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PII: S0046-8177(22)00167-8

DOI: https://doi.org/10.1016/j.humpath.2022.06.022

Reference: YHUPA 5324

To appear in: *Human Pathology*

Received Date: 14 June 2022

Accepted Date: 22 June 2022

Please cite this article as: Mirza H, Besse W, Somlo S, Weinreb J, Kenney B, Jain D, An Update on Ductal Plate Malformations and Fibropolycystic Diseases of the Liver, *Human Pathology*, https://doi.org/10.1016/j.humpath.2022.06.022.

An Update on Ductal Plate Malformations and Fibropolycystic Diseases of the Liver

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Running title: Ductal plate malformations

Competing interest: Not Declared

Funding: None

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Summary

A variety of cystic and fibrocystic lesions can occur in the liver, which may be single or multiple and etiologically can be acquired or have genetic underpinnings. While the morphology of ductal plate development and various associated malformations has been well described, the genetic etiologies of many of these disorders is still poorly understood. Multiple clinical phenotypes in the liver are proposed to originate from ductal plate malformations: congenital hepatic fibrosis, Caroli's disease, Von-Meyenburg complex and the liver cysts of autosomal dominant polycystic kidney and liver diseases. While many of the patients with these disorders, particularly with isolated liver involvement remain asymptomatic, some develop portal hypertension or symptoms from cyst enlargement. Development of hepatocellular malignancy is a risk in a small subset. Recent advances have made it now possible for some of these phenotypes to be genetically defined, and intriguingly animal models of adult polycystic liver disease suggest that abnormal organ development is not required. This review describes the current understanding, genetic underpinning and key clinicopathologic and imaging features of these fibro-polycystic liver diseases.

Key words: polycystic liver disease, ductal plate, congenital hepatic fibrosis, Caroli disease

Key points

- Polycystic liver disease is a diverse group of disorders, some of which are associated with cysts in other organs, especially kidney.
- The genetic alterations associated with various polycystic disorders have been elucidated in recent years. Concurrently, the genotype and phenotype correlations that include age of onset, disease severity, pathology and disease progression are also becoming clear.
- Each disease has characteristic clinical, imaging and pathologic features.
- Depending on the disorder, either the terminal, intermediate or proximal segments of the biliary system maybe involved, and the cysts may or may not be connected with the biliary system.
- The histology of various disorders in this group represents a varying combination of cyst formation, fibrosis and Von-Meyenburg complexes, which often results in a unique clinicopathologic phenotype for each disease, but some overlap between disorders also 4exists.

1. Introduction

A variety of cysts occur in the liver, which may be single or multiple. Etiologically they can be acquired or have genetic underpinnings (1). Genetic disorders with liver cysts are associated with defective development of the ductal plate and bile ducts in the liver and are referred to as ductal plate malformations (DPM). DPMs represent the majority of fibrocystic diseases involving the liver (2). These include congenital hepatic fibrosis, Caroli's disease, Von-Meyenburg complex (VMC) and polycystic liver disease (2, 3). Pathologically cystic phenotypes in the liver include both autosomal dominant as well as recessive disorders, some of which have associated renal cysts (3). While the embryology of DPM has been well described, genetic etiologies of these disorders is not completely understood (4) (Table 1). Gene discovery efforts for both the dominantly and recessively inherited fibrocystic liver diseases have made it now possible for these phenotypes to be genetically defined in at least 92% of autosomal dominant polycystic kidney disease (ADPKD), 30-50% of isolated polycystic liver disease (PCLD, also known as ADPLD), and 30-40% of nephronophthisis (5-7) (Table 1). Each of these disorders has been proposed to result from malformation of the ductal plate at different level of biliary system, although animal models of ADPKD—PCLD suggest that abnormal organ development is not required. Typically the distal most intralobular ducts (<20μ) are involved in the formation of VMCs, intralobular ducts between 20-50μ are involved in congenital hepatic fibrosis and ducts >50μ are involved in Caroli disease. In ADPKD the cysts develop form VMCs and lose their connection with the biliary system.

It will be of tremendous value to perform a genotype-phenotype correlation of these entities. This review describes the current understanding, genetic underpinning and key clinicopathologic features of these fibro-polycystic liver diseases(2).

2. Fibro-polycystic liver diseases

2.1 Autosomal dominant cystic phenotypes: PCLD and ADPKD

2.1.1 Introduction: Dominantly inherited polycystic liver disease (PCLD) is characterized by gradual accumulation and expansion of cysts arising from biliary epithelium (7). The gradual accumulation of these cysts through adulthood, and the enlarging nature of the cysts sets the dominantly inherited polycystic liver disease apart from recessive forms of fibrocystic liver disease (ARPKD, CHF, Caroli disease), which present earlier in life, have smaller or no cysts and fibrosis is the predominant feature (5-7).

2.1.2 Genetics: Typical ADPKD that can result in end stage kidney disease is caused by mutations in *PKD1* or *PKD2*, which encode polycystin-1 (PC1) and polycystin-2 respectively (8) (Table 1). These proteins are known to localize on the primary cilium of renal tubular and biliary epithelial cells, and the cilium is necessary for disease pathogenesis (9, 10). Cholangiocyte cilia are proposed to have mechanosensory and chemosensory roles in addition to regulating bile secretion. The unique consequences of polycystin loss appear different from the loss of cilia altogether, a finding described using mouse models (9).

PCLD is caused by mutations in any of at least the following 7 different genes which are necessary for the maturation of PC1 through the endoplasmic reticulum (ER): *PRKCSH, SEC63, GANAB, ALG8, SEC61B, DNAJB11,* and *ALG9 (11-17).* Identification of these genes and recent study of the associated phenotypes shows that ADPKD and PCLD exist as a phenotypic spectrum (ADPKD—PCLD) attributed to a range in overall PC1 dosage.

At the cellular level, ADPKD—PCLD patients have a germline heterozygous mutations in each of their cells, but cyst epithelium shows additional loss of the single remaining normal copy of the respective gene. This was shown by several studies decades ago, then more recently by next

generation sequencing (18-22). This suggests that somatic second hit mutations to the normal allele in individual cells, which result in the cellular recessive genotype within the tissue, are required for the cyst initiation. The resultant loss of polycystin function results in cell proliferation, flattening of typically cuboidal epithelial cells, and fluid secretion by incompletely understood mechanisms which together result in a cyst. This mechanism is consistent with the clinical findings of randomly sized and distributed fluid-filled cysts that grow in number and size with time, causing organ enlargement.

Mouse models studying liver cysts in ADPKD—PCLD caused by *Pkd1, Pkd2, Prkcsh,* or *Sec63* use conditional alleles to achieve the recessive genotype for the cyst epithelium which at the level of the whole organism would be embryonic lethal (23, 24). While bi-alleleic inactivation during development results in the rapid liver and kidney cyst progression, bi-alleleic inactivation in adulthood also has a robust cystic phenotype, albeit more slowly progressive(9, 10). This demonstrates that at least in mice, abnormal organ development is not required for cyst formation in the PC1-dependent mechanism. Mouse models of bi-allelic polycystin loss show a distinct and more severe phenotype than those that result from loss of the cilia structural components that cause the recessive fibrocystic diseases discussed below.

2.1.3 Clinical features: Polycystic liver disease is clinically characterized into two entities by whether it occurs with clinically relevant kidney cysts or without significant kidney cysts (25). The former is autosomal dominant polycystic kidney disease (ADPKD); the latter is autosomal dominant polycystic liver disease (ADPLD), also known as isolated polycystic liver disease (PCLD). According to autopsy studies half to two-thirds of cases of polycystic liver disease occur with kidney cysts (26-28). ADPKD prevalence is 1:400 to 1:1000, and more than half of such patients progress to end-stage kidney disease by the sixth decade of life (29). Polycystic liver

disease of varied severity is present in 94 percent of ADPKD patients by age 35 (8). PCLD is a relatively rare. The reported incidence clinically is in the 1:10,000 to 1:100,000 range, but this is suspected to significantly underrepresent the actual incidence as majority of the cases when broadly defined are asymptomatic (30, 31). This is also supported by a higher incidence of about <0.01% in autopsies (32, 33).

The disease typically presents in adulthood (5-7). In ADPKD and PCLD, liver cyst burden correlates with age and is significantly greater in women (8). This finding is correlated to duration of estrogen exposures from pregnancies or birth control pills (34-36). In general, the liver disease is less severe in PCLD than that seen with ADPKD, and there is, by definition, no associated significant clinical renal cystic disease. In the absence of significant renal involvement, most patients are asymptomatic with normal liver function tests, and the disease is often discovered incidentally (32). When present, symptoms are caused by cyst enlargement, usually manifesting as right upper quadrant discomfort/pain with associated nausea or early satiety (32). In severe cases, cyst infection, hemorrhage, or biliary obstruction may also occur.

Cysts can also more rarely be seen in the pancreas and possibly spleen(37). Clinical diagnostic criteria are not well defined, but in general the finding of 5 or more cysts in the liver should prompt further consideration for polycystic liver disease(32). Although cysts do not typically cause liver failure, their complications can necessitate surgical interventions ranging from cyst aspiration to cystectomy or cyst fenestration to partial hepatectomy, or in some cases, total hepatectomy with liver transplant. In general, a conservative clinical approach is appropriate (32).

2.1.4 Imaging features: Typical ADPKD—PCLD livers have a unique appearance on imaging due to the large number of randomly distributed discrete cysts of different sizes (Fig. 1A, B). Cyst size and number increase slowly with age, and they may have varying signal intensity (MRI),

attenuation (CT), and echogenicity (ultrasound) due to hemorrhage on imaging. Over time, the cyst walls may calcify. When large, the cysts can exert mass effect on adjacent organs, hepatic vascular and biliary structures.

The recent discovery of disease genes for ADPKD—PCLD has allowed for reframing the phenotypic characterization of genetically-defined cases. For example, PCLD cases attributed to heterozygous *ALG8* loss of function mutations ranged from having hepatomegaly with large liver cysts, to having a normal liver size with innumerable tiny cysts (15).

2.1.5 Pathologic findings*:* Hepatic cysts in ADPKD and PCLD arise from dilatation of Von-Myenburg complexes and generally involve the liver diffusely and histologically are similar (Fig. 2A, B) (32, 38). Generally, a large number of VMCs are present in the liver and show varying degrees of dilation and cystic change. The cyst lining epithelium is biliary-type, low columnar to cuboidal initially and becomes increasingly flattened as the cyst enlarges (Fig. 2C,D). In some of the larger cysts following rupture or hemorrhage the epithelial lining may get denuded and lost. In contrast to the cysts of ARPKD, which maintain their connection to the biliary tree, the cysts of ADPKD may lose their connection, grow in size and impinge on the hepatic parenchyma (32). The hepatic lobular architecture is generally intact (32). Fibrosis of the background liver is not a feature, and when seen is largely limited to areas surrounding the cyst. Fibrosis around the cyst occurs due to pressure atrophy of the surrounding parenchyma or following cyst rupture and subsequent inflammation. The cysts are non-dysplastic and there is no apparent increase in the risk of any hepatocellular or biliary malignancy. Biopsy of the cysts is generally not indicated in PCLD, and the pathologist is involved either when cysts are resected/unroofed, or when the patient undergoes transplantation.

2.2 Autosomal recessive fibrocystic diseases

2.2.1 Introduction: Recessive fibrocystic phenotypes in the liver or kidney are caused by germline recessive loss of proteins which are required for the function and maintenance of the primary cilium. Such diseases are thus termed the "ciliopathies". The resulting liver lesion is known as congenital hepatic fibrosis (CHF) (6). CHF results from ductal plate malformation during development that leads to progressive fibrosis of the portal tracts with time with or without macroscopic cysts. Cases where intrahepatic bile ducts are dilated are given the diagnosis of Caroli disease. These likely represent a continuum with each other and may co-exist when it is referred to as Caroli syndrome (39). Clinical suspicion for CHF is often based on imaging showing echogenic liver parenchyma with or without cysts, splenomegaly, and accompanying fibrocystic changes of the kidneys (40). Another non-cilium related recessive disease that could have similar phenotype to CHF is cystic fibrosis in a subset of patients with liver involvement. Most patients with cystic fibrosis are identified with newborn screening in the United States or when the diagnosis becomes clinically apparent due to pulmonary and pancreatic involvement. Other more common causes of cirrhosis such as viral hepatitis, alcoholic liver disease, autoimmune causes, and genetic disorderss such as alpha -1 antitrypsin deficiency and Wilson disease can be distinguished by disease time course and other clinical and genetic testing. However, as phenotypic overlap can exist, liver pathology can be an important part of the workup of fibrocystic liver disease.

2.2.2 Genetics: Autosomal recessive polycystic kidney disease (ARPKD) is caused by recessive loss of the *PKHD1* gene which encodes the ciliary protein fibrocystin (41). This disease is characterized by cystic dilations of the renal collecting duct which can lead to renal failure, and ductal plate malformation in the liver which can progress with time to congenital hepatic fibrosis. ARPKD may be clinically suspected as early as in utero, but typically in infancy or early childhood

and rarely in adults or elderly. Disease severity correlates with *PKHD1* genotype, where truncating variants are generally more severe than missense variants(42, 43). Patients with truncating variants on both alleles more often do not survive the perinatal period, often due to respiratory or renal failure. In contrast, those with milder mutations may survive childhood without significant kidney issues but are diagnosed by genetic testing during work-up for CHF in adolescence or at a later age(44).

2.2.3 Clinical features: CHF typically occurs in patients with manifestation in other organs, particularly the kidney, when involved. The most common of which is autosomal recessive polycystic kidney disease (ARPKD). Based on the occurrence of recessive conditions which typically have CHF, it is estimated that recessive fibrocystic disease is present in approximately 1:10,000 to 20,000 live births (39). Additional ciliopathies defined by their constellation of organ phenotypes have been termed Joubert syndrome and related disorders (JSRDs), Leber's congenital amaurosis (LCA), Bardet-Biedl syndrome (BBS), Meckel syndrome (MKS), cranioectodremal dysplasia, Ellis-van Creveld syndrome (EVC), Jeune asphyxiating thoracic dystrophy (JATD), oculomotor apraxia, renal dysplasia, reno-hepatic-pancreatic dysplasia (RHPD), and oro-facial-digital syndrome (X-linked)(39, 40, 45) Nephronophthisis, the name given to recessive fibrocystic kidney disease is seen approximately 80-90% in isolation and makes up 5% of pediatric end-stage renal disease. Approximately 10-20% of nephronophthisis cases occur as part of these ciliopathy syndromes. CHF is expected for the clinical diagnosis of ARPKD and MKS and has been reported with variable frequency in JSRDs, BBS, EVC, JATD, RHPD, and OFD1 including those caused by mutations in the following nephronophthisis-associated genes: *NPHP3, NPHP11, TMEM67, ANKS6, and DCDC2*.

Several clinical forms are recognized: portal hypertensive, cholangitic, mixed portal hypertensive-cholangitic and latent (46). The liver disease tends to manifest typically with portal hypertension. In general, baseline liver function tests are within normal range, although alkaline phosphatase may be slightly elevated and hepatocytic dysfunction is often a late manifestation. This can help distinguish it clinically from other biliary diseases such as primary biliary cirrhosis and primary sclerosing cholangitis, which consistently have high elevations in these laboratory values. Patients may come to clinical attention as a result of cholangitis, or variceal bleed and/or hepatosplenomegaly due to portal hypertension (47). CHF can sometimes progress to liver failure requiring liver transplantation(48). There is an increased risk of hepatocellular malignancies, particularly cholangiocarcinoma associated with both CHF and Caroli disease (49, 50).

2.3 Congenital hepatic fibrosis:

2.3.1 Imaging findings: Periportal fibrosis in CHF appears as high signal intensity on T2-weighted MR images in the liver parenchyma (Fig 3A,B) that may accompany the portal veins and as hyperechoic periportal thickening on ultrasound (51, 52). There may be multiple large enhancing regenerative nodules measuring up to 3.0 cm(53, 54). Many patients with CHF have one or more associated biliary abnormalities, including VMC, Caroli disease, and fusiform dilation of the extrahepatic bile ducts Vascular abnormalities include an enlarged hepatic artery, cavernous transformation of the portal vein, and portal vein thrombosis (55, 56). Signs of portal hypertension, such as varices, portosystemic shunts, splenomegaly, and ascites, are often present (Fig 3A) (53). Ciliopathy-associated renal abnormalities, such as fibrocystic kidney disease and medullary sponge kidney, provide additional support for the diagnosis of CHF. Some patient with CHF have macroscopic cysts, which may assist distinguishing it from other causes of liver fibrosis on imagingFig 3B).

2.3.2 Pathologic findings: The pathology of CHF is best described as DPM of the interlobular bile ducts with superimposed destructive and sclerosing cholangitis (38). Grossly the liver appear firm and depending upon the extent of fibrosis may appear indistinguishable from cirrhosis (Fig 4A,B). The histologic hallmark of CHF is broad dense fibrous septa with numerous embedded irregularly shaped bile duct structures(Fig 4C, D) (46). The ductular structures are uniformly distributed across these fibrous septa. These ducts are lined by bland biliary type epithelium that ranges from low columnar to flattened, often with a central lumen with inspissated secretions or bile (Fig 4D). These can be easily differentiated from bile ductular reaction seen with other biliary disorders which can also have irregular outlines, but lined by cuboidal epithelium, typically lack a lumen and very closely hug the limiting plate of hepatic lobules. In the so-called focal form, fibrous enlargement is predominantly relegated to the portal tracts, while in the so-called diffuse form adjacent portal tracts are linked by broad fibrous septa (46). The fibrosis may result in cirrhosis-like nodularity of the liver, although the septa tend to take on a "jigsaw puzzle" like appearance, cutting out hepatocytic nodules that have geographic shapes rather than the rounded profiles typically seen with cirrhosis (Fig 4C). Fibrosis, rather than cyst formation, is the hallmark of this DPM, hence the nomenclature. Although bridging fibrosis may be present, the hepatocyte lobules are generally normal in architecture and function. The central venules are still identifiable, although they may not remain centrally located in the nodules, and the hepatic cords remain mostly single cell thick; these features can be helpful in differentiation from typical cirrhosis.

It should be remembered that hepatic architecture may resemble cirrhosis in the presence of another concomitant liver disorder e.g., alcoholic liver disease or chronic viral hepatitis. This can be further complicated by the fact that some of these patients have a milder variant

of CHF, lack significant renal disease and tend to present at an older age (5-6th decade) (57). When superimposed abnormal bile ducts with cystic changes are present, the disease process is then referred to as Caroli syndrome (58).

2.4 Caroli disease:

2.4.1 Imaging Findings: On imaging, the presence of tiny dots with strong contrast enhancement within the dilated intrahepatic bile ducts (the "central dot" sign) is suggestive of Caroli disease This feature consists of fibrovascular bundles within dilated cystic intrahepatic ducts showing strong contrast enhancement on CT or MR imaging (Fig 6A,B) (51, 54). The central dot sign corresponds to a portal-vein radicle and an accompanying hepatic artery branch protruding into the lumen of a dilated bile duct(59). In the absence of the central dot sign, MRCP can be valuable in diagnosis of Caroli disease by demonstrating saccular dilation of large intrahepatic bile ducts that communicate with the biliary tree (Fig. 6). However, MRCP imaging may not always demonstrate communication between cystic lesions and draining bile ducts. In these instances, hepatobiliary contrast agents–enhanced MRI, may demonstrate the communications between cystic lesions allowing differentiation of Caroli disease from other cystic liver diseases. Bile stasis in Caroli disease may result in biliary calculi, cholangitis and liver abscesses, which can be depicted on imaging. There may be findings of secondary biliary cirrhosis due to biliary obstruction. In Caroli syndrome, there is evidence of CHF or other fibrocystic hepatic disease, and the bile duct dilatation is less prominent (Fig 3B).

2.4.2 Pathologic findings: Caroli Disease occurs as a component of ARPKD; however, it may also occur without associated renal abnormalities. The condition is defined by saccular or fusiform dilation of the large intrahepatic bile ducts, generally the right and left hepatic ducts

and their corresponding segmental larger ducts (38). Intraluminal protrusion of duct wall and the presence of intraductal vascular tracts are common findings. Caroli disease generally involves the entire liver, although it may be segmental in some cases. Grossly the cyst of variable size are seen involving the liver and seem to follow the portal tracts (Fig 6A). Biopsy is seldom performed and is often not useful in elucidation of the disease process; histologic examination of resection or hepatectomy specimens is more revealing. Dilated ducts may be associated with marked chronic inflammation, acute inflammatory infiltrates, and a variable degree of fibrosis. Inspissated bile and purulent material may be present within duct lumina (Fig 6B, C). As in ARPKD, the cysts are in continuity with the remainder of the biliary system, rather than being isolated. The cysts correspond to incompletely remodeled ductal plate remnants with a variable degree of dilation (38). In this setting presence of dysplasia or overt cholangiocarcinoma may be seen in some cases and is a known long-term risk.

Caroli Syndrome is defined by the presence of Caroli disease type changes superimposed with congenital hepatic fibrosis (Fig 6D) (58, 60). Caroli syndrome is more common than pure Caroli disease without other hepatic abnormalities (58). Caroli syndrome involves the entire intrahepatic biliary tree. Histologic findings are defined by overlapping features of CHF and Caroli disease.

2.5 Von-Meyenburg complexes

2.5.1 Introduction*: Von-Myenberg complexes (VMC)*, or biliary hamartomas, are typically small, often multiple, and are almost invariably asymptomatic (38). They may be seen in otherwise normal liver, or in association with many of the other fibropolycystic disorders/DPMs, including ADPKD, CHF and Caroli syndrome (38). The VMCs occurring without other associated DPMs, can be few or numerous in the liver and some cases are

associated with renal cysts. The nature of VMC remains controversial. Initially, due to their increased presence in patients with alcoholic cirrhosis an ischemic etiology was proposed. Subsequently their association with various DPMs was recognized and currently these are indeed considered a spectrum of DPM involving the most peripheral intrahepatic bile ducts (38).

2.5.2 Genetics: So far the genetic underpinning of VMC has been not studied, but a recent case report showed presence of germline *PKHD1* heterozygous mutation in a patient with multiple VMCs (16). This suggests a likely genetic etiology like other polycystic disorders, possibly requiring additional somatic hits for their development and progression to neoplasia. Interestingly *PKHD1* is the disease gene for autosomal recessive polycystic kidney disease (ARPKD), as discussed above. The carrier parents of ARPKD children were thought to be unaffected, however, a study in 2011 screened 110 such parents and found that 10 (9%) had polycystic livers, a dramatic enrichment over the general population (61). From a different perspective, *PKHD1* heterozygous loss of function mutations were found enriched, to an extent reaching genome-wide significance, in a cohort of clinically and radiographically-defined PCLD (62). Together, these studies identified the *PKHD1* heterozygous carrier genotype as an autosomal dominant cause of PCLD, although with only approximately 9% penetrance, and with cysts being typically small and in normal-sized livers. The genetic or environmental factors effecting penetrance are yet to be described. Cyst formation in *PKHD1* heterozygous carriers might require a somatic second hit mutation resulting in a focal recessive genotype of the cyst epithelium. One study showed that adult biallelic inactivation of *Pkhd1* was indeed sufficient to produce cysts in mice. Whether the PKHD1 heterozygous carrier genotype might share the same PC1-dependent mechanism of

cyst formation as ADPKD—PCLD is not yet definitively known, although the lack of large cysts may suggest against it. We suspect that individuals with heterozygous *PKHD1* mutations likely have VMCs, some of which enlarge and appear as cysts on imaging. This coincides with the incidence of about 5-7% for VMC in autopsies/ liver biopsies.

2.5.3 Clinical findings: VMC are asymptomatic and often incidentally detected, either on imaging or pathology. Their incidence increases with age, reaching a peak around the 6-7th decade, irrespective of gender (62). No therapy is required, however, presence of multiple VMCs may prompt one to look for cystic renal disease. The differential diagnosis for these includes a variety of simple hepatic cysts, developmental malformations, infectious cysts and abscesses, and cystic neoplasms. There are several case reports and small series reporting their association with bile duct adenomas and cholangiocarcinomas which suggests a pre-neoplastic potential.

2.5.4 Imaging findings: Because VMCs exhibit a range of histopathologic features, they have variable appearance on imaging, depending on their size, degree of ductal dilation, and density of surrounding fibrous stroma. They are usually identified when they appear as diffuse, uniform, tiny (1-15 mm) cystic lesions that do not communicate with the bile ducts, an appearance on T2-weighted MRI and MRCP termed the "starry sky" liver (Fig 7A,B)(8, 30, 63). Following administration of intravenous contrast, there might be thin, smooth, enhancing rim which is often more apparent on MRI compared to CT(8). However, when the lesions consist of primarily fibrous stoma and minimal ductal dilatation, they may demonstrate mild T2-hyperintensity or may not even be apparent on cross sectional imaging. On ultrasound, tiny individual lesions cannot be resolved and are instead interpreted as diffuse heterogenous liver echotexture. Larger ones (>10 mm) may appear hypoechoic or anechoic and comet-tail artifact may be seen (29).

2.5.5 Pathologic findings: VMCs are asymptomatic, commonly encountered during laparotomy and are frequently sent for frozen section to rule out metastasis. VMCs may be present throughout the liver, however, grossly they are most easily recognized on the capsular surface as 1-5mm whitish nodules (Fig 8A)(38). They are also discovered incidentally in liver biopsies, resections or at autopsy(62). VMCs consist of dilated irregular small ducts lined by biliary epithelium, which are embedded in a fibrous, and often hyalinized stroma (Fig 8B,C)(38). Often the background liver is unremarkable, but sometimes they can be seen associated with other liver diseases and cirrhosis (Fig 8D). The lining epithelium can be columnar but tends to become cuboidal or flat as the ducts become more dilated. Inspissated bile within dilated lumina is common. Secondary changes like hyaline fibrosis, extensive cystic change, inflammation, and calcification may occur. Some lesions may undergo extensive sclerosis or cystic change such that their underlying nature may become impossible to recognize. These need to be differentiated clinically form bile duct adenoma, cholangiocarcinoma and metastasis. Some VMCs show bile duct adenoma immediately adjacent to it, and some have overlapping features with it, where the differentiation is often arbitrary and subjective. In addition, their co-occurrence in some cases argues that these may be related.

2.6 Non-genetic liver cysts

The differential diagnosis of polycystic or fibrocystic liver disease includes benign, malignant, and infectious etiologies. Fungal micro-abscesses can have similar appearance to multiple biliary hamartoma but present with fever and signs of infection. Echinococcal liver cysts can have similar appearance to sporadic cysts or even PLD, although have a more focal distribution, and require exposure to areas of the world where this parasite lives. Several additional diagnoses can result in

lesions of similar appearance to fibrocystic disease. Many of these lesions such as hemangiomas, angiomyolipomas, or a primary biliary or hepatic malignancy should be easily distinguished radiographically from genetic causes of polycystic and fibrocystic liver disease as they are individual lesions with different imaging characteristics. Metastatic disease to the liver can in some cases require more careful imaging evaluation or biopsy to evaluate fully.

Figure legends.

Figure 1 (A) CT scan and (B) T1-weighted MRI of a 61-year-old male with autosomal dominant polycystic kidney disease (ADPKD) showing innumerable hepatic cysts, some with increased attenuation and signal intensity (arrows) indicative of hemorrhagic or proteinaceous material in the cysts.

Figure 2 (A) Gross photograph of a liver section in a patient with autosomal dominant polycystic kidney disease (ADPKD) showing multiple cysts of varying sizes involving, and enlarging the liver diffusely. The background liver parenchyma grossly looks unremarkable. The gross and histologic changes features are indistinguishable from autosomal dominant polycystic liver disease (ADPLD). (B). Histology of cysts in ADPLD at low magnification showing cysts of various sizes, few Von-Meyenburg complexes and dense fibrosis around the cysts. (C) Higher magnification of the cysts to show denudation of the lining epithelium in some of the cysts and dense hyalinized sclerosis around some of them. (D) Close-up of the cysts showing non-dysplastic low cuboidal to flattened epithelium lining the cysts.

Figure 3 (A) 38-year-old female with congenital hepatic fibrosis. Contrast-enhanced CT shows a cirrhotic-appearing liver along with manifestations of portal hypertension including splenomegaly and portosystemic shunts. (B) T2-weighted MRI in a 40-year-old female with congenital hepatic fibrosis and also showing fusiform dilatation of the intra- and extra-hepatic bile ducts consistent with Caroli syndrome.

Figure 4 (A) Gross photograph of the liver and its cut surface (B) showing diffuse nodularity which is indistinguishable from cirrhosis on gross examination. (C) Histology of congenital hepatic fibrosis at low magnification showing various sized irregular islands of hepatocytes separated by fibrous septa containing ductular profiles that have ductal plate like architecture. (D). Higher magnification showing the ductular structures are irregular in outline, show varying degree of luminal dilation of the and lining of low cuboidal to flattened epithelium. The surrounding connective tissue is very dense and hyalinized.

Figure 5 (A) Coronal contrast enhanced-CT in a 10-month-old male with Caroli disease, congenital hepatic fibrosis, and autosomal recessive polycystic kidney disease (ARPKD) showing portal veins in the center of markedly dilated bile ducts, resulting in the appearance of the "dot sign" on the axial images (B)

Figure 6 (A) Gross photograph of a liver in a patient with Caroli disease with the cut section features showing variable sized cysts that are dilated bile ducts and seem to follow the portal tracts. (B) Histology of cysts at low magnification showing various sized biliary cyst. The fibrosis is seen surrounding the cysts and follows the portal tracts, but the liver lacks the nodularity and extensive septa formations seen with Congenital hepatic fibrosis. (C) The cysts contain inspissated bile in the lumen and lined by low columnar to cuboidal biliary type benign epithelium. (D) A biopsy showing a fibrous septum with few irregular biliary structures on one side of the biopsy along with biliary type cysts on the other side of the biopsy suggestive of a Caroli syndrome.

Figure 7 (A) MRI showing diffuse, uniform, tiny cystic lesions that do not communicate with the bile ducts. (B) Appearance on T2-weighted MRI and MRCP termed the "starry sky" liver, is characteristic of Von-Meyenburg complexes.

Figure 8 (A) Gross photograph of a liver in a patient with multiple Von-Meyenburg complexes. The cut section fails to show the lesions as the lesions are very tiny and unless show secondary changes like extensive cystic change, calcifications or enlargement, these are not grossly visible on the cut section, but otherwise are easily seen (B) as tiny small while nodules on the capsular surface (inset). Von-Meyenburg complexes shows irregular ductular structures, often containing inspissated bile in the lumen and lined by low cuboidal to flattened epithelium. The surrounding connective tissue is very dense and frequently hyalinized. Adjacent liver parenchyma appears normal without any fibrosis. (C) A case of cirrhosis due to hepatitis C virus infection showing incidental multiple scattered Von-Meyenburg complexes in the fibrous septa. Finding incidental Von-Meyenburg complexes associated with any other liver disease is not uncommon.

References

1. Rawla P, Sunkara T, Muralidharan P, Raj JP. An updated review of cystic hepatic lesions. Clin Exp Hepatol. 2019;5(1):22-9.
2. Raynaud P, Tate J, Callens C, Cordi S, Vandersmissen P, Carpentier R, et al. A classification of ductal plate malformations based on distinct pathogenic mechanisms of biliary dysmorphogenesis. Hepatology. 2011;53(6):1959-66.
3. Gordon SC. Update on Polycystic Liver Disease. Gastroenterol Hepatol (N Y). 2020;16(1):39-41.
4. Lee-Law PY, van de Laarschot LFM, Banales JM, Drenth JPH. Genetics of polycystic liver diseases. Curr Opin Gastroenterol. 2019;35(2):65-72.
5. Besse W, Dong K, Choi J, Punia S, Fedeles SV, Choi M, et al. Isolated polycystic liver disease genes define effectors of polycystin-1 function. J Clin Invest. 2017;127(9):3558.
6. Hildebrandt F, Zhou W. Nephronophthisis-associated ciliopathies. J Am Soc Nephrol. 2007;18(6):1855-71.
7. Cornec-Le Gall E, Torres VE, Harris PC. Genetic Complexity of Autosomal Dominant Polycystic Kidney and Liver Diseases. J Am Soc Nephrol. 2018;29(1):13-23.
8. Bae KT, Zhu F, Chapman AB, Torres VE, Grantham JJ, Guay-Woodford LM, et al. Magnetic resonance imaging evaluation of hepatic cysts in early autosomal-dominant polycystic kidney disease: the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease cohort. Clin J Am Soc Nephrol. 2006;1(1):64-9.
9. Ma M, Tian X, Igarashi P, Pazour GJ, Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. Nat Genet. 2013;45(9):1004-12.
10. Fedeles SV, Gallagher AR, Somlo S. Polycystin-1: a master regulator of intersecting cystic pathways. Trends Mol Med. 2014;20(5):251-60.
11. Li A, Davila S, Furu L, Qian Q, Tian X, Kamath PS, et al. Mutations in PRKCSH cause isolated autosomal dominant polycystic liver disease. Am J Hum Genet. 2003;72(3):691-703.
12. Drenth JP, te Morsche RH, Smink R, Bonifacino JS, Jansen JB. Germline mutations in PRKCSH are associated with autosomal dominant polycystic liver disease. Nat Genet. 2003;33(3):345-7.
13. Davila S, Furu L, Gharavi AG, Tian X, Onoe T, Qian Q, et al. Mutations in SEC63 cause autosomal dominant polycystic liver disease. Nat Genet. 2004;36(6):575-7.
14. Porath B, Gainullin VG, Cornec-Le Gall E, Dillinger EK, Heyer CM, Hopp K, et al. Mutations in GANAB, Encoding the Glucosidase IIalpha Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. Am J Hum Genet. 2016;98(6):1193-207.
15. Besse W, Dong K, Choi J, Punia S, Fedeles SV, Choi M, et al. Isolated polycystic liver disease genes define effectors of polycystin-1 function. J Clin Invest. 2017;127(5):1772-85.
16. Cornec-Le Gall E, Olson RJ, Besse W, Heyer CM, Gainullin VG, Smith JM, et al. Monoallelic Mutations to DNAJB11 Cause Atypical Autosomal-Dominant Polycystic Kidney Disease. Am J Hum Genet. 2018;102(5):832-44.
17. Besse W, Chang AR, Luo JZ, Triffo WJ, Moore BS, Gulati A, et al. ALG9 Mutation Carriers Develop Kidney and Liver Cysts. J Am Soc Nephrol. 2019;30(11):2091-102.
18. Qian F, Watnick TJ, Onuchic LF, Germino GG. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. Cell. 1996;87(6):979-87.

19. Wu G, D'Agati V, Cai Y, Markowitz G, Park JH, Reynolds DM, et al. Somatic inactivation of Pkd2 results in polycystic kidney disease. Cell. 1998;93(2):177-88.
20. Watnick TJ, Torres VE, Gandolph MA, Qian F, Onuchic LF, Klinger KW, et al. Somatic mutation in individual liver cysts supports a two-hit model of cystogenesis in autosomal dominant polycystic kidney disease. Mol Cell. 1998;2(2):247-51.
21. Janssen MJ, Salomon J, Cnossen WR, Bergmann C, Pfundt R, Drenth JP. Somatic loss of polycystic disease genes contributes to the formation of isolated and polycystic liver cysts. Gut. 2015;64(4):688-90.
22. Tan AY, Zhang T, Michaeel A, Blumenfeld J, Liu G, Zhang W, et al. Somatic Mutations in Renal Cyst Epithelium in Autosomal Dominant Polycystic Kidney Disease. J Am Soc Nephrol. 2018;29(8):2139-56.
23. Piontek KB, Huso DL, Grinberg A, Liu L, Bedja D, Zhao H, et al. A functional floxed allele of Pkd1 that can be conditionally inactivated in vivo. J Am Soc Nephrol. 2004;15(12):3035-43.
24. Fedeles SV, Tian X, Gallagher AR, Mitobe M, Nishio S, Lee SH, et al. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. Nat Genet. 2011;43(7):639-47.
25. Pei Y, Obaji J, Dupuis A, Paterson AD, Magistroni R, Dicks E, et al. Unified criteria for ultrasonographic diagnosis of ADPKD. J Am Soc Nephrol. 2009;20(1):205-12.
26. Feldman M. Polycystic disease of the liver. Am J Gastroenterol. 1958;29(1):83-6.
27. Comfort MW, Gray HK, Dahlin DC, Whitesell FB, Jr. Polycystic disease of the liver: a study of 24 cases. Gastroenterology. 1952;20(1):60-78.
28. Karhunen PJ, Tenhu M. Adult polycystic liver and kidney diseases are separate entities. Clin Genet. 1986;30(1):29-37.
29. Torres VE, Rossetti S, Harris PC. Update on autosomal dominant polycystic kidney disease. Minerva Med. 2007;98(6):669-91.
30. Qian Q, Li A, King BF, Kamath PS, Lager DJ, Huston J, 3rd, et al. Clinical profile of autosomal dominant polycystic liver disease. Hepatology. 2003;37(1):164-71.
31. Van Keimpema L, De Koning DB, Van Hoek B, Van Den Berg AP, Van Oijen MG, De Man RA, et al. Patients with isolated polycystic liver disease referred to liver centres: clinical characterization of 137 cases. Liver Int. 2011;31(1):92-8.
32. Arnold HL, Harrison SA. New advances in evaluation and management of patients with polycystic liver disease. Am J Gastroenterol. 2005;100(11):2569-82.
33. Kwok MK, Lewin KJ. Massive hepatomegaly in adult polycystic liver disease. Am J Surg Pathol. 1988;12(4):321-4.
34. Srivastava A, Patel N. Autosomal dominant polycystic kidney disease. Am Fam Physician. 2014;90(5):303-7.
35. van Aerts RMM, Bernts LHP, Gevers TJG, Kievit W, Koopmans L, Nieboer TE, et al. Estrogen-Containing Oral Contraceptives Are Associated With Polycystic Liver Disease Severity in Premenopausal Patients. Clin Pharmacol Ther. 2019;106(6):1338-45.
36. D'Agnolo HM, Drenth JP. Risk factors for progressive polycystic liver disease: where do we stand? Nephrol Dial Transplant. 2016;31(6):857-9.
37. Luciano RL, Dahl NK. Extra-renal manifestations of autosomal dominant polycystic kidney disease (ADPKD): considerations for routine screening and management. Nephrol Dial Transplant. 2014;29(2):247-54.

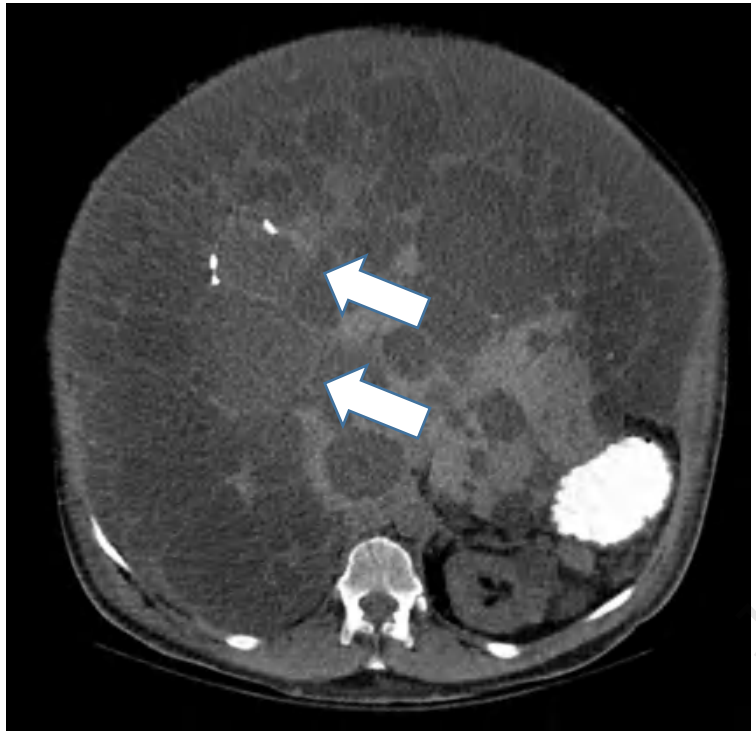
38. Desmet VJ. [Cystic diseases of the liver. From embryology to malformations]. Gastroenterol Clin Biol. 2005;29(8-9):858-60.
39. Gunay-Aygun M, Gahl WA, Heller T. Congenital Hepatic Fibrosis Overview horizontal line RETIRED CHAPTER, FOR HISTORICAL REFERENCE ONLY. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, et al., editors. GeneReviews((R)). Seattle (WA)1993.
40. Schueler M, Braun DA, Chandrasekar G, Gee HY, Klasson TD, Halbritter J, et al. DCDC2 mutations cause a renal-hepatic ciliopathy by disrupting Wnt signaling. Am J Hum Genet. 2015;96(1):81-92.
41. Guay-Woodford LM, Desmond RA. Autosomal recessive polycystic kidney disease: the clinical experience in North America. Pediatrics. 2003;111(5 Pt 1):1072-80.
42. Furu L, Onuchic LF, Gharavi A, Hou X, Esquivel EL, Nagasawa Y, et al. Milder presentation of recessive polycystic kidney disease requires presence of amino acid substitution mutations. J Am Soc Nephrol. 2003;14(8):2004-14.
43. Gunay-Aygun M, Tuchman M, Font-Montgomery E, Lukose L, Edwards H, Garcia A, et al. PKHD1 sequence variations in 78 children and adults with autosomal recessive polycystic kidney disease and congenital hepatic fibrosis. Mol Genet Metab. 2010;99(2):160-73.
44. Jiang C, Zhou Q, Jin M, Niu J, Gao Y. Congenital hepatic fibrosis with polycystic kidney disease: Two case reports. Medicine (Baltimore). 2019;98(20):e15600.
45. Waters AM, Beales PL. Ciliopathies: an expanding disease spectrum. Pediatr Nephrol. 2011;26(7):1039-56.
46. Desmet VJ. What is congenital hepatic fibrosis? Histopathology. 1992;20(6):465-77.
47. Zhu B, Du Z, Wang Z, Li Y, Zhang J, Zhu H. Congenital Hepatic Fibrosis in Children and Adults: Clinical Manifestations, Management, and Outcome-Case Series and Literature Review. Gastroenterol Res Pract. 2020;2020:8284274.
48. Wu WK, Ziogas IA, Izzy M, Pai AK, Hafberg ET, Matsuoka LK, et al. Liver transplantation for congenital hepatic fibrosis. Transpl Int. 2021;34(7):1281-92.
49. Bauman ME, Pound DC, Ulbright TM. Hepatocellular carcinoma arising in congenital hepatic fibrosis. Am J Gastroenterol. 1994;89(3):450-1.
50. Scott J, Shousha S, Thomas HC, Sherlock S. Bile duct carcinoma: a late complication of congenital hepatic fibrosis. Case report and review of literature. Am J Gastroenterol. 1980;73(2):113-9.
51. Venkatanarasimha N, Thomas R, Armstrong EM, Shirley JF, Fox BM, Jackson SA. Imaging features of ductal plate malformations in adults. Clin Radiol. 2011;66(11):1086-93.
52. Veigel MC, Prescott-Focht J, Rodriguez MG, Zinati R, Shao L, Moore CA, et al. Fibropolycystic liver disease in children. Pediatr Radiol. 2009;39(4):317-27; quiz 420-1.
53. Zeitoun D, Brancatelli G, Colombat M, Federle MP, Valla D, Wu T, et al. Congenital hepatic fibrosis: CT findings in 18 adults. Radiology. 2004;231(1):109-16.
54. Brancatelli G, Federle MP, Vilgrain V, Vullierme MP, Marin D, Lagalla R. Fibropolycystic liver disease: CT and MR imaging findings. Radiographics. 2005;25(3):659-70.
55. Bayraktar Y, Balkanci F, Kayhan B, Uzunalimoglu B, Ozenc A, Ozdemir A, et al. Congenital hepatic fibrosis associated with cavernous transformation of the portal vein. Hepatogastroenterology. 1997;44(18):1588-94.
56. Odievre M, Chaumont P, Montagne JP, Alagille D. Anomalies of the intrahepatic portal venous system in congenital hepatic fibrosis. Radiology. 1977;122(2):427-30.

57. Averback P. Developmental arachnoid cysts of the posterior fossa-an analysis of 13 cases. Acta Neurochir (Wien). 1977;39(3-4):181-6.
58. Wang ZX, Li YG, Wang RL, Li YW, Li ZY, Wang LF, et al. Clinical classification of Caroli's disease: an analysis of 30 patients. HPB (Oxford). 2015;17(3):278-83.
59. Mamone G, Carollo V, Cortis K, Aquilina S, Liotta R, Miraglia R. Magnetic resonance imaging of fibropolycystic liver disease: the spectrum of ductal plate malformations. Abdom Radiol (NY). 2019;44(6):2156-71.
60. Turkbey B, Ocak I, Daryanani K, Font-Montgomery E, Lukose L, Bryant J, et al. Autosomal recessive polycystic kidney disease and congenital hepatic fibrosis (ARPKD/CHF). Pediatr Radiol. 2009;39(2):100-11.
61. Gunay-Aygun M, Turkbey BI, Bryant J, Daryanani KT, Gerstein MT, Piwnica-Worms K, et al. Hepatorenal findings in obligate heterozygotes for autosomal recessive polycystic kidney disease. Mol Genet Metab. 2011;104(4):677-81.
62. Redston MS, Wanless IR. The hepatic von Meyenburg complex: prevalence and association with hepatic and renal cysts among 2843 autopsies [corrected]. Mod Pathol. 1996;9(3):233-7.
63. Giambelluca D, Caruana G, Cannella R, Lo Re G, Midiri M. The starry sky liver: multiple biliary hamartomas on MR cholangiopancreatography. Abdom Radiol (NY). 2018;43(9):2529-30.

Table 1. Liver phenotypes associated with ductal plate malformations

Lesion/ disease	Age/Gender	Genes	Chromosome	Mode of inheritance	Other organs involved	Associated VMC	Comments
CHF	Neonates to Young adults (<30Y)	*PKHD1*	6p12.2	AR (mostly), AD and X-linked rarely	Kidney (ARPKD)	Yes	Cholangiocarcinoma, Portal hypertension, rarely HCC
Caroli disease	Adolescents (<30Y)	*PKHD1*	6p12.2	AR (mostly), AD and X-linked rarely	Kidney (ARPKD)	Yes	Cholangiocarcinoma, Cholangitis
Multiple VMCs	Adults to elderly	*unknown*		No established inheritance pattern	Sometimes Kidney	Yes	Cholangiocarcinoma, rarely HCC
PCLD	30-50Y	*PRKCSH* *SEC63*	19p13.2 6q21	AD	some genes with variable	Yes	?

(Isolated liver, also known as ADPLD)		*LRP5* *ALG8* *ALG9* *GANAB* *SEC61B* *DNAJB11* *PKHD1*	16q13.2		kidney/liver cyst distribution		
PLD in association with ADPKD	30-50Y	*PKD1* *PKD2*	16p13.3 4q21	AD	Kidney	Yes	?



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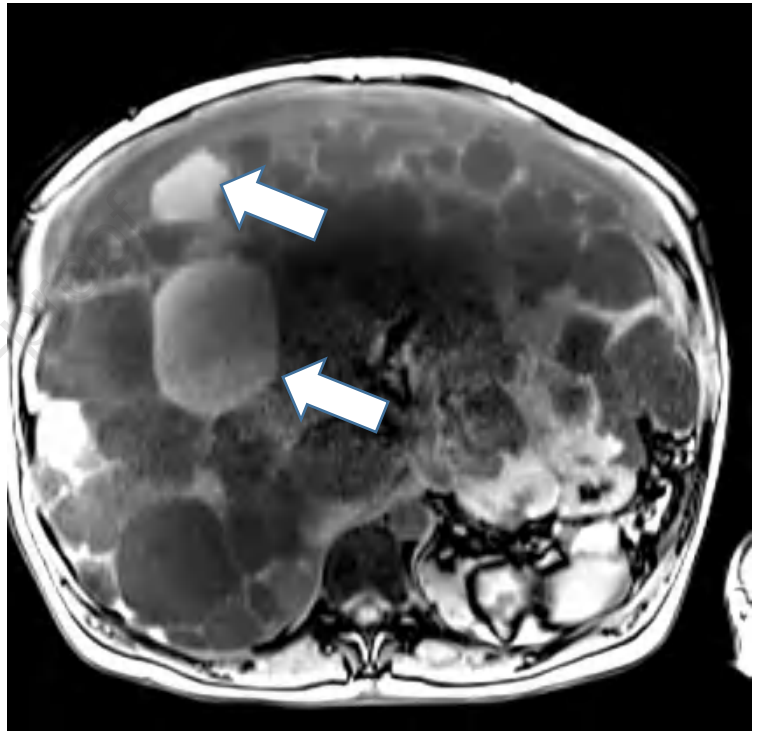
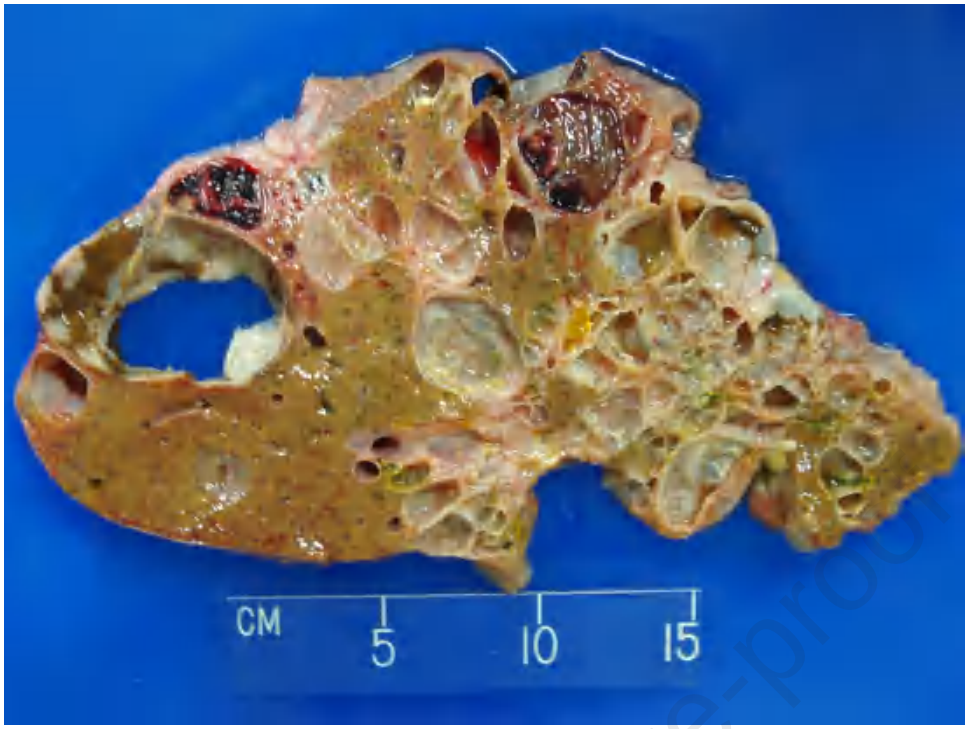
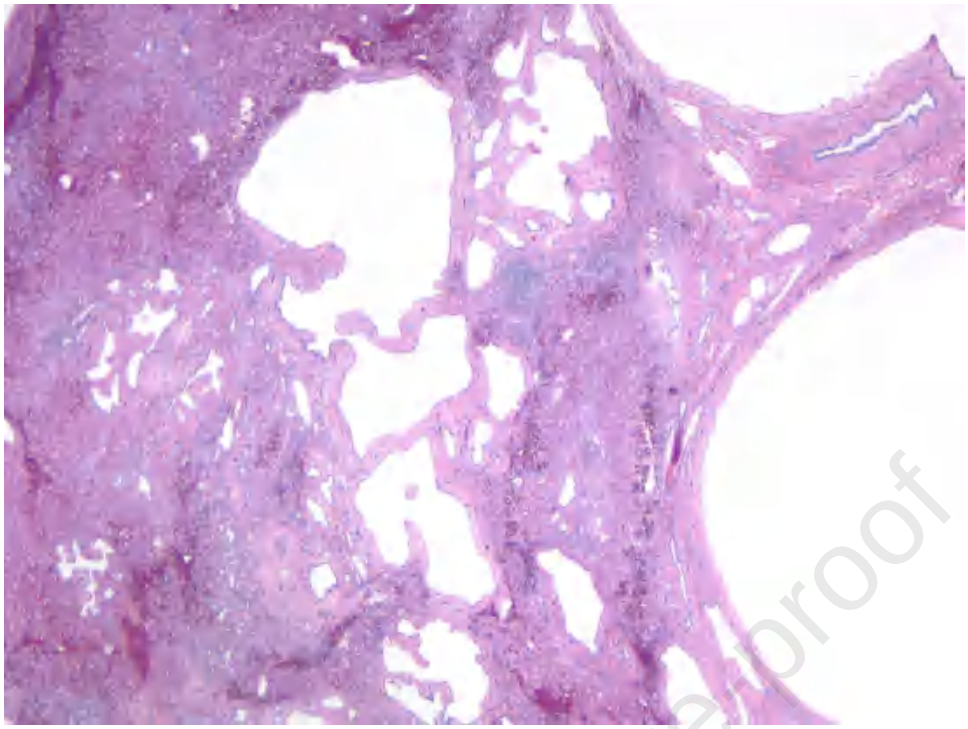


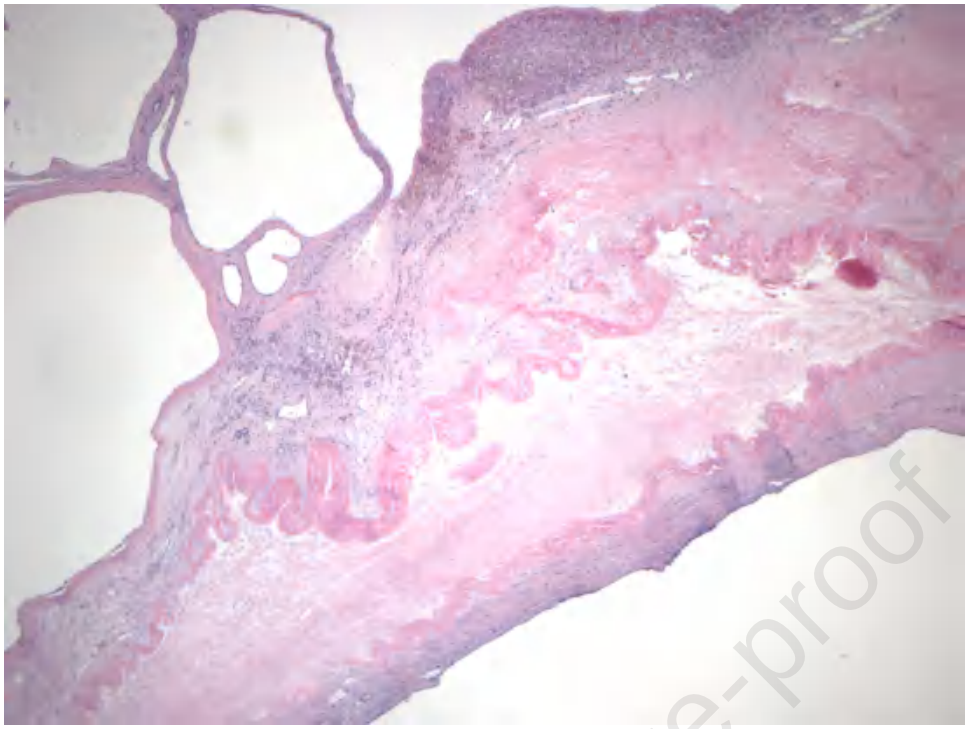
B

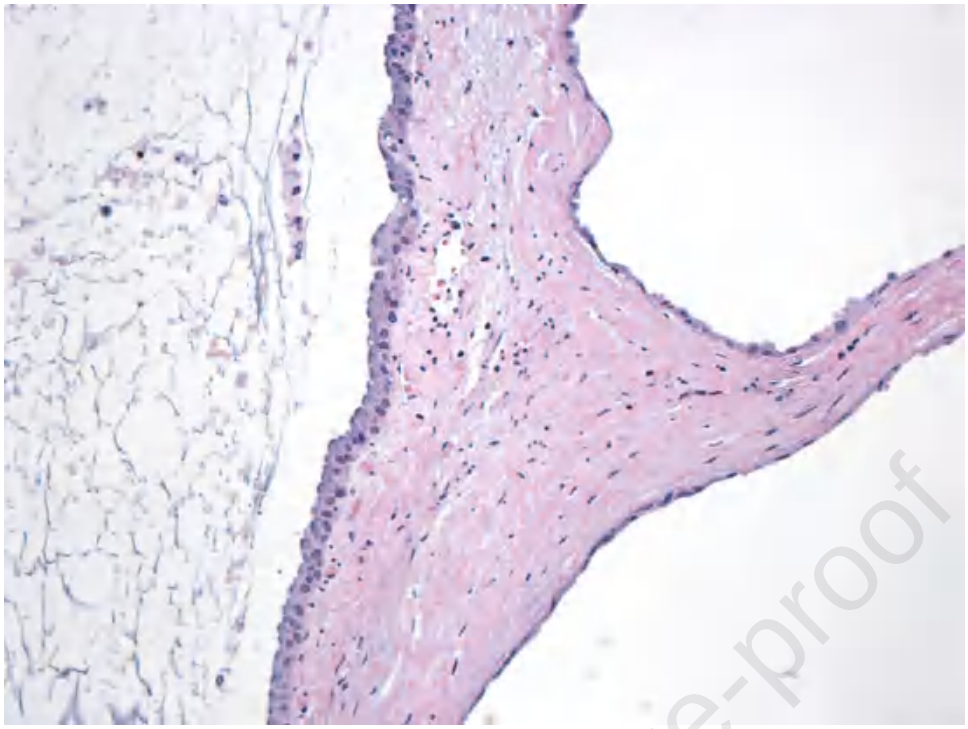
61 year old male with APCKD

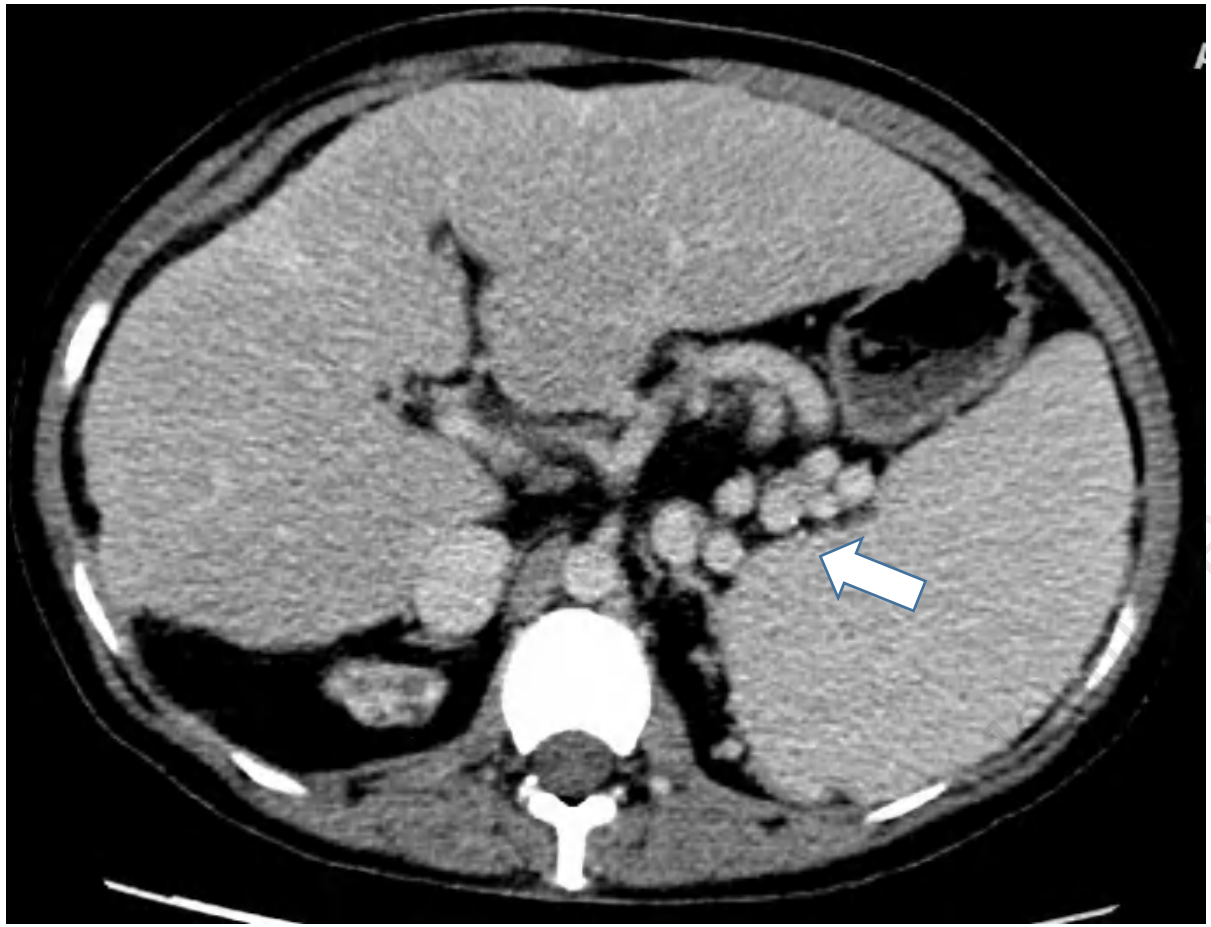
CT (A) and T1-weighted MRI (B) show innumerable hepatic cysts, some with increased attenuation and signal intensity (arrows).











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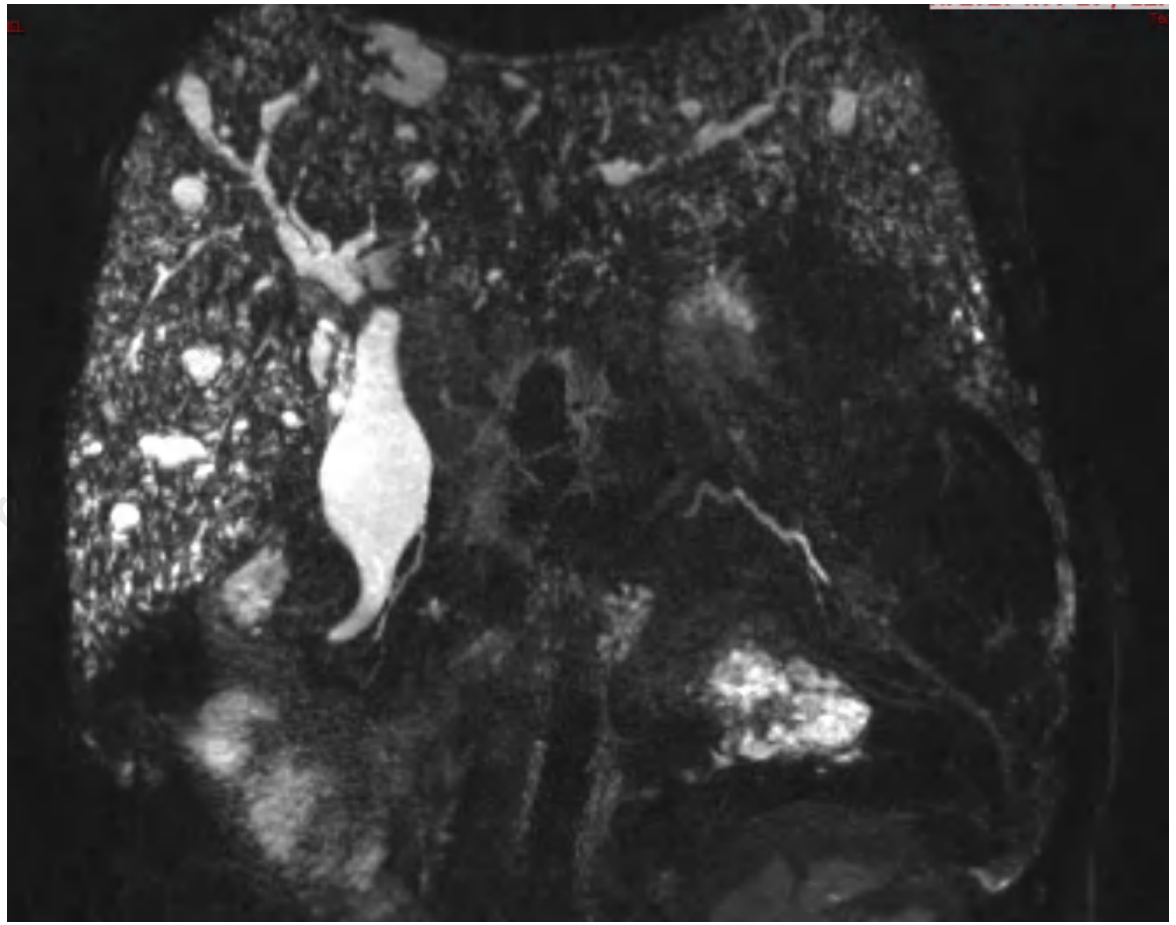

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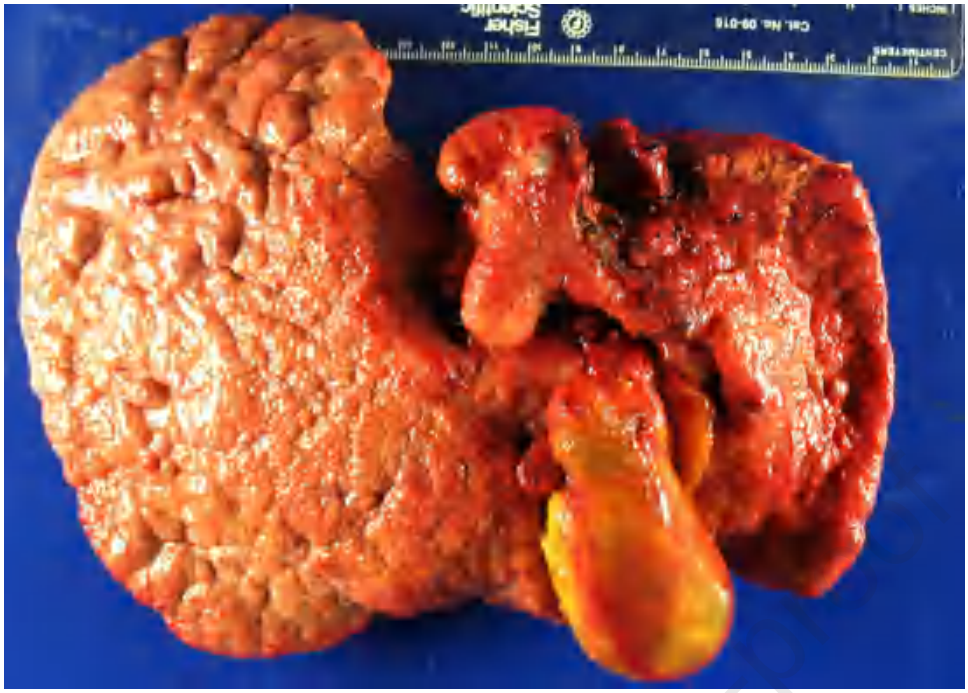

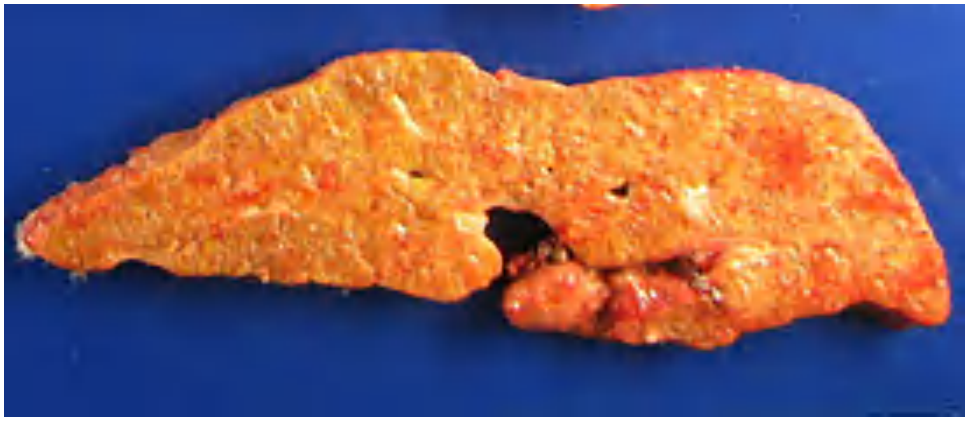

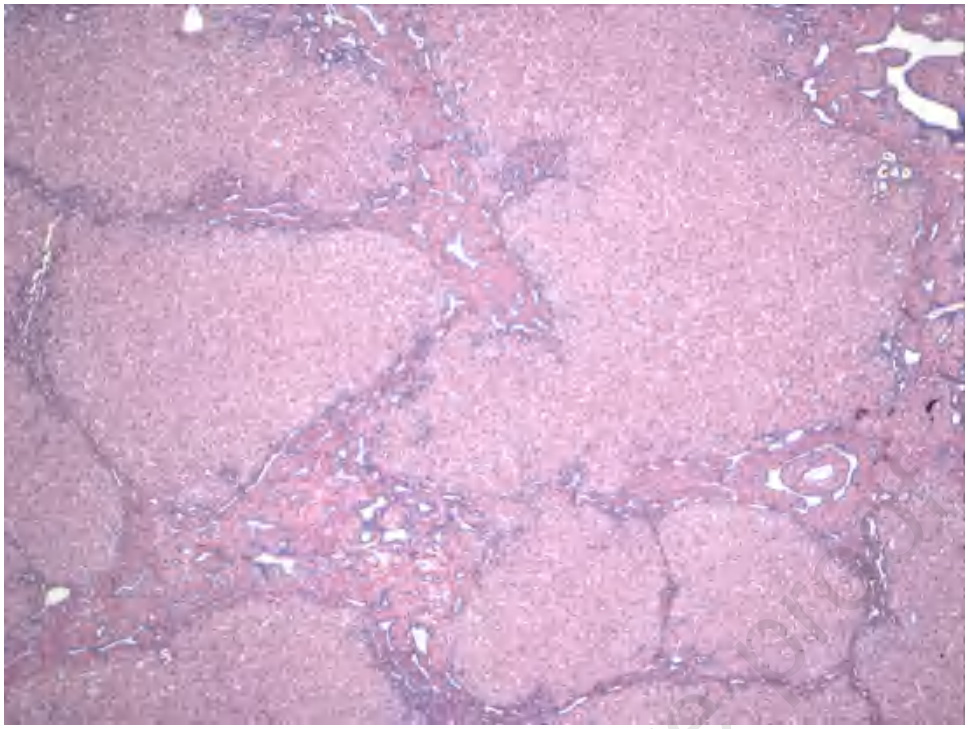

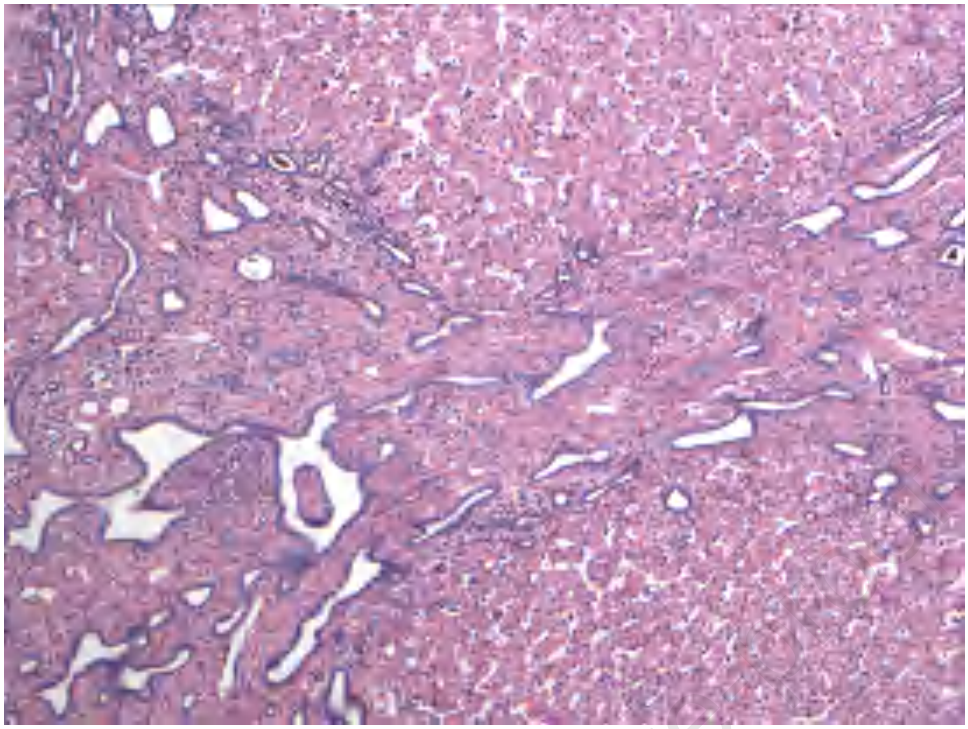

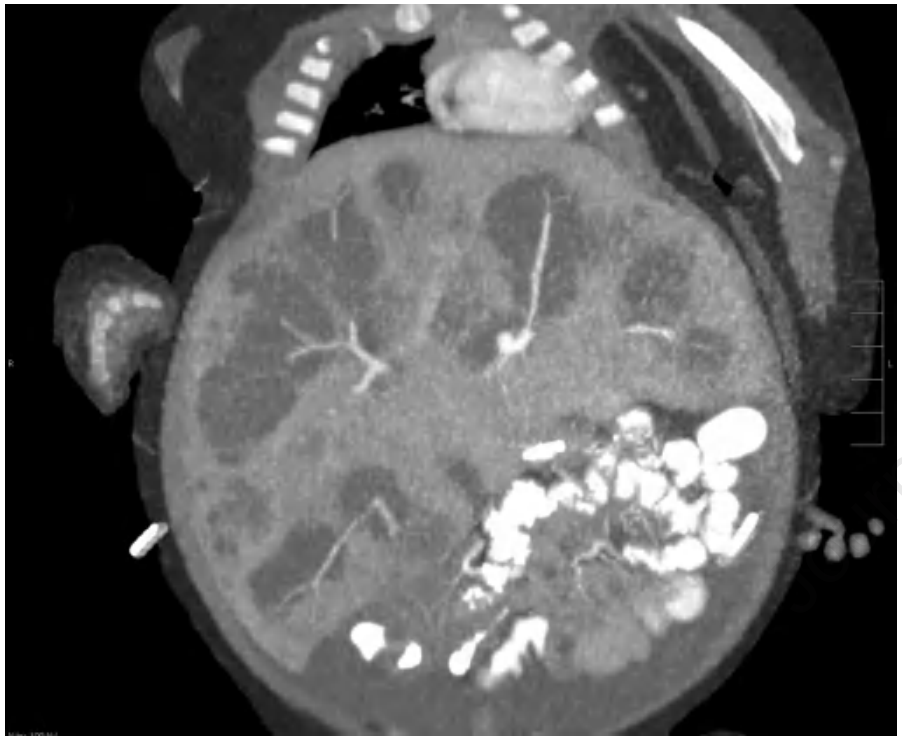

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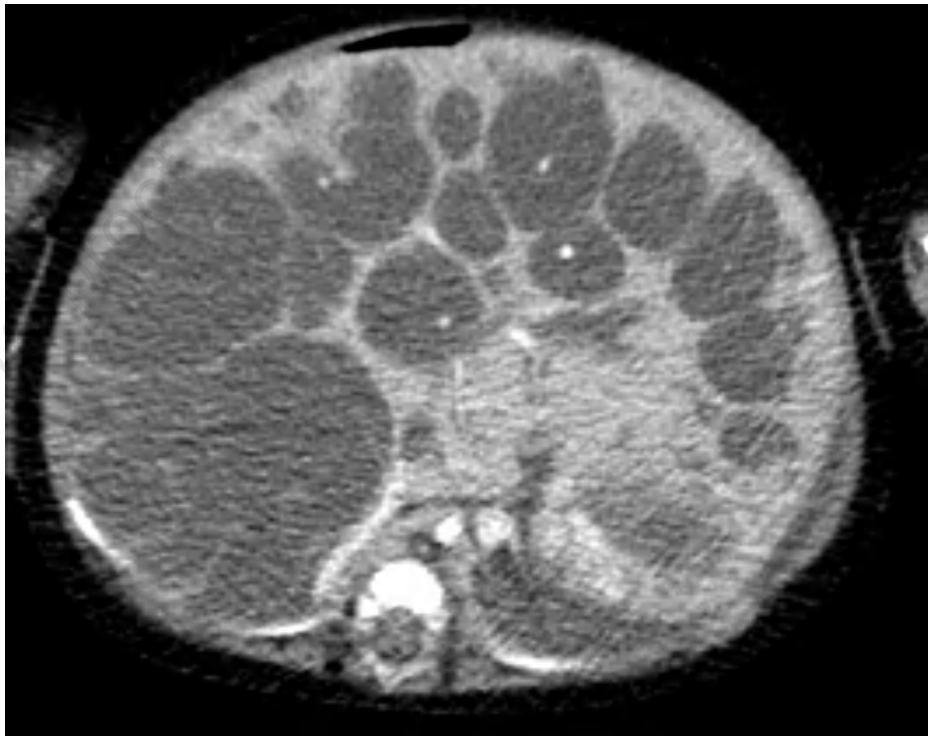

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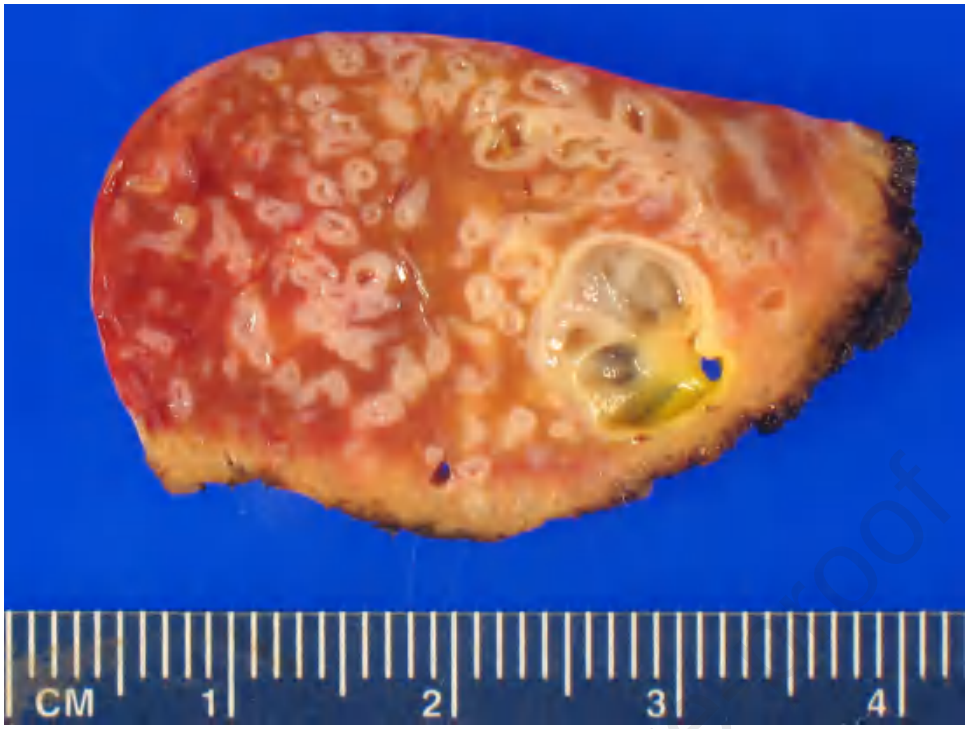

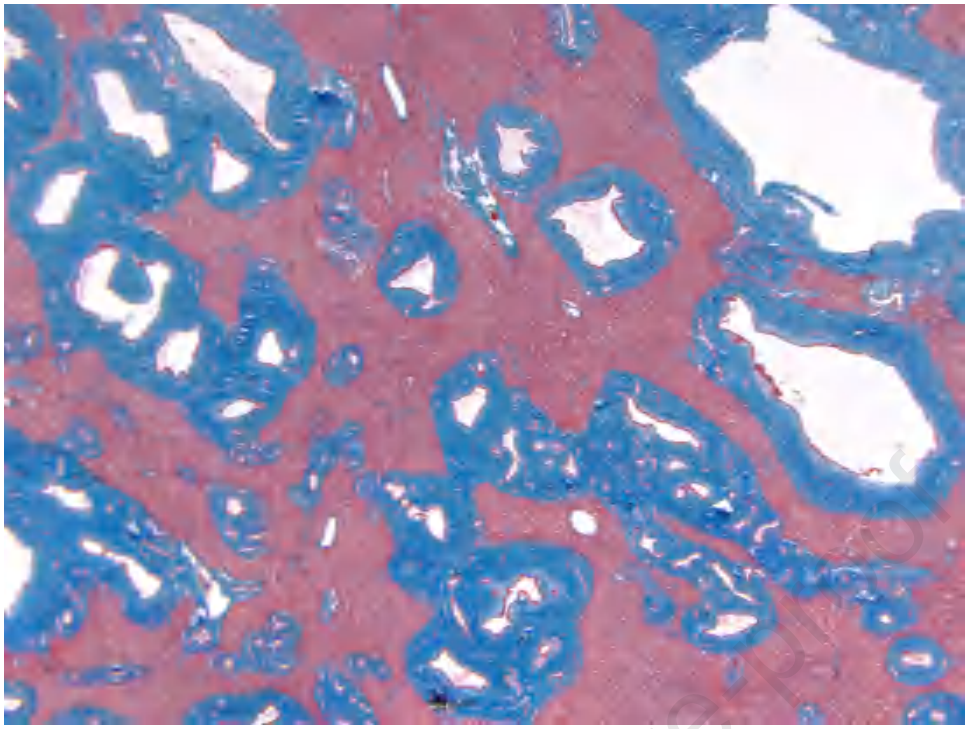

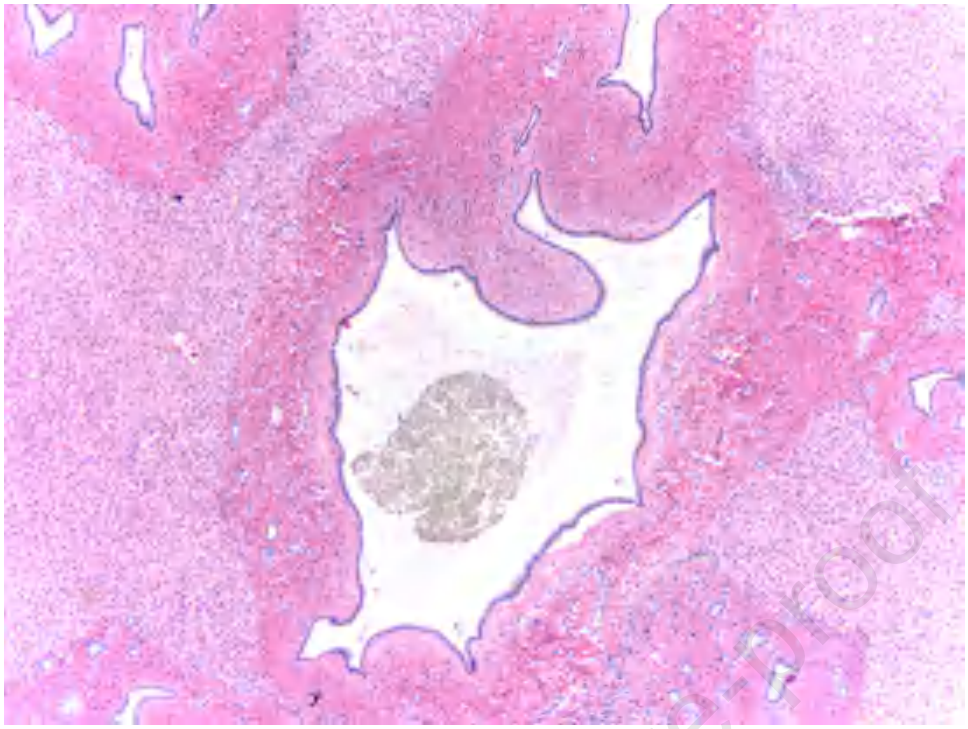

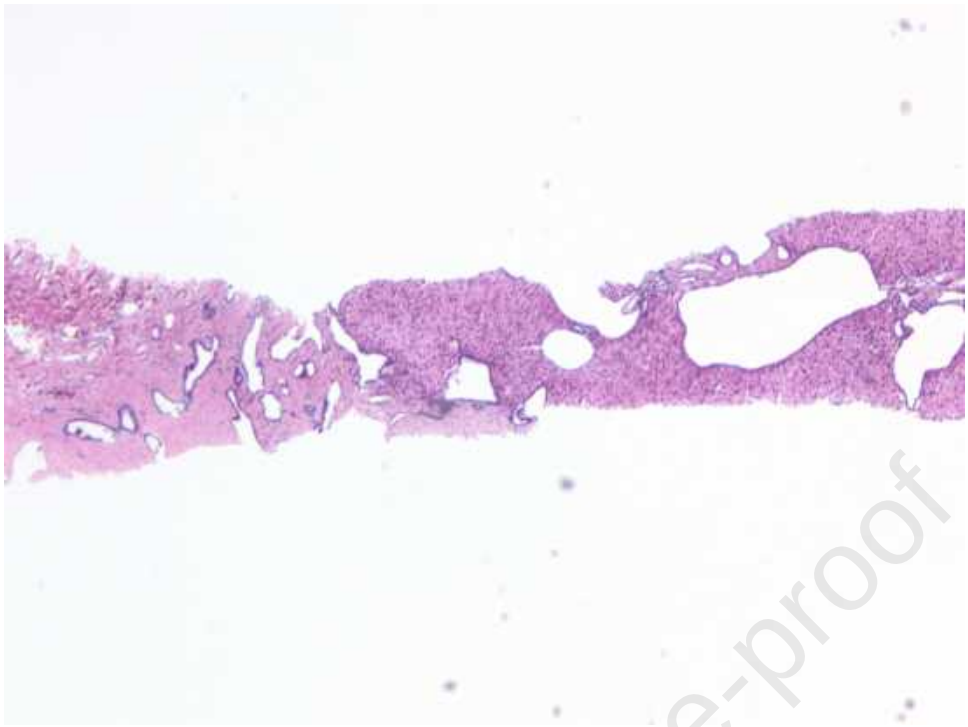

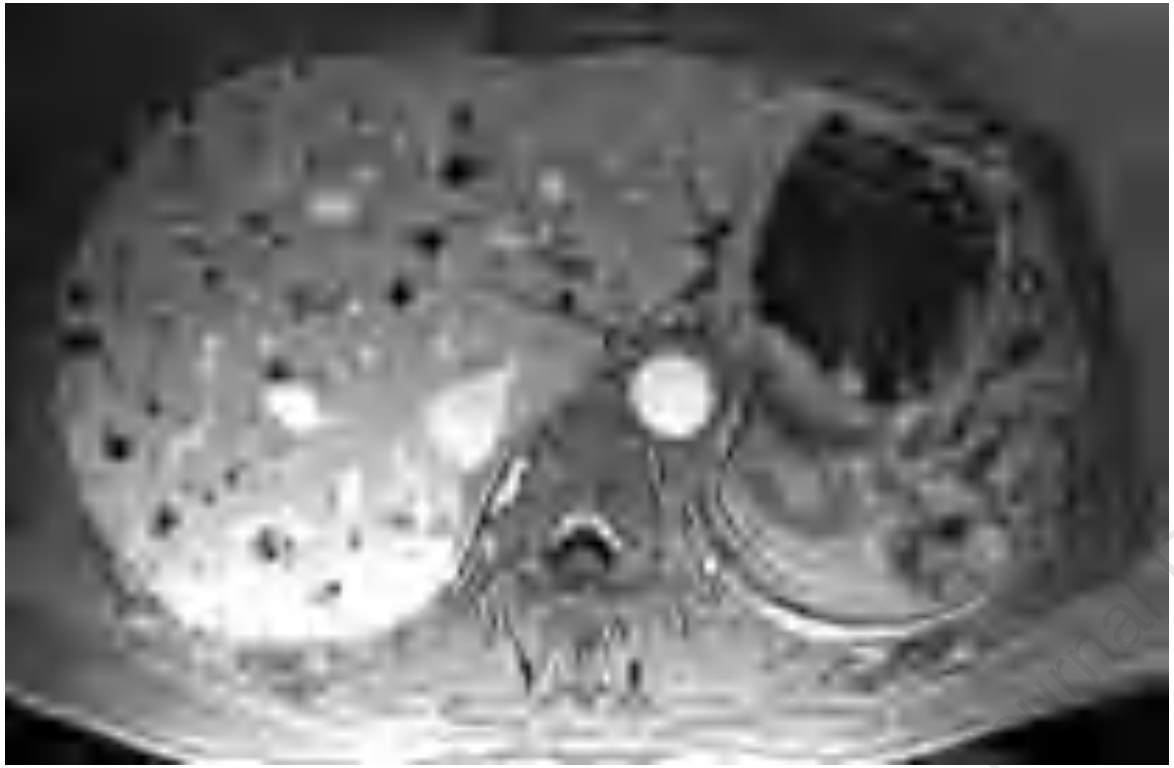

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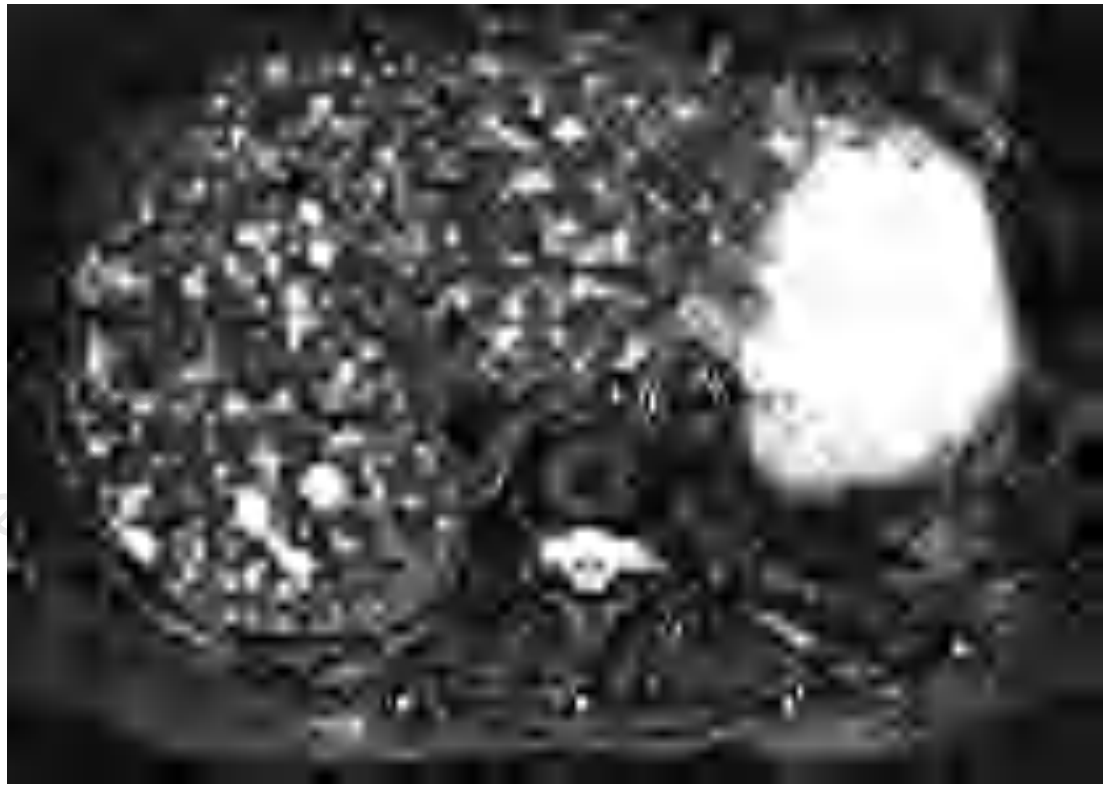

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