the quantification of intracystic CEA and CA19-9 may be useful to rule out a benign liver cyst, which is characterized by very low levels of these markers [61, 62]. Particularly, the detection of elevated intracystic CA19-9 levels has been reported in only one case of benign liver cyst [63]. The role of cyst fluid analysis has been recently studied by Koffron in 22 patients with BCA and in 8 controls with simple cysts and polycystic liver disease [7]. Cyst fluid CA19-9, but not CEA, demonstrated a striking 100% sensitivity and specificity for BCA with very high levels in the cyst fluid, ranging from 1,757 to 2,247 U/ml (normal serum values <33). On the contrary all patients with simple cysts had intracystic CA19-9 within the range of normal serum values. These findings prompted the authors to propose a practical algorithm for the diagnosis of biliary cystic tumors. In the case of a large asymptomatic cyst they advocate a fine needle aspiration with measurement of cyst fluid CA19-9 and CEA. If both markers are within normal range, BCA can be reasonably excluded and serial follow-up started. In the setting of elevated levels of one or both of these markers it is suggested to perform laparoscopy with intraoperative multiple sampling of the cyst wall. Radical surgery should be applied in case of histology consistent with BCA, while simple marsupialization could suffice in cases of simple cysts [7]. The weak point of this algorithm is that frozen section biopsies are not always reliable and false negatives may occur even after multiple biopsies [26]. To date the reliability of frozen sections in ruling out malignant transformation has not yet been assessed and only the final histological report after total excision is considered diagnostic [26, 64].

In summary a presumptive diagnosis of biliary cyst tumor can be made by imaging alone in the majority of cases, with no need of fine needle aspiration. This procedure (FNA) should be reserved to doubtful cases – e.g., large asymptomatic cysts where septa cannot be easily demonstrated on imaging or when a complicated benign cyst simulates a biliary tumor. The most challenging differential diagnosis is the hemorrhagic cyst, where US visualizes internal clots as papillary excrescences and pseudonodules. In these cases MRI may establish the correct diagnosis by demonstrating a fluid–fluid level inside the cyst

with the typical T1 hyperintensity in its dependent portion [57, 65]. Though hepatic abscesses and echinococcal cysts may appear similar to BCA and BCAC on imaging, they can be easily differentiated on clinical grounds. Hepatocellular carcinoma and cholangiocarcinoma may rarely present as large hypoechoic and hypodense mass and thus simulate BCAC, but their contrastographic behavior on dynamic CT and MRI is quite characteristic [44].

THERAPY

Once a diagnosis of BCA has been made or in cases of multiloculated cysts with a high index of suspicion for BCA, surgery should be performed without delay [6, 44]. It should again be emphasized that the preoperative differential diagnosis with BCAC is unreliable and that BCA itself has malignant potential; therefore, a "wait and see" attitude cannot be recommended. Even small tumors should not be followed up in order to detect their enlargement or degeneration, but should be excised right away. Excision must be complete, as it may occur when BCA has been erroneously diagnosed as a benign complex cyst and subjected to marsupialization or simple drainage, otherwise recurrence is the rule [64]. BCA was treated in the past with a number of procedures such as marsupialization, internal Roux-en-Y drainage, repeated needle aspirations, sclerosing therapy, fenestration, or partial resection. None of these methods is acceptable nowadays due to their high rate of complications and recurrences [6, 7, 64]; however, the surgical technique of choice has yet to be defined.

Liver resection with clear margins is advocated by some surgeons because of possible foci of carcinoma in situ at the borders of the cyst [64]. Others think that, especially in the case of large tumors, complete removal could be safely achieved by simple enucleation [41]. In these cases the surgeon exploits the pseudocapsule originated by the compressed liver parenchyma, but it is sometimes difficult to precisely define the borders and to minimize the blood loss because of an abundance of compressed vessels around the lesion. These problems more often arise when the mesenchymal stroma is absent making it more difficult for the surgeon to establish a dissection plane within the densely fibrotic wall [1]. In addition, simple enucleation does not guarantee adequate free margins and should not be performed when there is a suspicion of BCAC [41]. This is frequently the case, since BCA and BCAC can be considered as two faces of the same disease [14]. If simple excision is chosen, it is mandatory to perform intraoperative ultrasound to disclose possible small foci of BCA at the periphery of the cyst that may have gone undetected in the preoperative work-up

[66]. Very large, centrally located BCAs are a special problem as they may impinge on the hilum and hepatic veins, and in these cases the surgeon faces the difficult task of trying to develop an avascular plane of cleavage. Insurmountable problems may arise if the tumor is communicating with the hepatic duct, which makes complete enucleation unsafe or unfeasible. In these cases, more aggressive procedures such as plurisegmentectomy can be envisaged [15]. If complete resection of the tumor cannot be achieved during surgery, ablation of the residual cyst wall should be performed using electrocautery or better with argon beam coagulation [7, 43] possibly followed by omentopexy [68]. As mentioned previously a communication of BCA with the biliary system should always be sought preoperatively with MRCP and confirmed intraoperatively with cystography [68]. If the biliary fistula is small, it can be closed at surgery with suture, but in cases where a postoperative bile leak is suspected the affected bile duct should be resected and biliary reconstruction performed [7, 69]. Rarely, a centrally located BCA is directly connected to the main bile duct: in this case and when it is feasible, complete excision of the tumor with bile duct resection and biliary anastomosis should be attempted [70].

In recent times open surgery has often been replaced by laparoscopic surgery in the treatment of accessible benign liver lesions. The advantages of the latter are reduced perioperative blood loss, shorter operative time, and shorter hospital stay [67, 71]. Reduction of abdominal wall damage and cosmetic advantage are additional benefits of this type of surgery. Provided that the tumor is located in a favorable position and is of reasonable size, laparoscopic surgery can be safely and successfully performed by an experienced surgeon and has similar outcomes to open surgery [7].

PROGNOSIS

Due to the slow growth of these tumors, prognosis after adequate resection is fairly good and prolonged survival has been unanimously reported [2, 16, 55, 72–74]. Even in case of cystadenocarcinoma, long-term survival can be achieved, provided that the tumor is discovered and resected prior to development of distant foci or metastasis [16]. Follow-up after surgery should be performed with yearly MRI [74], especially when the risk of recurrence is high – e.g., incomplete resection with remnant cyst, fulguration with argon laser beam [7], or invasion of the adjacent hepatic parenchyma [73]. In the other cases follow-up examinations can be less stringent, particularly in the BCA/BCAC variant with mesenchymal stroma which has a better prognosis [6].

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21 Cholangitis

Shayan Irani, MD and Richard Kozarek, MD

CONTENTS

Summary

Acute cholangitis is a common complication in certain of the fibrocystic disorders especially Caroli's disease and choledochal cysts, with recurrent episodes potentially leading to progressive liver failure. It is a clinical syndrome characterized by fever, jaundice, and abdominal pain (Charcot's triad) that develops as a result of stasis and an infection of the biliary tree. The additional symptoms of hypotension and a change in mental status (Reynolds' pentad) can occur in some patients with suppurative cholangitis and is still associated with a high morbidity and mortality rate. The differential diagnosis includes hepatic abscess, cholecystitis, hepatitis, right lower lobe pneumonia, and other less common disorders. Hepatic abscess and renal failure are complications associated with cholangitis that convey a poor prognosis. Diagnosis is made on clinical grounds, routine blood work, and imaging studies of the biliary tree. Magnetic

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resonance cholangiopancreatography (MRCP) is especially useful in the fibrocystic diseases, in that it provides a useful anatomic map prior to undertaking any definitive therapy. Blood cultures and bile cultures should always be obtained.

Antibiotics, and if possible, appropriate biliary drainage are the two arms used to treat this condition. Eighty percent of patients will respond to initial empiric antibiotics and conservative management with only a few requiring emergent biliary decompression. If, however, after 6–12 h of conservative management, there is decline in clinical status with increased pain, fever, hypotension, or confusion, emergent drainage should be performed. Quinolones, beta-lactam/beta-lactamase inhibitors, third-generation cephalosporins, or carbepenams are acceptable initial choices for antibiotics, with changes made according to the biliary and/or blood culture data.

Biliary drainage can be achieved in order of preference with endoscopic, percutaneous, or very rarely surgical intervention. Endoscopic stent placement is probably as effective as nasobiliary drain placement without the associated discomfort of the nasobiliary drain or its accidental dislodgement. Cholangioscopy can have a role in identifying and treating some of the causes (biliary stones) and other complications of fibrocystic diseases, including indeterminate strictures. Occasionally, recurrent bouts of cholangitis can lead to progressive liver failure necessitating liver transplantation as the only remaining management option.

Key Words: Fibrocystic disease, Cholangitis, MRCP, ERCP, PTBD

INTRODUCTION

Acute bacterial cholangitis refers to a bacterial infection of the biliary tree manifested by a clinical syndrome characterized by fever, jaundice, and abdominal pain. Jean-Martin Charcot (1825–1893), a French neurologist, was the first to describe this triad as a serious life-threatening illness; however, it is now known that its severity can vary from mild to life threatening [1]. The hepatic fibrocystic diseases are particularly prone to recurrent bouts of bacterial cholangitis due to anatomic abnormalities that predispose to bile stasis. In this chapter, we will discuss the pathogenesis, clinical features, diagnosis, and management of this fairly common complication in the different fibrocystic diseases of the liver.

PATHOGENESIS

Under normal conditions, human bile is a sterile fluid protected from intestinal bacterial flora by several mechanisms. The sphincter of Oddi and the tight junctions between hepatocytes serve as anatomic barriers, the bile flow and biliary mucus provide a physical barrier, bile salts provide a chemical barrier, and the Kupffer cells and immunoglobulins provide an immunological barrier [2].

The two prerequisites needed to develop cholangitis are obstruction and colonization (bactobilia). The normal intraductal biliary pressure is 7–14 cm H_2O. The pressures in an obstructed biliary tree can rise upward of 20–30 cm of H_2O. This rise in pressure with bacterial colonization can lead to cholangiovenous reflux of infected bile leading to bacteremia and also septicemia [3, 4]. In a study by Lau et al., a significant correlation was demonstrated between biliary and serum levels of endotoxins and the clinical severity of acute cholangitis [5]. High intrabiliary pressures also encourage the migration of bacteria from the portal circulation into the biliary tract leading to subsequent colonization. This high intrabiliary pressure also disrupts the normal hepatic host defense by adversely affecting the hepatic tight junctions, bile flow, Kupffer cell function, and immunoglobulin A production [2].

The hepatic fibrocystic diseases are a group of rare, predominantly autosomal recessive, disorders which share a number of common pathologic features including the presence of cysts lined by biliary epithelium [6]. An understanding of the pathophysiology of these disorders provides an understanding of the breakdown in the natural host defenses and the risk of cholangitis. These disorders have varying degrees of biliary dilation and increased hepatic fibrosis. In the combined cystic disorders of the liver and kidney (autosomal dominant and recessive polycystic kidney disease) (Fig. 21.1), the defective proteins have been at least partially localized to the cilia in the renal tubular cells and the cholangiocytes [7]. Congenital hepatic fibrosis (CHF) and Caroli's syndrome probably result from an abnormality in remodeling of the embryonic ductal plate of the liver. Ductal plate malformation (DPM) refers to the persistence of excess embryonic bile duct structures in the portal tracts [8]. The choledochal cysts (types I–V) consist of dilated portions of the extra- and or intra-hepatic portions of the biliary tree (see Chapters 14 and 15). The cyst wall is composed of dense fibrous tissue with little or no muscle or elastic tissue and often without an epithelial lining. The lack of muscular tissue, dilated ducts, and lack of epithelial lining leads to a breakdown in the host defenses at many levels (Fig. 21.2a–c). There is an association in up to 90% of these cases with anomalous pancreaticobiliary anatomy [9–11]. In addition

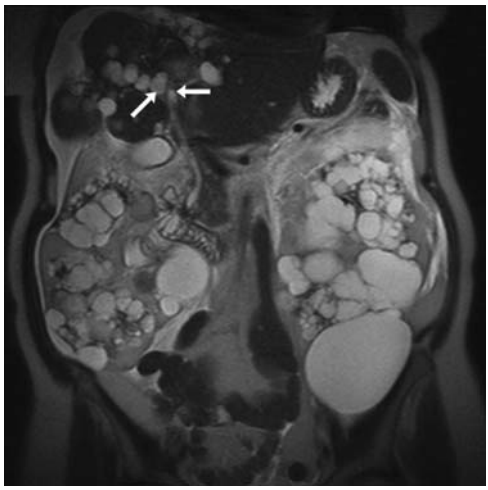

Fig. 21.1. Autosomal dominant polycystic kidney disease (ADPKD) with intrahepatic cysts causing biliary compression at the hilum (*arrows*).

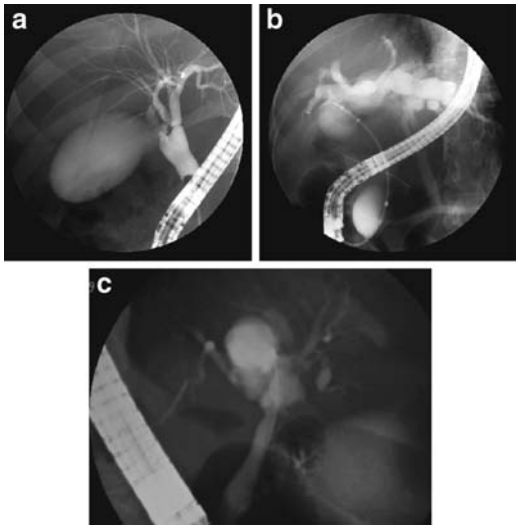

Fig. 21.2. Endoscopic retrograde cholangiographic images of a Type Ia choledochal cyst (**a**), a Type IVa **c**holedochal cyst (**b**), and a patient with Caroli's disease (**c**).

to the inherent risk of stasis in hese conditions, there is an associated risk of stone formation within the cysts. The stones can migrate from the cysts within the liver and present as bile duct stones with cholangitis (Fig. 21.3a, b). Strictures, both benign and malignant, can

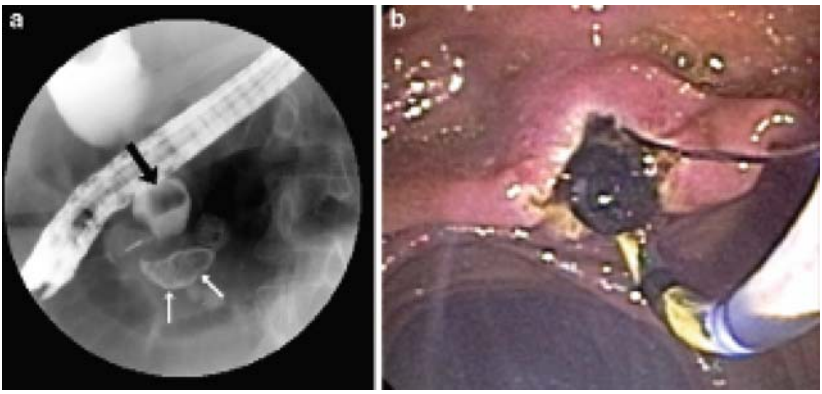

Fig. 21.3. (**a**) Choledochal cyst with common bile duct stone (*large arrow*) and chronic pancreatitis with pancreatic duct stones (*small arrows*); (**b**) common bile duct stone being extracted in same patient with choledochal cyst.

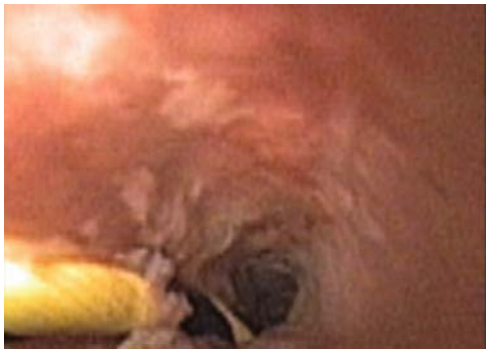

Fig. 21.4. Benign-appearing stricture seen on digital cholangioscopy in a patient with recurrent stones and choledochal cyst.

complicate these conditions and further add to the risk of cholangitis. Benign strictures can occur from recurrent bouts of cholangitis and/or stone formation and passage (Fig. 21.4). Malignancy in the form of cholangiocarcinoma can occur in 10–30% of these patients and the risk increases with age and has been reported as high as 50% in older patients (Fig. 21.5a, b) [12, 13].

Bacterial colonization (bactobilia) can occur in an ascending manner from the duodenum through the sphincter of Oddi or from hematogenous spread from the portal vein. The latter is a much less common source for biliary colonization. There are several causes leading to breakdown in the protective barrier mechanism of the sphincter – biliary

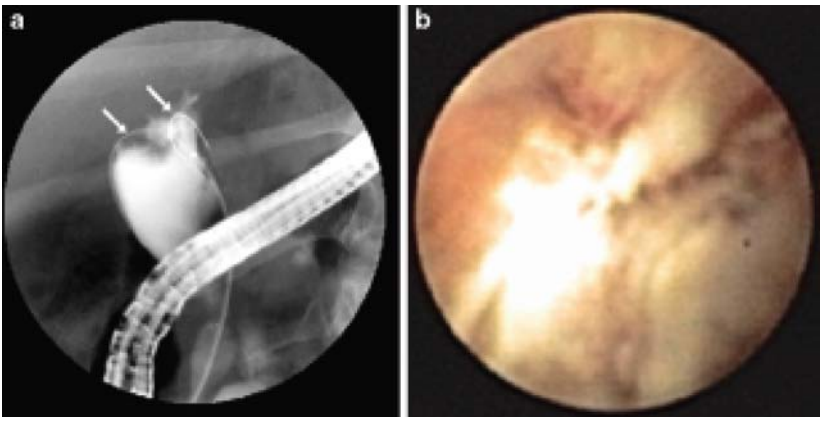

Fig. 21.5. (**a**) Completely obstructing squamous cell carcinoma which developed in patient with Type I choledochal cyst; (**b**) a squamous cell carcinoma in a choledochal cyst seen on digital cholangioscopy.

sphincterotomy and stenting being two of the most common reasons in which bacteria in high concentrations enter the biliary tree. In the absence of effective drainage, there is a high risk of developing bacterial cholangitis. Foreign bodies such as stones and stents in the biliary tree, even with an intact sphincter of Oddi, can serve as a nidus for bactobilia. Choledocholithiasis carries a much higher rate of bile culture positivity than cholelithiasis alone [14, 15]. Bacteria can also be cultured from gallstones. In one study, brown pigment stones had 80% culture positivity, and 84% showed scanning electron microscopic evidence of bacterial structures [16]. The organisms grown in culture were those commonly seen in cholangitis (enterococci – 40%; *Escherichia coli* – 17%; *Klebsiella* spp. – 10%), although clinically *E. coli* tends to be twice as common as enterococci.

The bacteriology of cholangitis may have to do with the pathogenic features of some of the bacteria. The gram-negative Enterobacteriaceae family of bacteria have external pili which facilitate the organism's ability to adhere to foreign bodies such as stones and stents. In addition, an exopolysaccharide matrix produced by certain bacteria protects them from the host's bile salts, immunoglobulins, and may hinder antibiotic penetration as well [16]. Coliform bacteria are by far the most common pathogens isolated. Of the gram-negative organisms, *E. coli* is the major organism (25–50%), followed by *Klebsiella* (15–20%) and *Enterobacter* species (5–10%). Enterococcal species are the most common gram-positive organism (10–20%). The role of anaerobic organisms such as *Bacteroides* and *Clostridia* in bacterial cholangitis

is not clearly understood. They are isolated occasionally, but this is usually in the setting of a mixed infection. There is a slightly higher propensity to see them in the setting of repeated infections, surgery on the biliary tree, or possibly in the elderly patient [17]. Cultures from the bile stones or occluded stents are positive in over 90% of cases with mixed cultures of coliforms being the most common finding. Blood cultures, on the other hand, are less commonly positive and less frequently polymicrobial [18, 19]. Resistant organisms and candidal infections can be seen and are difficult to treat, especially in patients with malignant biliary obstruction [20].

CLINICAL MANIFESTATIONS

The clinical presentation of acute bacterial cholangitis varies from a mild illness to septic shock. Fever is the most common clinical symptom and is almost universal, followed by abdominal pain and jaundice (Table 21.1). This classic triad of Charcot – fever, right upper quadrant pain, and jaundice – occurs in only 50–75% of patients with acute cholangitis [18]. In the older patient with choledochal cyst, this classic presentation even is less frequent [21]. Hypotension and altered mental status occur in less than 14% of patients and can be seen with suppurative cholangitis. Charcot's triad with hypotension and altered mental status, also known as Reynolds' pentad, is associated with significant morbidity and mortality [22]. Hypotension may be the only presenting symptom in the elderly patient or those on corticosteroids. Hepatomegaly can be seen in patients with Caroli's syndrome. Associated splenomegaly usually signifies portal hypertension. This late complication of the hepatic fibrocystic diseases is not uncommon, especially when associated with congenital hepatic fibrosis.

The two most common complications of cholangitis are acute renal failure and intrahepatic abscess. Intrahepatic abscesses usually occur

Table 21.1
Clinical presentation of acute bacterial cholangitis

Symptom	*Percent*
Fever	90
Abdominal pain	70
Jaundice	60
Hypotension	30
Altered mental status	20

late in the disease course [23]. The 30-day mortality in patients with cholangitis has been reported to be around 10% [24]. In a study of 449 episodes of cholangitis over 20 years, seven factors were found to independently predict mortality: acute renal failure, female gender, age, cholangitis associated with liver abscess or cirrhosis, and cholangitis secondary to high biliary strictures or after transhepatic cholangiography [25].

The differential diagnosis includes liver abscess, cholecystitis, hepatolithiasis, biliary leaks, Mirizzi syndrome, hepatitis, and right lower lobe pneumonia/empyema. Laboratory testing and imaging studies are helpful in distinguishing cholangitis from these conditions.

DIAGNOSIS

Laboratory results are often helpful in guiding one to the biliary tract as the source of the sepsis. The white blood count (WBC) is elevated in over 80% of patients, and in many patients who have a normal WBC count, examination of the peripheral blood smear reveals a dramatic shift to immature neutrophil forms. Serum bilirubin level exceeds 2.0 mg/dL in 80% of patients. When the bilirubin level is normal initially, the diagnosis of cholangitis may not be suspected [26]. A cholestatic pattern of liver test abnormalities with elevations in the serum alkaline phosphatase and gamma-glutamyl transpeptidase (GGT) is seen in majority of the patients. Rarely, a pattern of severely elevated aminotransferases, as high as 1,000 IU/L, may be seen, consistent with acute hepatocyte necrosis. This pattern reflects microabscess formation within the liver and a liver biopsy in such cases would show neutrophils in the cholangioles with small abscesses and associated hepatocyte necrosis. The hepatic synthetic function is usually well preserved, but repeated episodes of obstruction and cholangitis within cystic bile ducts eventually may lead to hepatic failure [27].

All patients with suspected cholangitis should have blood cultures drawn. The empiric administration of antibiotics after blood cultures have been drawn is appropriate. Cultures should also be obtained from bile and/or stents removed at endoscopic retrograde cholangiopancreatography (ERCP). Antibiotic therapy should then be tailored to the organisms and their sensitivities based on the culture data [28].

Imaging of the biliary tree is the next step in making the diagnosis and managing this illness. Transabdominal ultrasonography is a quick, easily available, non-invasive first test to image the patient. As mentioned previously, the stasis associated with the various cystic disorders of the liver and the recurrent bouts of cholangitis puts them at risks for developing brown pigment stones. Dilation of the biliary tree and/or the

presence of choledocholithiasis can be detected. However, ultrasonography may be negative in patients with small stones, non-dilated ducts due to the acuity of the obstruction, and the limitations in scanning secondary to a large body habitus.

Magnetic resonance cholangiopancreatography (MRCP) is another non-invasive way to image the biliary tree. In addition to being more sensitive and specific than ultrasound for biliary dilation and choledocholithiasis, it gives the endoscopist a very useful map prior to ERCP. This is especially important in patients with fibrocystic disease which could be associated with intrahepatic segments of biliary obstruction. In addition, it helps evaluate for hepatic abscesses, to which therapy can be directed, if needed. In a recent meta-analysis, MRCP was shown to have an excellent overall sensitivity and specificity for demonstrating the presence and level of biliary obstruction, but a diminished sensitivity for detecting choledocholithiasis or differentiating benign from malignant obstruction in comparison to endoscopic ultrasound (EUS) and ERCP [29]. In the presence of a dilated common bile duct (CBD), an MRCP has a 90–95% concordance with ERCP in diagnosing CBD stones over 1 cm in diameter [30, 31]. In cases of mild cholangitis, and if the risks of ERCP are high, an MRCP may be useful, especially if no stones or high-grade strictures are demonstrated that need to be addressed with an ERCP. In more severe cases of cholangitis with sepsis or impending sepsis, a therapeutic ERCP with drainage of the obstruction should not be delayed (Fig. 21.6).

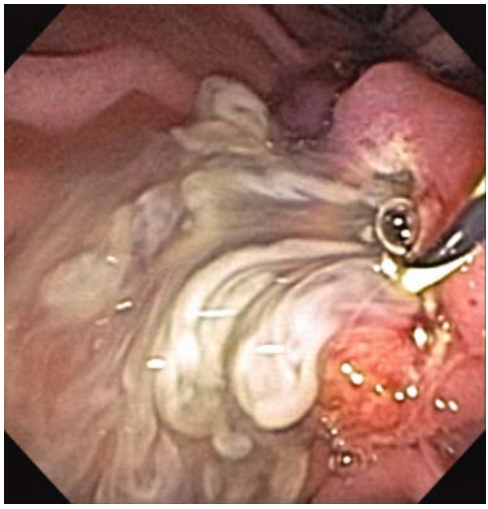

Fig. 21.6. Suppurative cholangitis.

Computerized tomographic scans (CT) may not have the same sensitivity in defining the anatomy of the biliary tree as an MRCP, but are more often obtained because of better availability and lower cost. CT scans are very useful in evaluating the differential diagnosis and associated complications of acute bacterial cholangitis and can be particularly helpful in cases of liver abscesses and associated pancreatitis. One group of investigators also evaluated the ability of abdominal CT to help differentiate suppurative from non-suppurative cholangitis. In this study, papillitis and marked early inhomogeneous enhancement of the liver were found to be the most discriminative CT findings for the diagnosis of acute suppurative cholangitis and the differentiation between suppurative and non-suppurative cholangitis [32]. In patients with Caroli's disease, CT scanning will identify low-density saccular structures within the liver, which may be diffuse or localized. When localized, the left lobe is more typically involved. Radiographic "dots" within cysts correspond to portal vein branches, which protrude into the cyst lumen. Intracystic stones can also be seen in some cases.

Endoscopic ultrasound may play a role in the diagnosis of the cause of cholangitis in certain cases. Two separate meta-analyses of 31 and 27 studies, respectively, have found very high sensitivities (89–94%) and specificities (94–95%) in detecting choledocholithiasis when compared with ERCP and intra-operative cholangiogram as a gold standard [33, 34]. EUS is also useful in the diagnosis of cholangiocarcinoma that can complicate choledochal cysts [35].

ERCP remains the gold standard for the diagnosis and management of acute cholangitis. In the right clinical setting, this could and should be the first step in the evaluation and treatment of cholangitis. There are distinct risks associated with an ERCP, such as pancreatitis, bleeding, bacteremia, perforation, risks of sedation, and death. There is an increased risk of bleeding associated with a sphincterotomy [36]. Overfilling of the biliary tree and/or rapid contrast injection should be avoided due to the increased risk of cholangiovenous reflux and sepsis.

TREATMENT

The treatment of cholangitis is twofold; antibiotics and decompression of the biliary tree. Supportive care should be provided in the form of fluids to maintain urine output, correction of coagulopathy, and close monitoring of vital signs for evidence of sepsis. In cases of suspected sepsis, monitoring for multi-organ failure from endotoxemia is essential.

There is no established consensus on the initial choice of antibiotic use due to a lack of prospective data [17, 19, 37, 38]. The choice of intravenous versus oral antibiotics should be based on the severity of the disease. Clearly, in the setting of bacteremia and/or sepsis, intravenous antibiotics should be used until the bacteremia is resolved. A switch to oral antibiotics is appropriate at that time. The optimal duration of antimicrobial therapy after biliary decompression has not been studied in a prospective randomized fashion [39]. In a small retrospective study, a 3-day course of antibiotics was as effective as a longer course in patients who had a short interval between the onset of cholangitis and biliary drainage, resolution of fever, and successful biliary drainage [40]. An application of this study's results to clinical practice should be cautioned due to the inherent limitations of a retrospective study. In the case of mild to moderate cholangitis in a younger, otherwise healthy patient in which no organism(s) are isolated, a duration of 5–10 days seems appropriate [39]. Variables such as Caroli's disease, malignant biliary obstruction, immunosuppression, older age, prosthetic valves, and other co-morbidities probably warrant longer durations of antibiotics.

The choice of initial antibiotic use should be based on local data on bacterial sensitivities. Beta-lactam-based monotherapy appears to be as effective as a beta-lactam such as ampicillin and aminoglycoside (gentamycin), and monotherapy has been associated with less toxicity [41]. Fluoroquinolones have excellent rates of biliary excretion [19]. In a prospective randomized study, ciprofloxacin alone was found to be as effective as triple therapy with ceftazidime, ampicillin, and metronidazole [37]. The initial use of a fluoroquinolone alone (levofloxacin 500 mg daily) is our clinical practice. The other options for initial antibiotic use include a fluoroquinolone (ciprofloxacin 500 mg every 12 h or levofloxacin 500 mg daily) with metronidazole 500 mg every 8 h, a beta-lactam/beta-lactamase inhibitor (ampicillin–sulbactam 3 g every 6 h, piperacillin/tazobactam 4.5 g every 6 h, or ticarcillin/clavulenate 3.1 g every 4 h), a third-generation cephalosporin (ceftriaxone 1 g daily) with or without metronidazole 500 mg every 8 h, or monotherapy with a carbapenem (imipenem 500 mg every 6 h, meropenem 1 g every 8 h, or ertapenem 1 g daily). Sensitivity data from the blood and bile cultures should be followed and the antibiotics should be modified accordingly.

The vast majority of patients with acute cholangitis will respond to antibiotic therapy and other conservative measures. The patient's condition should improve within 6–12 h in most cases, with defervescence of fever, relief of discomfort, and a decline in the WBC count. In these cases, definitive therapy can be planned on an elective basis in 2–3

days. If, however, after 6–12 h of careful observation, the patient's clinical status declines with worsening fever, pain, mental confusion, or hypotension, the biliary tree must be decompressed immediately.

The method of biliary drainage provided in patients with cystic disorders of the liver depends on the underlying condition, the site of suspected obstruction, and its cause. The available methods of biliary drainage in order of preference are ERCP, percutaneous drainage of the biliary tree (percutaneous transhepatic biliary drain – PTBD), or open surgical decompression.

The safety and efficacy of endoscopic drainage have been proved by many studies [42]. ERCP is usually performed under moderate sedation with fluoroscopy. In pregnant patients and in the setting of hemodynamic instability, the procedure can be performed in the intensive care unit without fluoroscopy with aspiration of bile to confirm proper location [43]. Two randomized controlled trials comparing external drainage (nasobiliary drain) versus internal drainage (biliary stents) showed no significant difference in the success rate, effectiveness, or morbidity [44, 45]. In one of these studies, the incidence of tube complications, such as removal by the patient and nasal discomfort, was higher with nasobiliary drains, as would be expected [44]. There are no randomized controlled trials comparing endoscopic sphincterotomy (EST) alone versus EST with stent placement versus placement of stent alone. There are two case series which examined whether or not EST should be added to nasobiliary drainage or stent placement. They showed no added success or effectiveness of EST, with an increase in complications, especially bleeding [46, 47]. Accordingly, for critically ill patients requiring emergent drainage, nasobiliary drainage or stent placement without sphincterotomy is preferable, postponing definitive treatment to a later date. The success rates of removal of common bile duct stones is 90–95% after sphincterotomy. Minimal contrast injection or wire access only canulation is advisable to minimize the risk of cholangiovenous reflux and bacteremia during the procedure. Aspiration of bile and/or pus, prior to completion of the cholangiogram, without overfilling the ducts is advisable for the same reason. Risks associated with an ERCP include pancreatitis, perforation, risks associated with sedation, and post-sphincterotomy bleeding. Although ERCP has not been well studied in the pediatric age group, it is safe and successful as shown in a retrospective study on 125 children undergoing ERCP for various indications with successful canulation in over 95% of children. Complications were reported as one episode of bleeding and four cases of mild pancreatitis [48].

Patients with choledochal cysts, and Caroli's disease especially, are at increased risk of formation of bile duct stones, intra-hepatic stones,

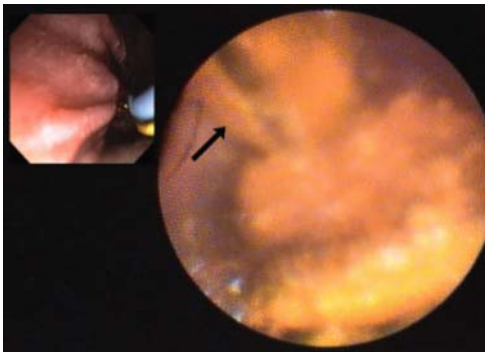

Fig. 21.7. Electrohydraulic lithotripsy (EHL) of large common bile duct stone in a choledochal cyst. The endoscopic view of the spyglass entering the papilla is seen in the upper *left* corner of the image. The *arrow* depicts the EHL probe as it makes contact with a large common bile duct stone.

malignant biliary strictures from cholangiocarcinoma, and benign strictures from recurrent bouts of cholangitis. Cholangioscopy can aid in the diagnosis and treatment of some of these conditions once the acute infection has been controlled. A number of reports have described the value of choledochoscopy with electrohydraulic lithotripsy for removal of bile duct stones that could not be extracted during ERCP (Fig. 21.7) [49–51]. Indeterminate strictures that can arise in these settings can also be evaluated with cholangioscopy. Despite the potential for direct visualization and directed biopsies of the stricture, however, the diagnostic yield may still be low due to several underlying factors. The biology of bile duct cancer, especially hilar cancers, tends to expand in a subepithelial fashion, making superficial biopsies unrevealing. Difficulty opening a biopsy forceps in a tight stricture is another inherent limitation [52, 53]. This yield can be improved with brush cytology, and, if needed, a transhepatic approach.

Percutaneous drainage of the biliary tree is performed under local and/or intravenous sedation through the abdominal wall into a dilated intrahepatic bile duct. The risks associated with the procedure include intra-abdominal and puncture site bleeding, catheter occlusion and dislodgement, bile leak, pneumothorax, and hemothorax. Retrospective studies on PTBD have shown high technical and clinical success rates. Chen et al. reported a clinical success rate of 82% and Pessa et al. reported a technical success rate of 100% with a 7% morbidity rate and a 5% mortality rate [54, 55]. As there is no randomized, controlled trial comparing endoscopic and percutaneous drainage, a definitive conclusion on the better procedure has not been reached. However,

considering the occurrence of some severe complications, such as intraperitoneal bleeding and biliary peritonitis, and the increased discomfort associated with an external drain, endoscopic drainage is the preferred option when available.

Surgical decompression, via a choledochotomy and T-tube placement, is and should be the last option to drain the bile duct. It is becoming an operation rarely performed in these days of endoscopic and percutaneous drainage. It is the most expensive of the three options to decompress the biliary system and has the highest complication rates, including T-tube malfunction and delayed bile duct stricture. A randomized controlled trial has been conducted to compare endoscopic drainage versus open drainage in 82 patients with severe acute cholangitis. As expected, morbidity and mortality were significantly lower in the endoscopic group, with a higher need for prolonged ventilation in the surgical group [42].

The ability to intervene surgically is limited in patients with Caroli's disease, unless there is localized disease and the involved lobe can be resected. In patients with Caroli's disease, hepatolithiasis can be treated with extracorporeal shock wave lithotripsy (ESWL) or intraductal electrohydraulic lithotripsy. The latter was used in a case series of six patients, with complete clearance of hepatolithiasis in four of these patients and partial clearance in the other two [56]. A percutaneous approach and direct cholangioscopy is often preferred in this setting and allows for multiple re-interventions, as needed. Ursodeoxycholic acid, at a dose of 10–20 mg/kg/day, has been used to treat intrahepatic lithiasis. In a group of 12 patients, 3 patients had a complete resolution and 9 patients had a partial resolution of stones [57]. It is more likely that ursodeoxycholic acid acts primarily by improving bile flow and not by direct dissolution of stones, since these stones are brown pigment stones. Orthotopic liver transplantation following control of sepsis has been successful in management of some of these patients with recurrent cholangitis [58].

In patients with polycystic liver disease, cysts can grow to cause biliary obstruction and cholangitis. There is no effective medical therapy for liver cysts. Percutaneous cyst aspiration combined with sclerosis has occasionally been done for cysts causing biliary and/or portal vein compressions [59]. Cyst fenestration, partial hepatectomy, and liver transplantation have been successfully done in some patients to treat recurrent obstructive cholangitis [59].

In the setting of choledochal cysts, complete cyst excision is essential in reducing the recurrence of cholangitis as well as the other complications associated with the cysts, including gallbladder cancer, cholangiocarcinoma, and choledocholithiasis. The surgery described

first by McWhorter which includes cyst excision followed by choledochostomy or hepaticojejunostomy with a Roux-en-Y anastomosis has stood the test of time. It is the preferred method for most type I, IVa, and IVb cysts [60, 61]. Cholangitis may still occur in up to 15% of patients after definitive surgery [62].

With effective antibiotics and timely biliary drainage, the prognosis for mild to moderate cholangitis is much improved. However, the mortality rate can be as high as 50% for patients with severe cholangitis (Reynolds' pentad).

SUMMARY

Acute cholangitis is a fairly common complication in certain of the fibrocystic diseases. It is characterized by fever, jaundice, and abdominal pain (Charcot's triad) as a result of stasis and an infection of the biliary tree. The differential diagnosis includes hepatic abscess, cholecystitis, hepatitis, right lower lobe pneumonia, and less common disorders. Hepatic abscess and renal failure are complications associated with cholangitis that convey a poor prognosis. Diagnosis is made on clinical grounds, routine blood work, and imaging studies of the biliary tree. MRCP is especially useful in the fibrocystic diseases, providing a very useful anatomic map prior to undertaking definitive therapy. Blood and bile cultures should always be obtained.

Eighty percent of patients will respond to initial empiric antibiotics and conservative management with only a few requiring emergent biliary decompression. Quinolones, beta-lactam/beta-lactamase inhibitors, third-generation cephalosporins, or carbepenams are acceptable initial choices for antibiotics, with changes made according to the biliary and/or blood culture data. Biliary drainage can be achieved in order of preference with endoscopic, percutaneous, or very rarely surgical intervention. Endoscopic stent placement is probably as effective as nasobiliary drain placement. Cholangioscopy can have a role in identifying and treating some of the causes (biliary stones) and other complications of fibrocystic diseases, including indeterminate strictures. Occasionally, recurrent bouts of cholangitis can lead to progressive liver failure necessitating liver transplantation as the only remaining management option.

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V Surgical Management

22 Surgical Management of Fibrocystic Liver Disease

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CONTENTS

Summary

Fibrocystic liver diseases comprise a large spectrum of mostly congenital disorders, all of which have varying degrees of cystic transformation of the liver and disease severity. This chapter will briefly discuss diagnostic strategies as well as current surgical

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treatment options for both non-infective (congenital cystic diseases, choledochal cysts, etc.) and infective (echinococcal cysts, amoebic or pyogenic abscesses) processes. We will discuss therapies for the most commonly seen forms of disease in greater detail. Many of the non-infective conditions present as a disease spectrum involving multiple organs (such as renal and hepatic involvement in polycystic syndromes) – we shall limit the discussion to management of hepatic conditions insofar as possible. It is, of course, clearly recognized by the author that management of these syndromes is often predicated by degree of involvement of other associated organs. There are a variety of cystic neoplasms of the liver, whose surgical treatment will also be discussed.

Key Words: Fibrocystic liver disease, Caroli's syndrome, Polycystic liver disease, Congenital hepatic fibrosis

INTRODUCTION

The rapidly expanding availability of a variety of sophisticated imaging techniques such as ultrasound, computerized tomography (CT), and magnetic resonance imaging (MRI) has led to increased frequency of diagnosis of cystic conditions of the liver and biliary tract. A large majority of these conditions are congenital and are associated with other organ involvement. Infective processes also contribute to the spectrum of disorders that comprises fibrocystic disease of the liver. The majority of the conditions discussed in this chapter are discussed in detail (incidence, diagnosis, and medical management) elsewhere in the volume. Here, we will focus primarily on the surgical management of some of the more commonly seen conditions.

SIMPLE HEPATIC CYSTS

These cysts are usually found incidentally on imaging studies. Patients with large cysts (>10 cm in diameter) may present with non-specific symptoms such as vague abdominal discomfort, nausea, or pain. Ultrasonographic findings of simple cysts present as an anechoic unilocular space with posterior acoustic enhancement. On CT scan, simple cysts are defined by a characteristic appearance of a well-demarcated water-attenuation lesion that does not enhance following the administration of intravenous contrast.

Magnetic resonance imaging (MRI) demonstrates a well-defined water-attenuation lesion that does not enhance following the administration of intravenous gadolinium. On T1-weighted images, simple

cysts show a low signal, whereas a high-intensity signal is seen on T2-weighted images. Large cysts (>6 cm in size) may present with complications such as spontaneous hemorrhage, rupture into the peritoneal cavity or bile duct, and infection [1].

The majority of asymptomatic simple cysts require no treatment. In patients presenting with symptoms, it is important to rule out other, more common, causes of abdominal pain such as gastritis, cholelithiasis, gastroesophageal reflux disease, and peptic ulcer disease. Symptomatic cysts as well as cysts that increase in size over time require intervention. Percutaneous needle aspiration is associated with a high failure rate and usually rapid recurrence. This approach is usually only presented as an option in patients reluctant to undergo surgery or in whom tolerance of general anesthesia is in question (i.e., advanced cardiopulmonary disease). Other treatment options include unroofing of the cyst ("marsupialization"), internal drainage with cystojejunostomy, and liver resection. The cyst fluid should always be sent for cytology and assay of certain tumor markers such as carcinoembryonic antigen, as there is a small incidence of associated cancer [2]. The laparoscopic approach is demonstrated to be safe with the ability to achieve a wide unroofing or resection without a large incision and provides quicker recovery time.

CHOLEDOCHAL CYSTS

Choledochal cysts comprise a relatively small proportion of benign biliary disease – with as much as 60% of patients presenting before 10 years of age. The most commonly used classification that is currently used is the Alonso-Lej classification, which typically divides choledochal cysts into four groups [3]. The most common type (type I) presents as a fusiform or saccular dilatation of the extrahepatic biliary tree. Less common variants include bile duct diverticula or choledochocele (type II), which may also be contained within the intraduodenal portion of the duct (type III). Type IV usually involves both the intrahepatic and the extrahepatic ductal system. When the intrahepatic ductal system is involved in the disease process, it is difficult to differentiate this from Caroli's syndrome – it is probably reasonable to consider choledochal cyst as part of a continuum in this group of conditions.

Choledochal cysts may remain asymptomatic for many years. The majority, however, will present with one or more of the classical triad of pain, obstruction, and a palpable mass, often in association with cholangitis. Less commonly, these patients can present with ascites or peritoneal rupture [4]. Diagnosis is made mainly by endoscopic retrograde cholangiopancreatography (ERCP), although current

imaging technologies in the form of CT scan, MRI, and magnetic resonance cholangiopancreatography (MRCP) are also gaining traction as diagnostic modalities.

The management of choledochal cysts is directed, where possible, toward resection of the involved biliary tree. Carcinoma is well recognized as a long-term complication of choledochal cysts, with adenocarcinoma predominating in >90% of cases. Given the high incidence of carcinoma in patients who were treated with non-resectional surgery, it is reasonable to state that choledochal cysts should always be excised with as complete a resection of the (extrahepatic) biliary tree as possible. At the time of resection, it is important to ensure that resection of the involved biliary tree is complete – this is usually accomplished by frozen section pathologic examination of the distal remnant of the specimen. Re-establishment of biliary drainage after the resection is completed is typically accomplished by roux-en-Y hepaticojejunostomy.

CAROLI'S DISEASE AND CAROLI'S SYNDROME

Caroli's disease is a congenital disorder characterized by multifocal, segmental dilatation of large intrahepatic bile ducts [5]. Caroli's syndrome is the more commonly known variant, in which bile duct dilatation is associated with congenital hepatic fibrosis [6]. Biliary stasis in the dilated bile ducts leads to cholangitis, which is a familiar presentation for these patients [7]. Intrahepatic stones may develop and are usually not amenable to surgical removal. Treatment is dependent on the extent of liver involvement. Hepatic resection is successful in patients with symptomatic disease affecting one lobe of the liver [8]. Patients with bilateral hepatic disease should be referred for liver transplantation owing to the high risk of malignancy (cholangiocarcinoma) as well as the technical considerations in being able to remove all the diseased segments [9]. In the setting of advanced Caroli's disease, refractory cholangitis, and complications related to portal hypertension, liver transplantation may be required [10, 11].

CONGENITAL HEPATIC FIBROSIS

Congenital hepatic fibrosis is frequently associated with the recessive form of polycystic kidney disease. Patients typically present at a younger age with clinical evidence of non-cirrhotic portal hypertension. Variceal bleeding is a common presentation. Some patients may present in adulthood, but this is uncommon [12]. Patients who are asymptomatic do not require any definitive therapy but should

be closely monitored. Patients with evidence of progressive hepatic dysfunction with signs of portal hypertension should be evaluated as candidates for liver transplantation, even in the absence of documented cirrhosis. Consideration may be given to porto-systemic shunting to alleviate symptoms in patients with preserved hepatic synthetic function and portal hypertension.

POLYCYSTIC LIVER DISEASE

There are two modes of presentation of this disease, both of which have distinct clinical and genetic presentations and should be elucidated prior to further management.

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Patients with ADPKD often present with multiple hepatic cysts (in addition to the usual primary manifestation of polycystic kidney disease) which may be derived from but are not necessarily in continuity with the biliary tract. Patients may develop innumerable hepatic cysts with resultant enlargement of the liver. The cysts are typically asymptomatic, but patients with marked hepatomegaly may complain of intermittent or continuous abdominal pain [13]. Remarkably, hepatic synthetic function is preserved even in patients with large cysts. Complications typically arise from displacement or pressure on adjacent structures by large cysts or even compression of hepatic veins and bile ducts. Infection and hemorrhage (either intracystic or intraperitoneal) are additional known complications of large cysts and must be treated aggressively. Cyst infection may be treated with intravenous antibiotics and percutaneous drainage of the infected cyst. Depending on the size and location of the infected cyst, some patients may benefit from surgical excision of the cyst wall, which may be done either by the laparoscopic or by the open approach. Intracystic hemorrhage may be managed with supportive care and monitoring – in some cases selective angiography may be necessary to isolate the bleeding vessel. Patients who present with free intraperitoneal hemorrhage will need to be closely monitored with appropriate supportive care. The majority of these patients present with self-limiting bleeding. If there is clinical evidence of ongoing hemorrhage, some patients may require operative exploration and control of bleeding by intra-operative coagulation and suture of bleeding vessels. This may be notoriously difficult to achieve, and patients who are hemodynamically unstable with ongoing hemorrhage may require damage control laparotomy with packing

and planned re-exploration. Patients with huge hepatomegaly (liver volumes in excess of 5,000 cc) and marked malnutrition and wasting as determined by nutritional parameters can be considered candidates for liver transplantation.

Autosomal Dominant Polycystic Liver Disease

Autosomal dominant polycystic liver disease (ADPLD) is a distinct clinical and genetic entity in which multiple cysts (derived from bile ducts) develop without associated polycystic kidney disease [14]. The cysts are typically diagnosed when complications due to local compression, hemorrhage, infection, or rupture arise [15].

Infected cysts require aggressive antibiotic therapy and may require percutaneous drainage. Symptomatic cysts may be treated by cyst fenestration or even partial hepatectomy when cysts are localized to one segment or lobe of the liver. In extreme cases when liver volumes are so great as to cause disabling pain and malnutrition, patients may be considered for liver transplantation.

OTHER CONGENITAL SYNDROMES ASSOCIATED WITH FIBROCYSTIC LIVER DISEASE

Alagille's syndrome. Alagille's syndrome is an autosomal dominant disorder that is characterized by the paucity of interlobular bile ducts and features of chronic cholestasis in association with other defined cardiac and vertebral anomalies [16]. Increased plasma bile acid levels may cause severe pruritus, necessitating biliary diversion procedures. Liver transplantation may be required in patients with progressive hepatic synthetic dysfunction, intractable pruritus, or evidence of portal hypertension [17].

Mitochondrial disorders. Infants with abnormalities in the mitochondrial respiratory chain enzymes can present with conjugated hyperbilirubinemia and hepatic dysfunction, with associated elevation in serum aminotransferases, hypoglycemia, coagulopathy, and lactic acidosis. Liver transplantation has been a successful treatment option in some patients [18].

Alpha-1 antitrypsin (AAT) deficiency. Infants with AAT deficiency can present with neonatal cholestasis and/or hepatitis. The diagnosis is based on a protease inhibitor (PI) phenotyping system which identifies the AAT alleles. The most common allele associated with deficiency and risk for liver disease is Z. The diagnosis is based upon identification of PiZZ homozygotes. Liver transplantation has improved the prognosis

of severe liver disease and should be considered the treatment of choice [19, 20].

Nephronophthisis (NPHP). NPHP is an autosomal recessive disorder discussed in detail elsewhere, which is characterized by reduced urinary concentrating ability, chronic interstitial nephritis, and progression to end-stage renal disease (ESRD). There are several other syndromes in which NPHP can present as a main clinical finding such as Joubert, Meckel, and Cogan syndromes. Hepatic involvement may be seen in some patients with specific gene mutations and is characterized by hepatosplenomegaly and portal fibrosis with mild ductal proliferation [21]. These patients may benefit from liver transplantation, in addition to renal transplantation.

BILIARY ATRESIA

Biliary atresia (BA) is characterized by inflammation of the extrahepatic bile ducts leading to progressive obliteration of the biliary tract. The exact cause is not known; however, several theories have been proposed defining infectious and genetic etiologies [22]. Infants typically present with icteric sclera, jaundice, hepatosplenomegaly, and acholic stools. Early diagnosis is crucial to providing timely treatment, which may halt the progression of hepatic injury and subsequent development of secondary biliary cirrhosis. The primary treatment for biliary atresia is surgical. The Kasai procedure or portoenterostomy involves anastomosis of a roux-en-Y loop of small bowel directly to the liver capsule after excision of the biliary remnant and portal fibrous plate. The procedure is most successful in infants diagnosed and treated before 10–12 weeks of age [23]. In a series of 349 infants diagnosed and treated for biliary atresia in Canada, older age at Kasai operation (defined as <30 days, 31–90 days, or >90 days) was associated with progressive decrease in success of the operation in halting progression of biliary disease [24]. There is controversy regarding whether treatment with glucocorticoids after the Kasai procedure improves outcomes. A randomized trial found that adjuvant treatment with glucocorticoids improved bilirubin levels at 1 month postoperatively, but not at the 6- and 12-month time points [25]. A definitive placebo-controlled trial of corticosteroids post-Kasai is currently underway in the United States by the Cholestatic Liver Disease Research and Education Network, funded by the National Institute of Digestive Diseases and Kidney. In those infants who do not achieve bile flow following the Kasai procedure, treatment options are limited to liver transplantation. Since the outcomes of liver transplantation improve for infants with weights >10 kg, transplantation of these infants may be delayed if growth can be achieved. Supplemental

feedings may be necessary to attain adequate growth. Children who undergo liver transplantation have a good prognosis with overall survival of approximately 70–80% at both 5 and 10 years post-transplant [24, 26, 27]. Malnutrition is associated with increased mortality either while waitlisted for transplantation or in the post-transplant period, again emphasizing the need for aggressive nutritional support of these infants [28].

ECHINOCOCCAL CYSTS/HYDATID CYSTS OF THE LIVER

Echinococcal (hydatid) cysts of the liver are caused by the larval form of *Echinococcus granulosus*, a parasitic infection usually acquired from dogs. Most patients are asymptomatic and present only when complications, such as intraperitoneal leakage, bacterial superinfection, or biliary obstruction from mass effect of enlarging cysts, occur. These cysts can rupture into the biliary tree causing biliary colic, cholangitis, and pancreatitis. Bacterial superinfection of the cysts can result in liver abscesses. They may also rupture into the peritoneum causing peritonitis and in some cases anaphylactic shock due to the release of antigenic material and secondary immunologic reactions. Imaging modalities such as ultrasound (90–95% sensitivity), computed tomography (95–100% sensitivity), and magnetic resonance imaging are used to diagnose hydatid cystic disease [29].

Treatment of hydatid cysts consists of medical therapy with albendazole in combination with surgery or percutaneous drainage/treatment approaches [30]. Surgical therapy is the preferred option in patients with large liver cysts (>10 cm) or complicated cysts (accompanied by obstruction, compression, or infection) [31]. The main goal of surgical treatment is to evacuate the cyst without intraperitoneal spillage of its contents and obliteration of the residual cavity [32]. This can be achieved by the excision of the intact cyst or cyst evacuation (in the case of large cysts) followed by cyst excision. If there are a large number of daughter cysts seen with localization to one segment or lobe of the liver, partial hepatic resection may be necessary to remove all of the disease. Approaches such as marsupialization, omentoplasty, and internal drainage are rarely performed due to the high recurrence rates. Both open and laparoscopic techniques yield good results, although allergic reactions due to peritoneal spillage are more commonly seen in laparoscopic intervention [33]. Although it is still common practice to instill a protoscolicidal agent (hypertonic saline, cetrimide, or ethanol) into the cyst during surgery, this has not been shown to reduce the incidence of secondary recurrence. Percutaneous techniques such

as PAIR (*p*uncture, *a*spiration, *i*njection, and *r*easpiration) are gaining wider acceptance because of its low morbidity and low incidence of recurrence. Percutaneous puncture of the cysts under ultrasound or CT guidance is followed by aspiration of cyst fluid and injection of a protoscolicidal agent into the cyst cavity. The cyst is then reaspirated after a (minimum) period of 15 min [34]. Patient selection for this modality is of paramount importance, and the procedure should not be used in patients with superficial cysts (increased risk of spillage into the abdominal cavity), inactive or calcified cysts, cysts with biliary communication, or cysts with solid material or echogenic foci.

CYSTADENOMA AND CYSTADENOCARCINOMA

Cystadenomas of the liver are rare cystic tumors that can grow to a large size and usually require surgical intervention. The most common presenting symptoms described include abdominal discomfort or pain, anorexia, and, in some cases, a palpable mass-like effect. The differential diagnosis includes simple hepatic cyst, echinococcal cyst, and cystadenocarcinoma. The preferred treatment for cystadenomas is resection which can be done by enucleating it from the surrounding liver. It is essential to remove the entire cyst wall as malignant transformation has been described in up to 15% of patients. Partial excision is invariably associated with recurrence and should be avoided whenever possible [35]. Aspiration leads to rapid recurrence of fluid and is not recommended as definitive therapy [36]. In the rare patients with cystadenocarcinoma, aspiration may inadvertently lead to peritoneal seeding with malignant cells.

Cystadenocarcinomas likely arise from malignant transformation in previously existing cystadenomas. In contrast to cystadenoma, however, treatment should consist of lobar hepatic resection. Enucleation is not recommended due to high risk of recurrence.

CONCLUSION

The surgical treatment of fibrocystic diseases of the liver is varied, depending in many cases on patients presenting with symptoms prior to the initiation of definitive diagnosis and treatment. Also, the presence of associated organ dysfunction as seen in patients with polycystic kidney disease may drive decisions for management. Multi-disciplinary management aids in the appropriate decision-making regarding the timing and scope of definitive surgical therapy.

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