Adult onset of bile transport defects & small-duct PSC

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Outline

physiology of canalicular transport

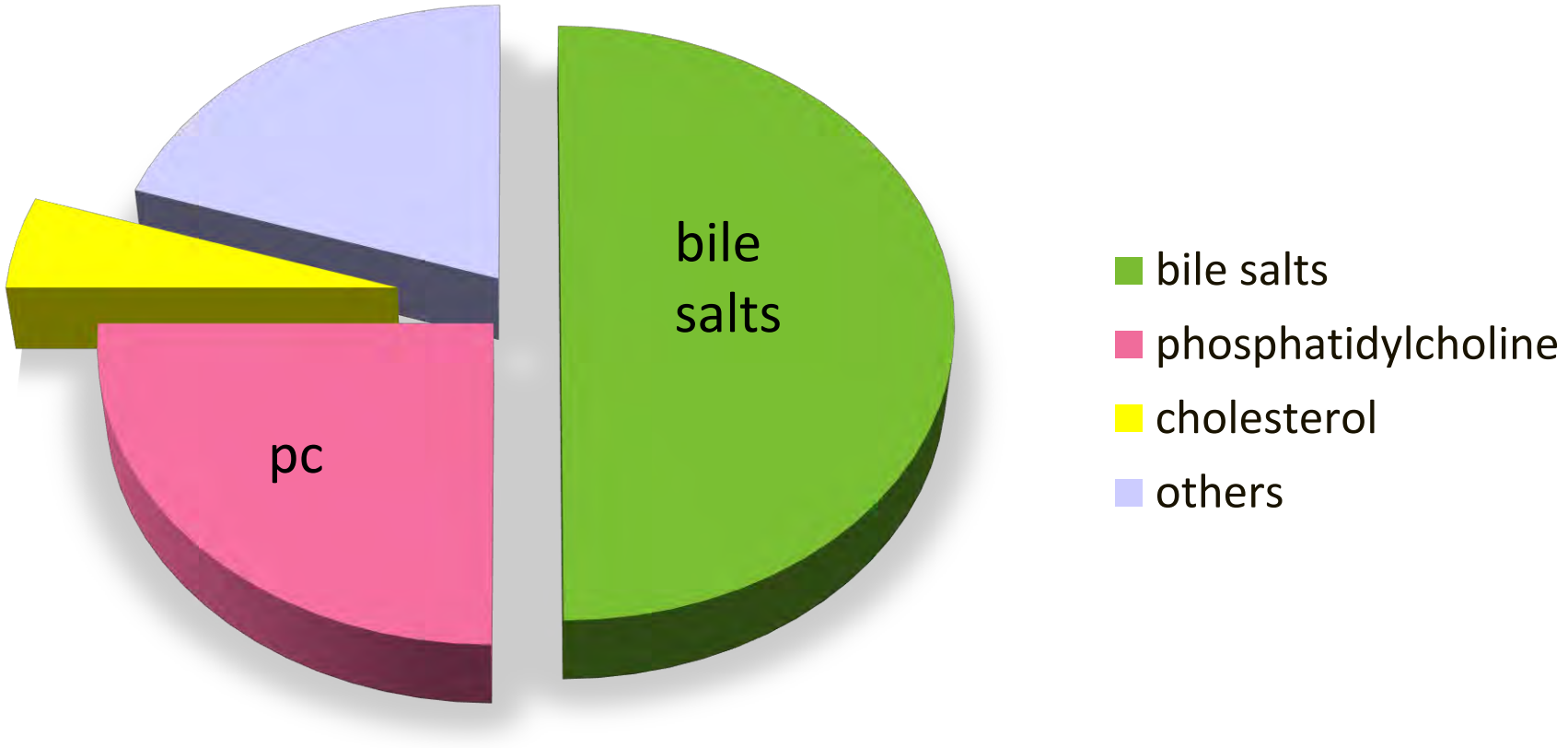
clinical changes in mild transporter deficiency

histology

extending the genetic causes

small-duct primary sclerosing cholangitis

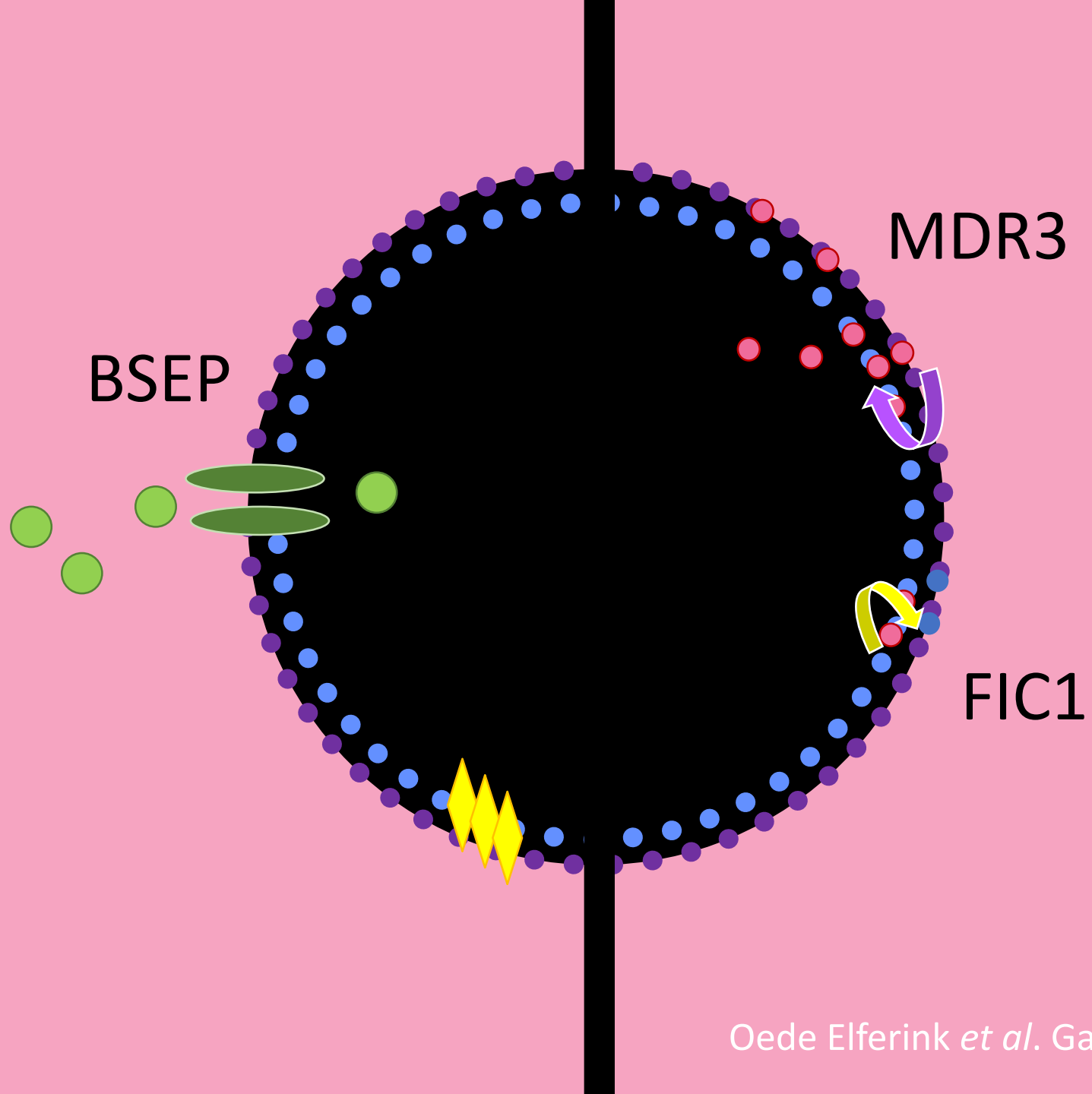
Components of bile



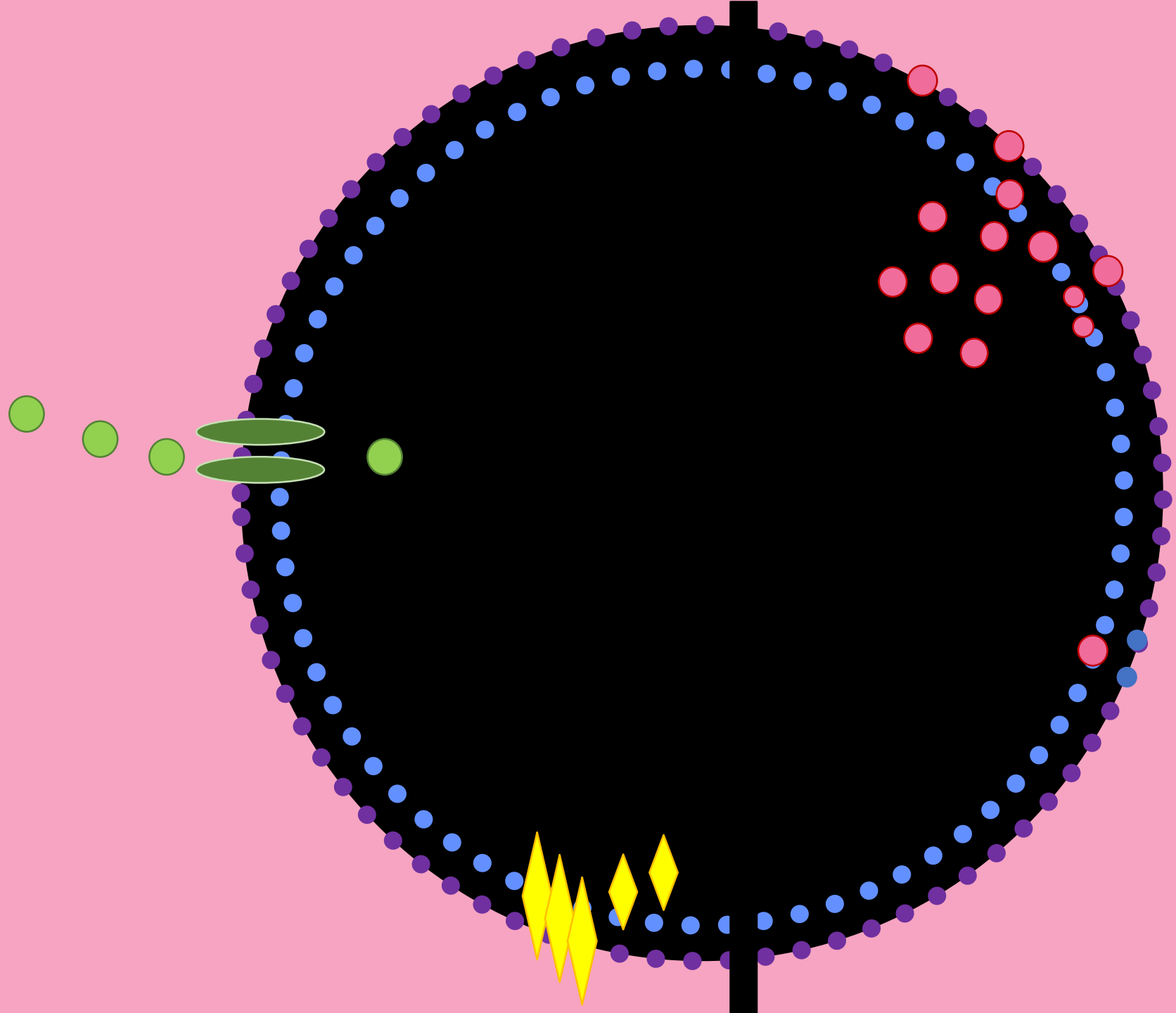
modified from Linton KJ. Biochem Soc Trans 2015; 43(5):1003-10

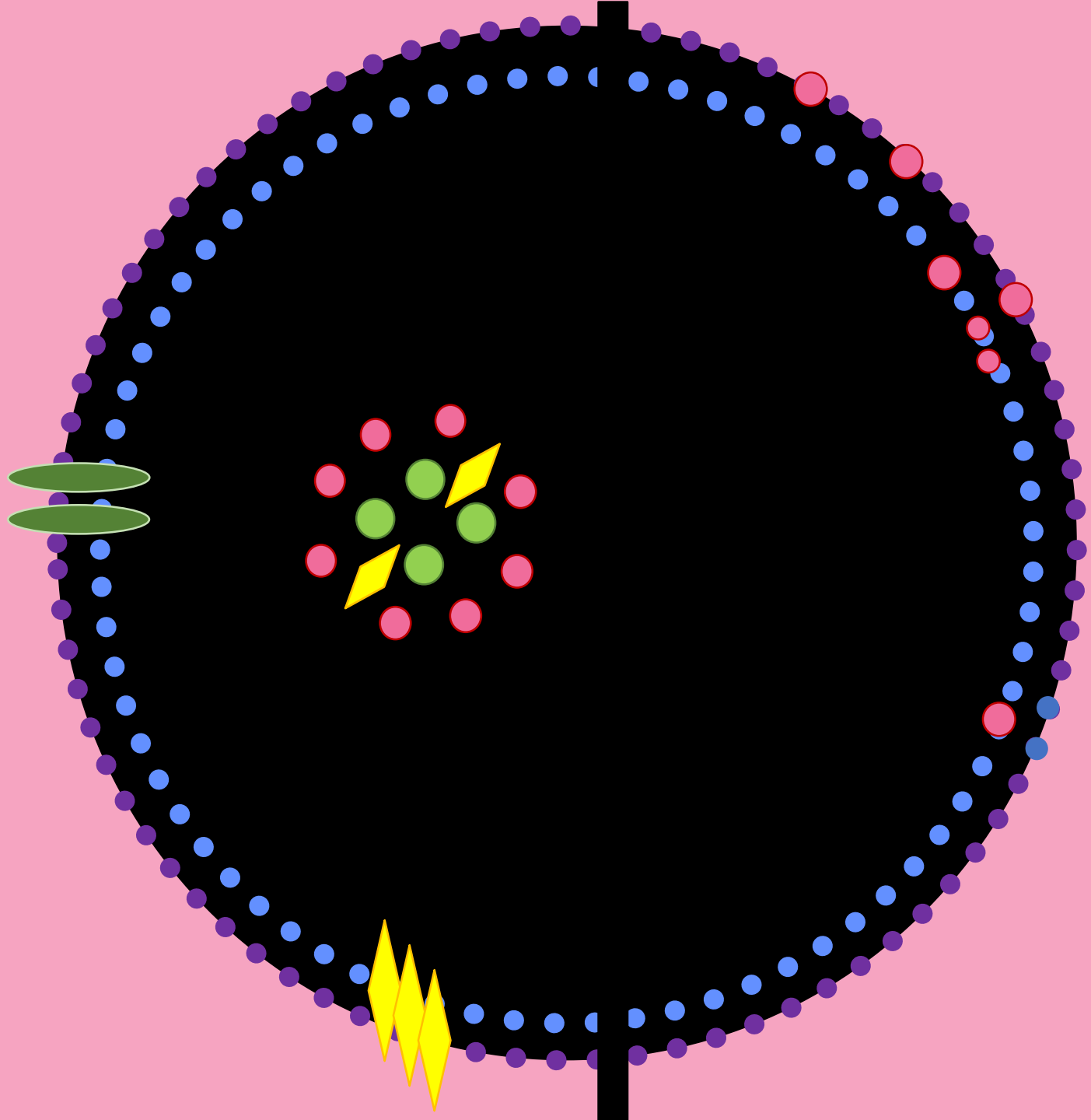
Inherited cholestasis – 3 key canalicular transporters

transporter	*gene*	syndrome	action
FIC1	*ATP8B1*	PFIC1	flips phosphatidylserine (ps)
BSEP	*ABCB11*	PFIC2	transports bile salts
MDR3	*ABCB4*	PFIC3	flops phosphatidylcholine (pc)



Oede Elferink *et al*. Gastroenterology 2006





What could possibly go wrong?

Severe disease – pediatric

biallelic mutation - both genes

- *ATP8B1* (FIC1) PFIC1
- *ABCB11* (BSEP) PFIC2
- *ABCB4* (MDR3) PFIC3

What could possibly go wrong?

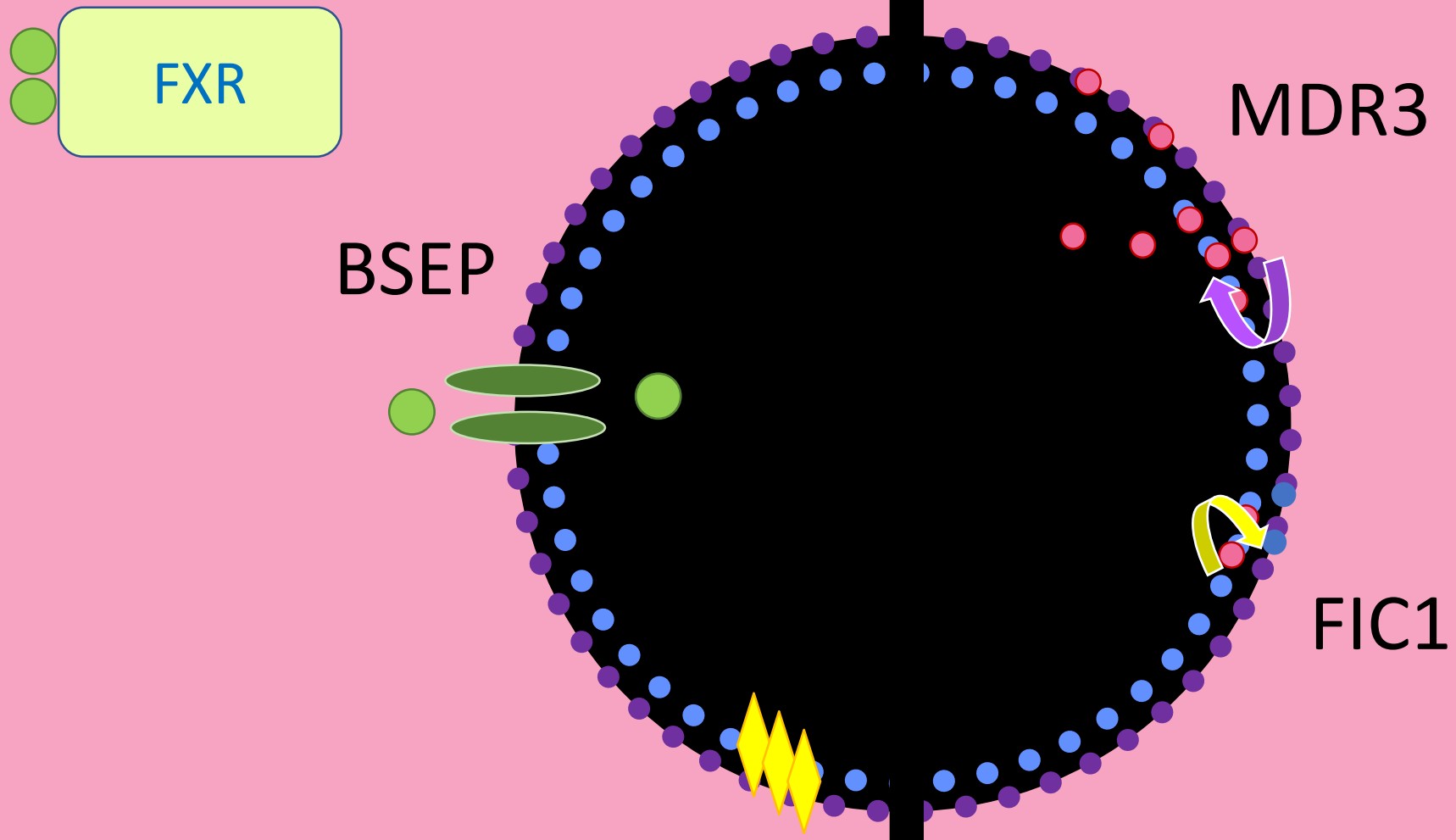
Mild disease – adult

❖ heterozygous mutation

- *ATP8B1* (FIC1) — cholestasis
- *ABCB11* (BSEP) — insufficient bile salt osmosis
  > leads to cholestasis
- *ABCB4* (MDR3) — micelle formation fails
  > bile salt toxicity
  > cholesterol stones

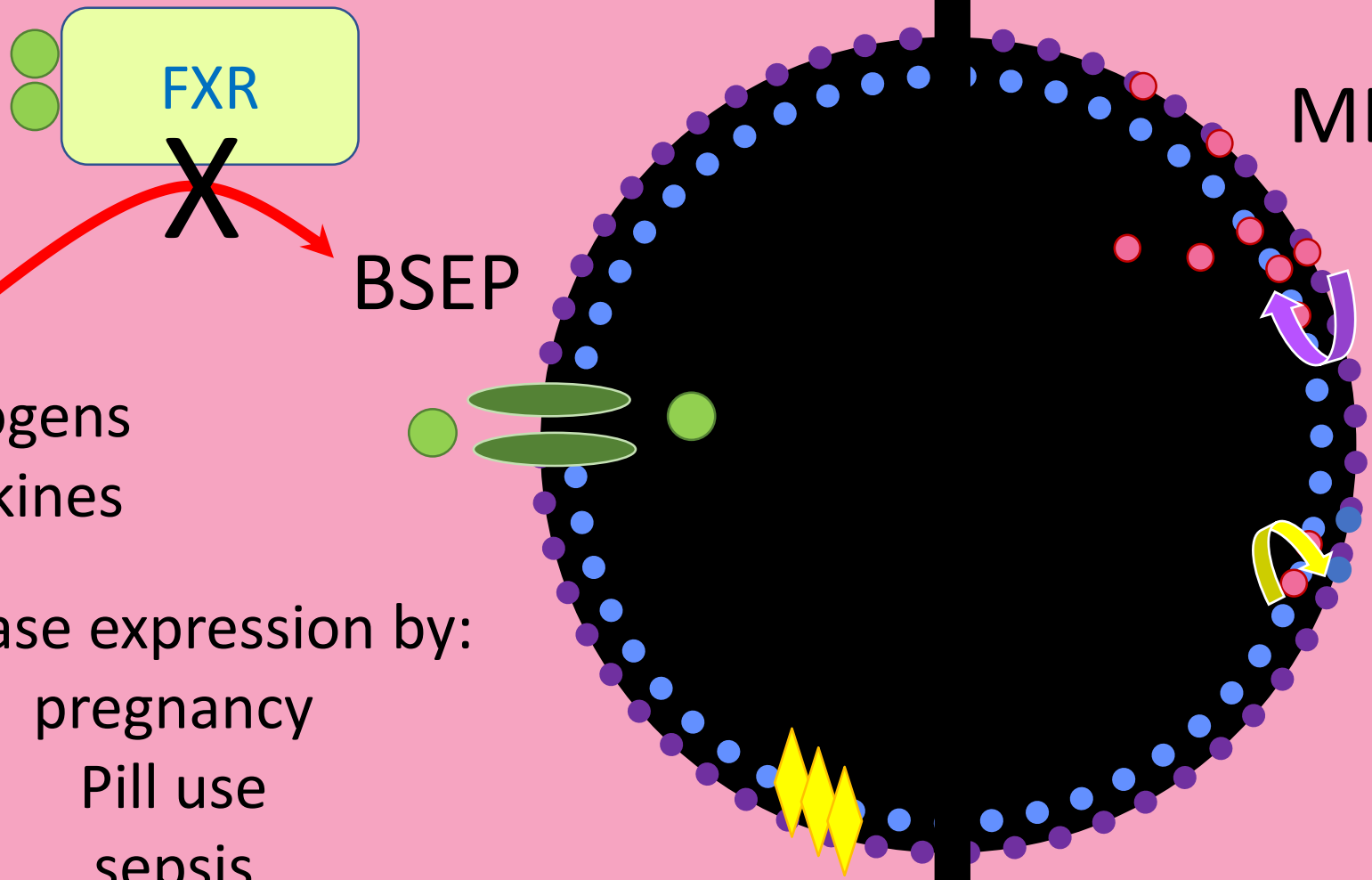
❖ often needs precipitating event

FXR = masterswitch



Bull LN & Thompson RJ. Clin Liver Dis 2018; 22:657-69

FXR = masterswitch



estrogens
cytokines

disease expression by:
pregnancy
Pill use
sepsis

Bull LN & Thompson RJ. Clin Liver Dis 2018; 22:657-69

A spectrum of severity

GASTROENTEROLOGY 2001;120:1448–1458

The Wide Spectrum of Multidrug Resistance 3 Deficiency: From Neonatal Cholestasis to Cirrhosis of Adulthood

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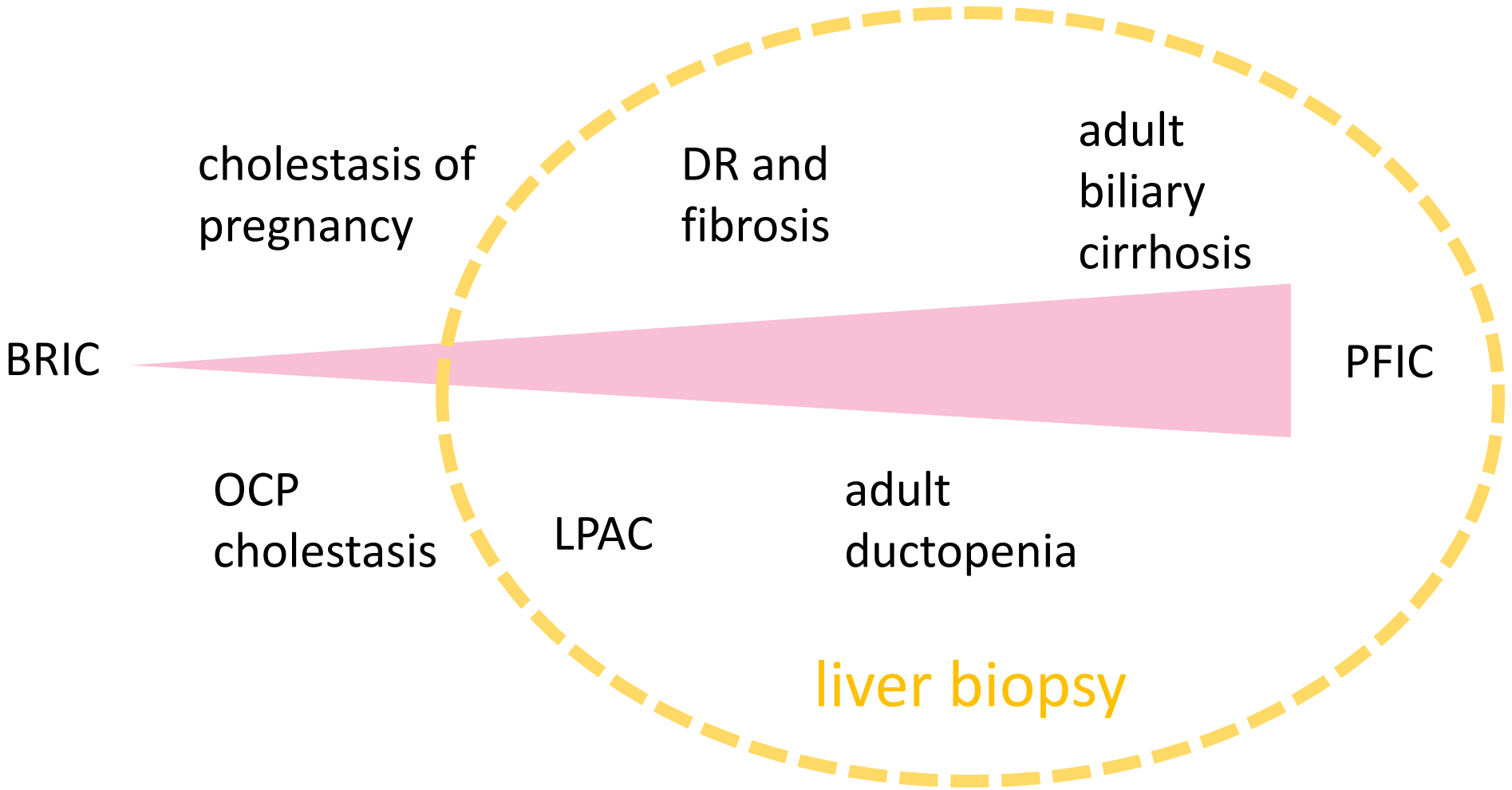
ABCB4 mutations (ABCB4 / MDR3 deficiency)

- PFIC & BRIC (idiopathic episodic jaundice)

- adult cryptogenic cirrhosis with biliary features
- drug-induced jaundice (some cases)
- cholestasis of pregnancy (15%)
- chronic cholestatic LFTs (34%)
- adult ductopenia
- low phospholipid-associated cholelithiasis (LPAC) syndrome

Balistreri WF & Bezerra JA. Clin Liver Dis 2006; 10:27-53
Reichert MC & Lammert F. Semin Liver Dis 2018; 38:299-307
Poupon R et al. Hepatology 2013; 58:1105-1110

ABCB4 mutations (ABCB4 / MDR3 deficiency)



Have we been missing them?

- PFIC 1 in 50,000 - 100,000 births

Gonzales E *et al*. Eur J Hum Genet 2014; 22:doi 10.1038

- >> at least 1 / 300 carrier

How can we find these cases?

- awareness of clinical presentations
- importance of family history
- know different patterns
- genetic testing increasingly available

Histology – *ABCB4* mutation (MDR3)

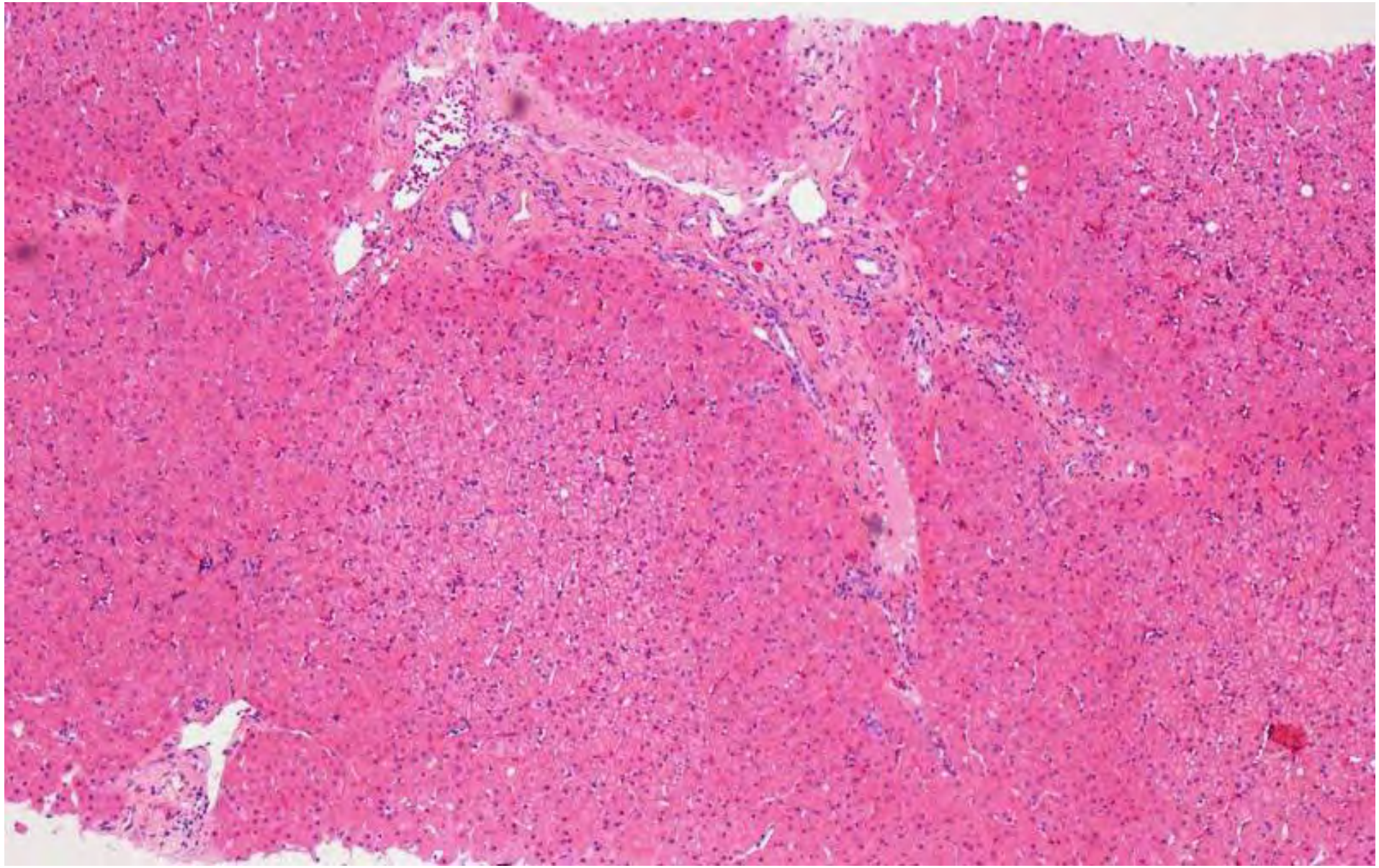
- 13 patients – heterozygous *ABCB4* mutation

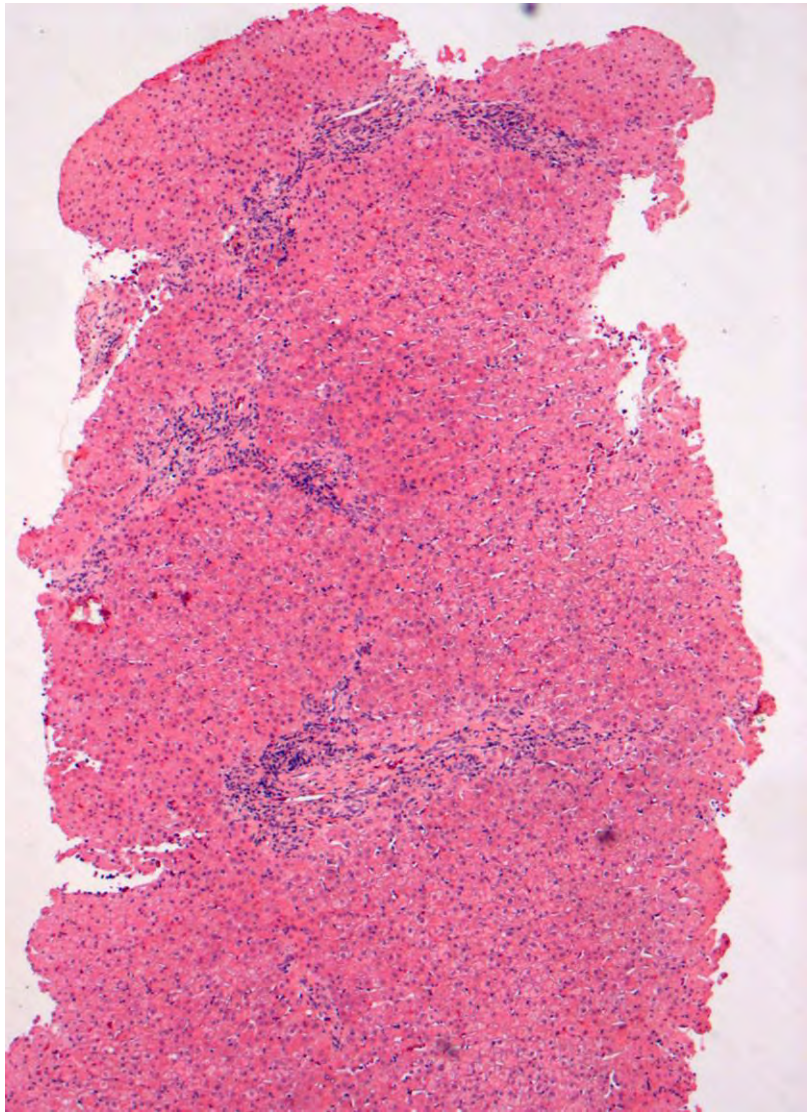
- ductular reaction
- mild portal inflammation — MOST
- fibrosis (variable)

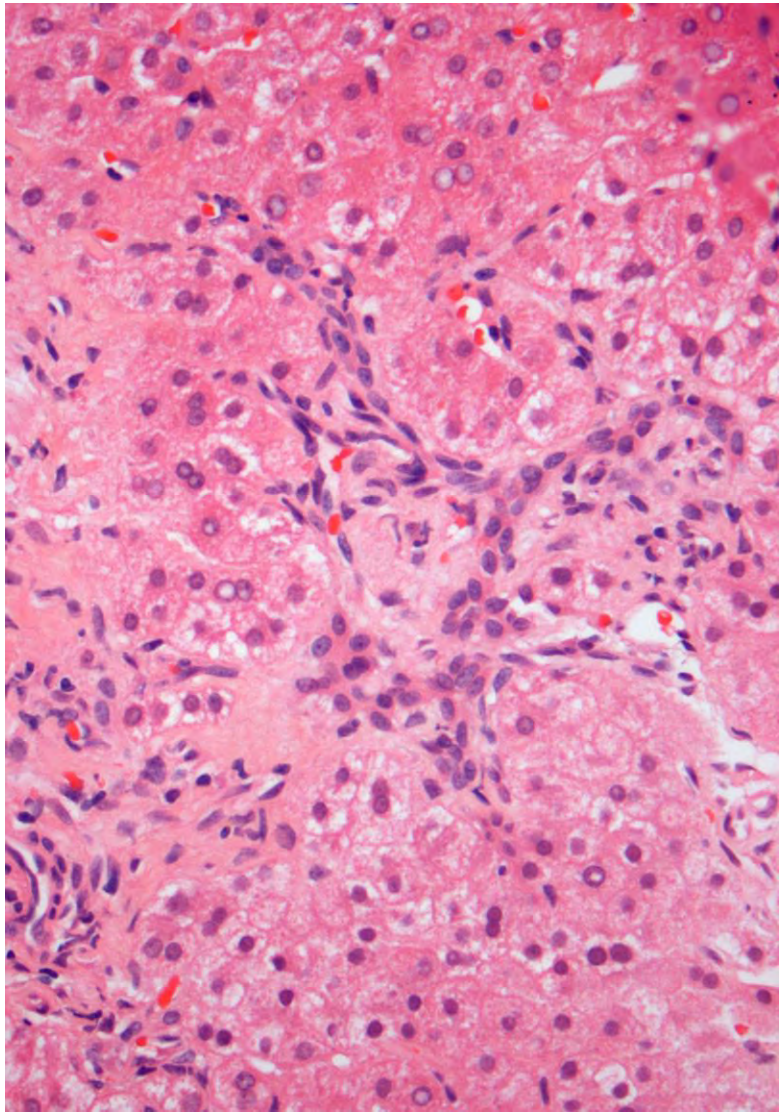
- cholesterol crystals or spaces in ducts
- bile duct injury — SOME
- periductal onion-skinning fibrosis

Wendum D et al. Virchows Arch 2012; 460:291-8
Ziol M et al. Gastroenterology 2008; 135:131-141

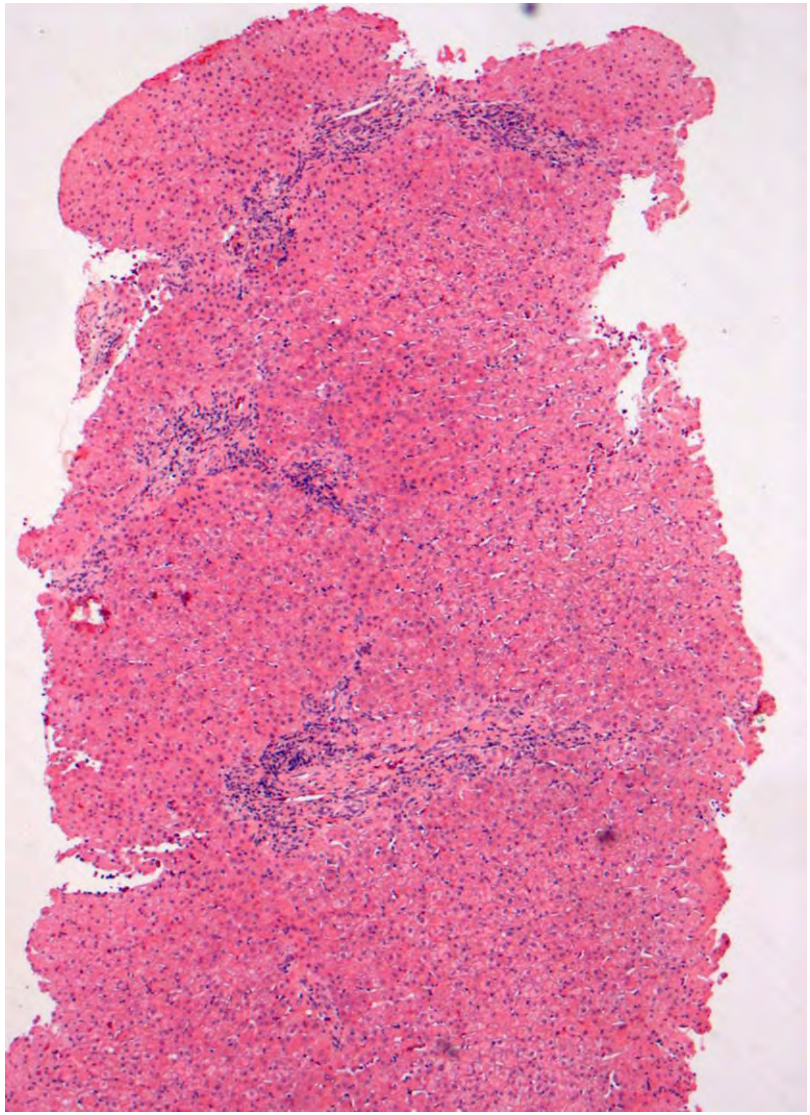
My 1st case: Cholestasis of pregnancy – didn't resolve

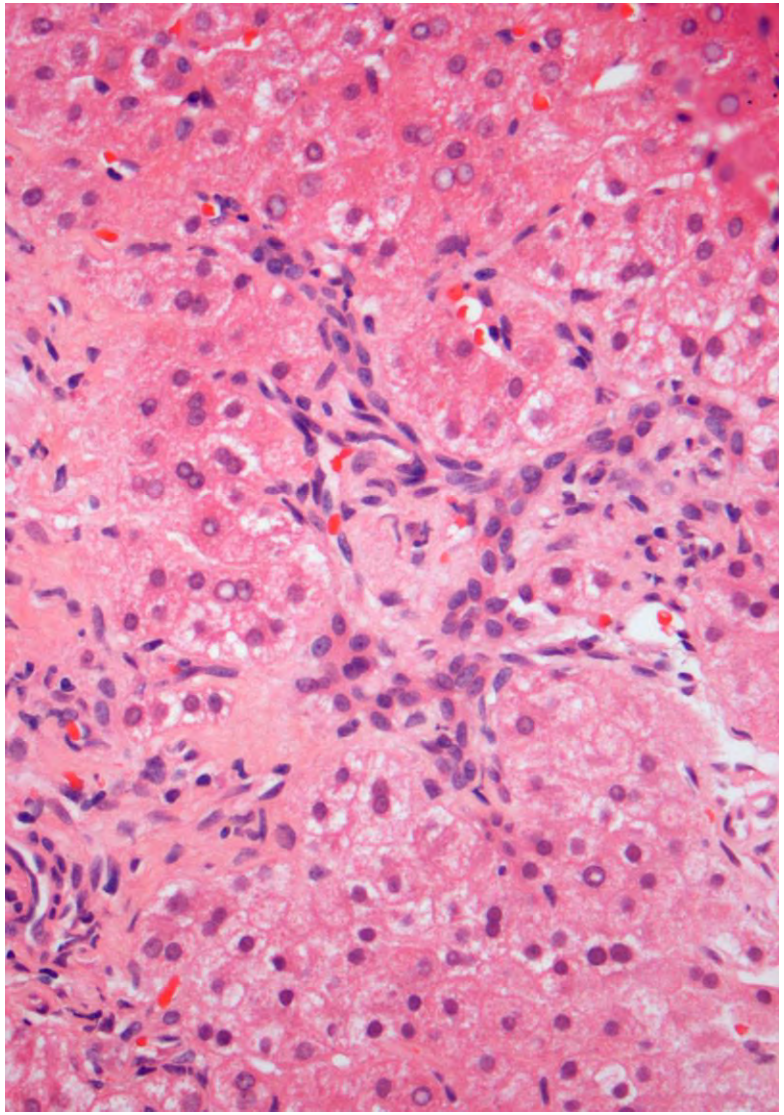






“That’s funny, her sister was transplanted with unexplained biliary cirrhosis”





Case 2 – contraceptive cholestasis

- 17 yo female
- severe pruritis 8 wks after commencing OCP
- not clinically jaundiced (liver bx because of ANA)

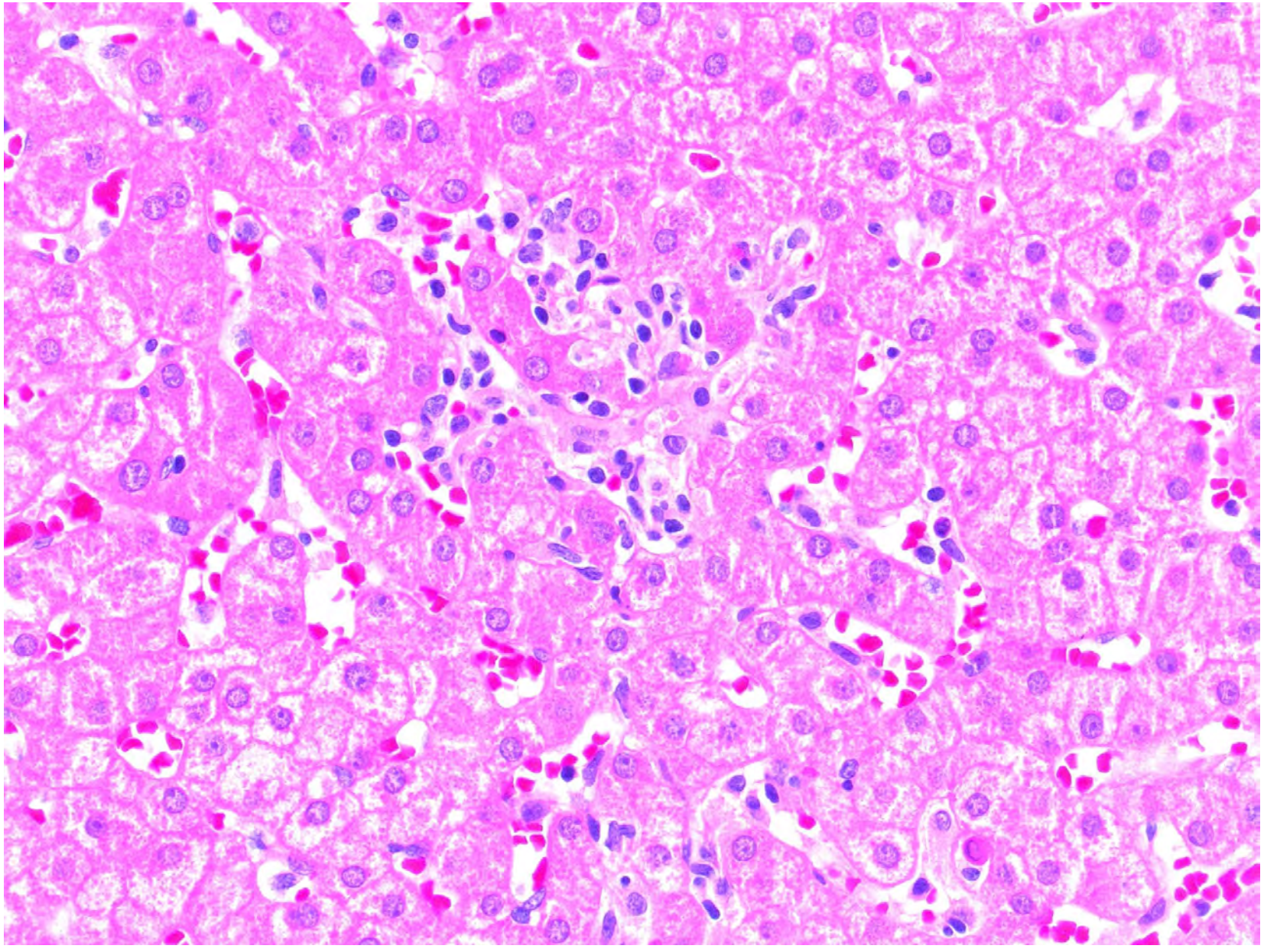
- FHx: mother had early cholecystectomy & intrahepatic cholestasis of pregnancy

Case 2 – contraceptive cholestasis

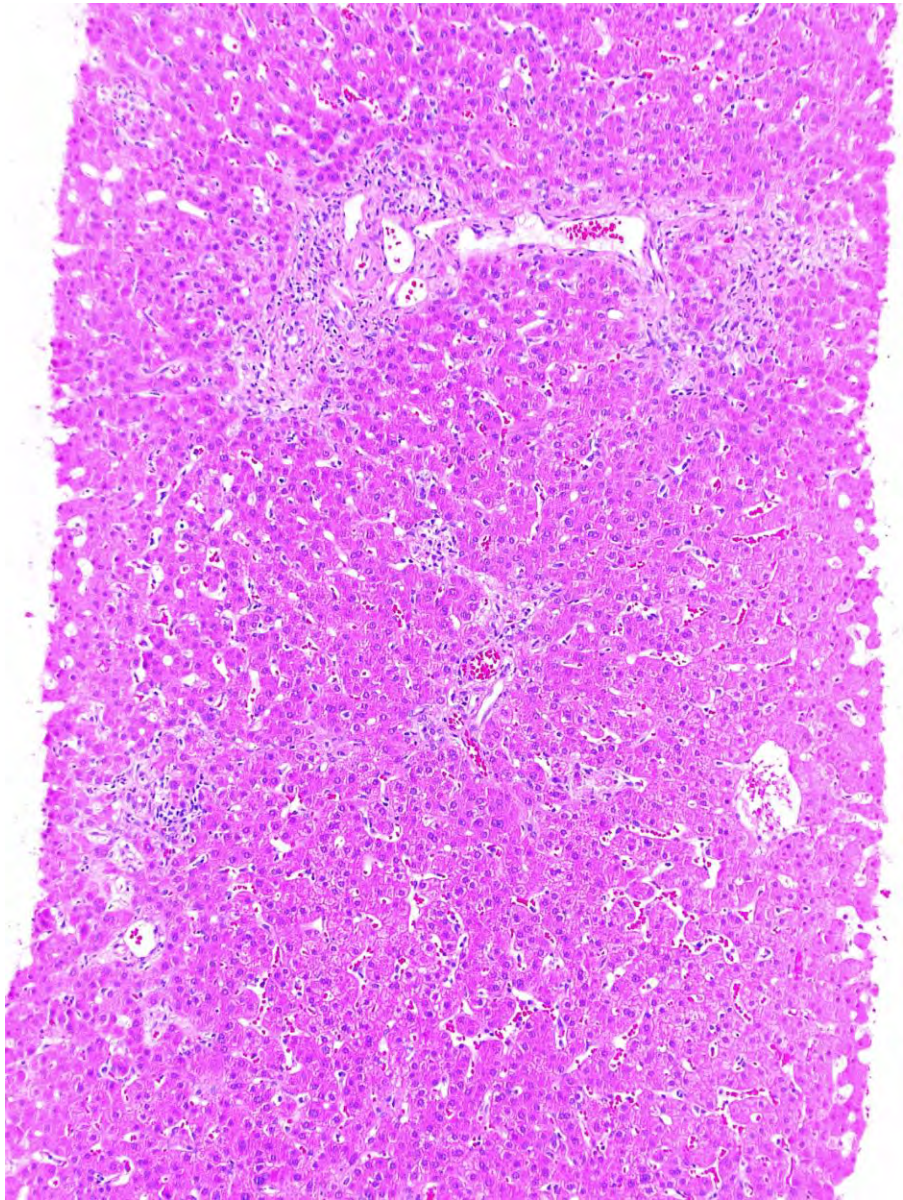
- *Audience response question*:
- Which of the following is true (may be more than one)?

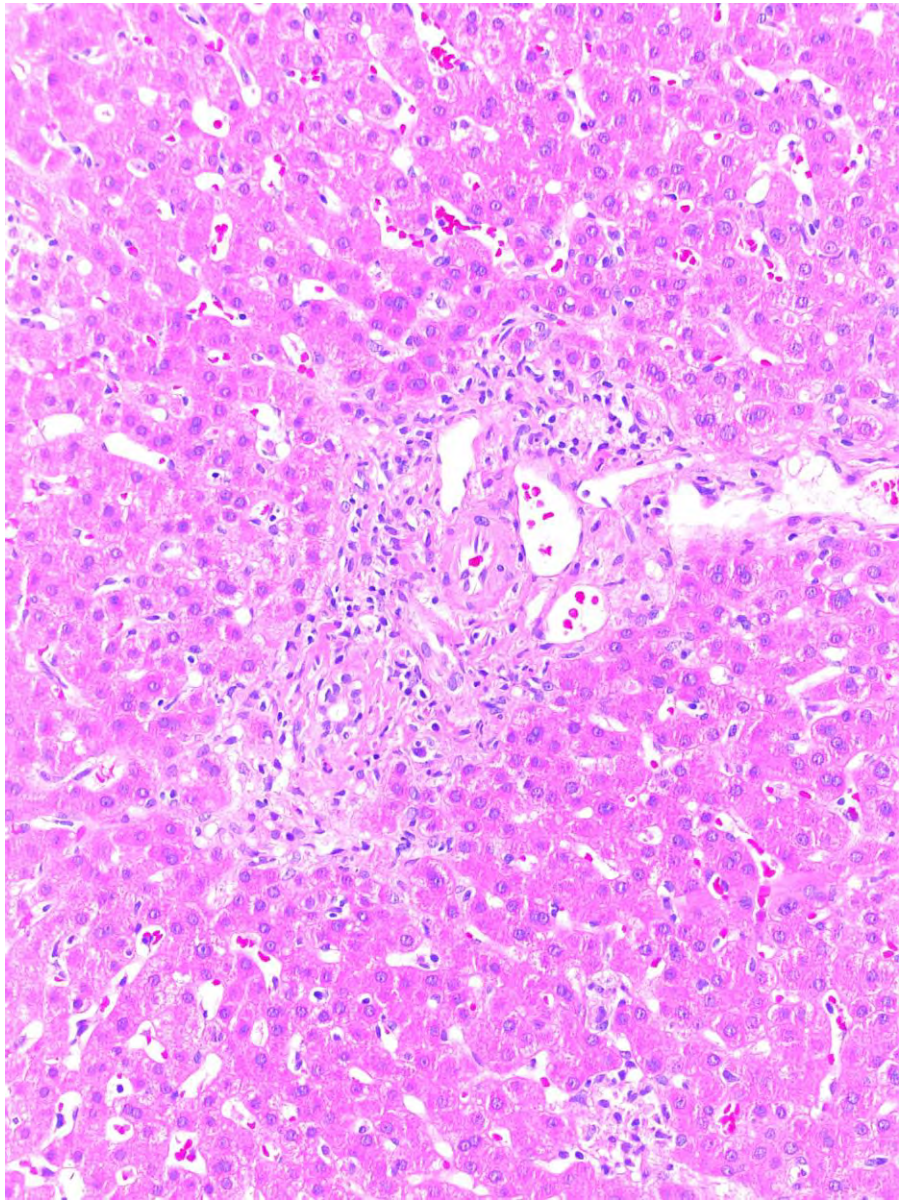
A. the biopsy will show canalicular cholestasis
B. the maternal history of early gallstones suggests *ABCB11* mutation (BSEP) is more likely than *ABCB4* mutation (MDR3)
C. periductal onion-skinning fibrosis can be a rare histological feature

No canalicular cholestasis

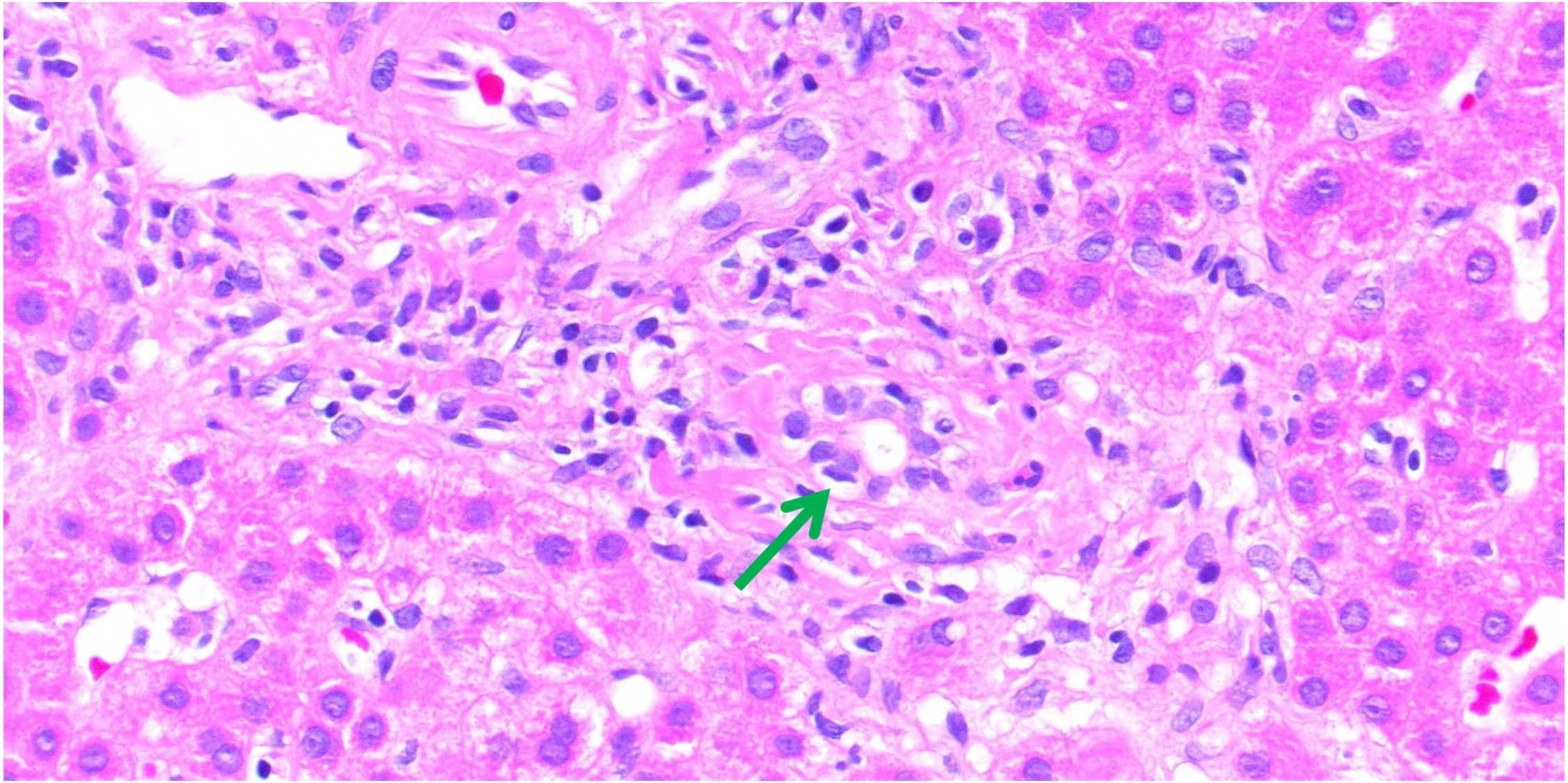


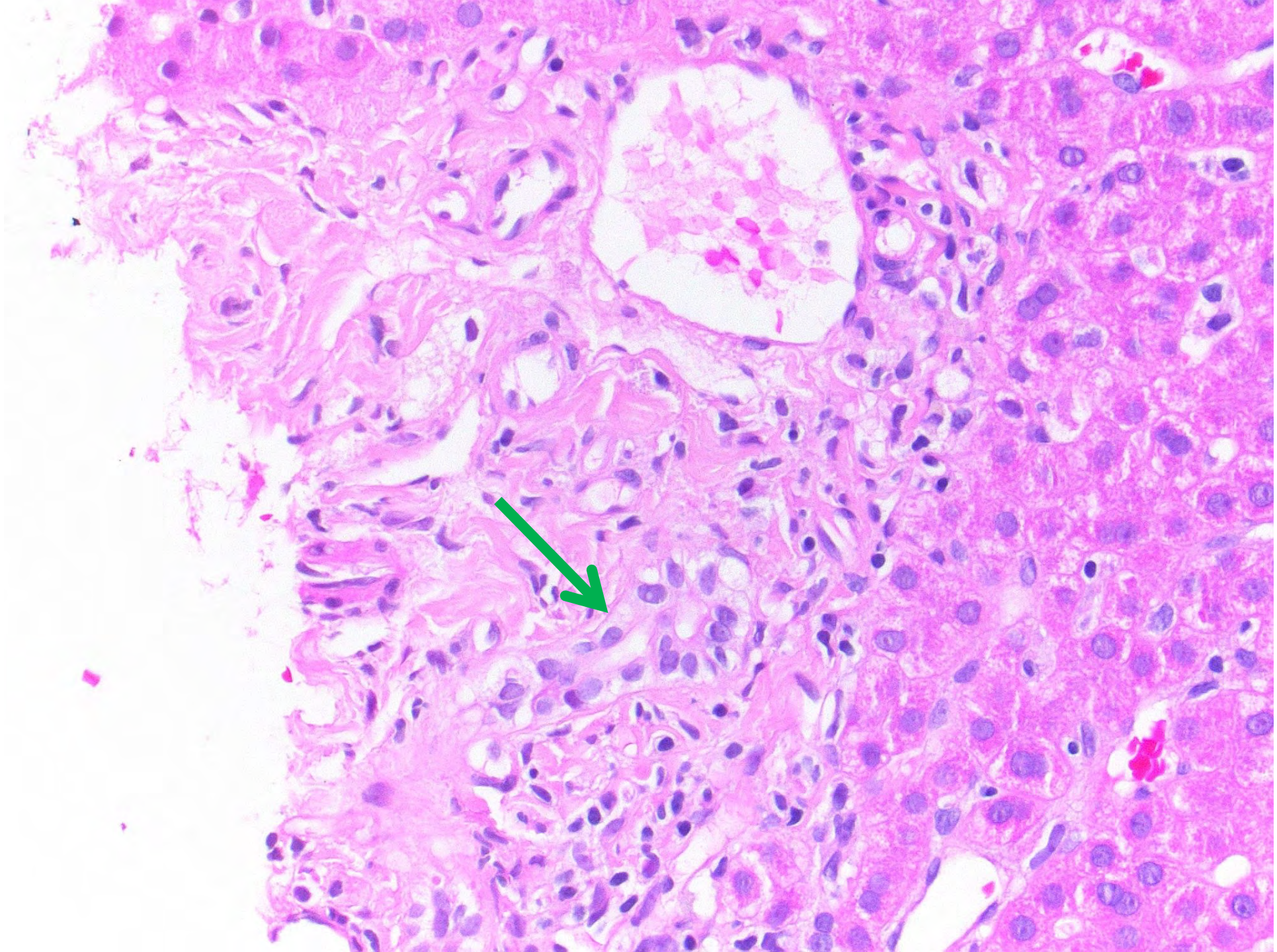
Mild portal fibrosis



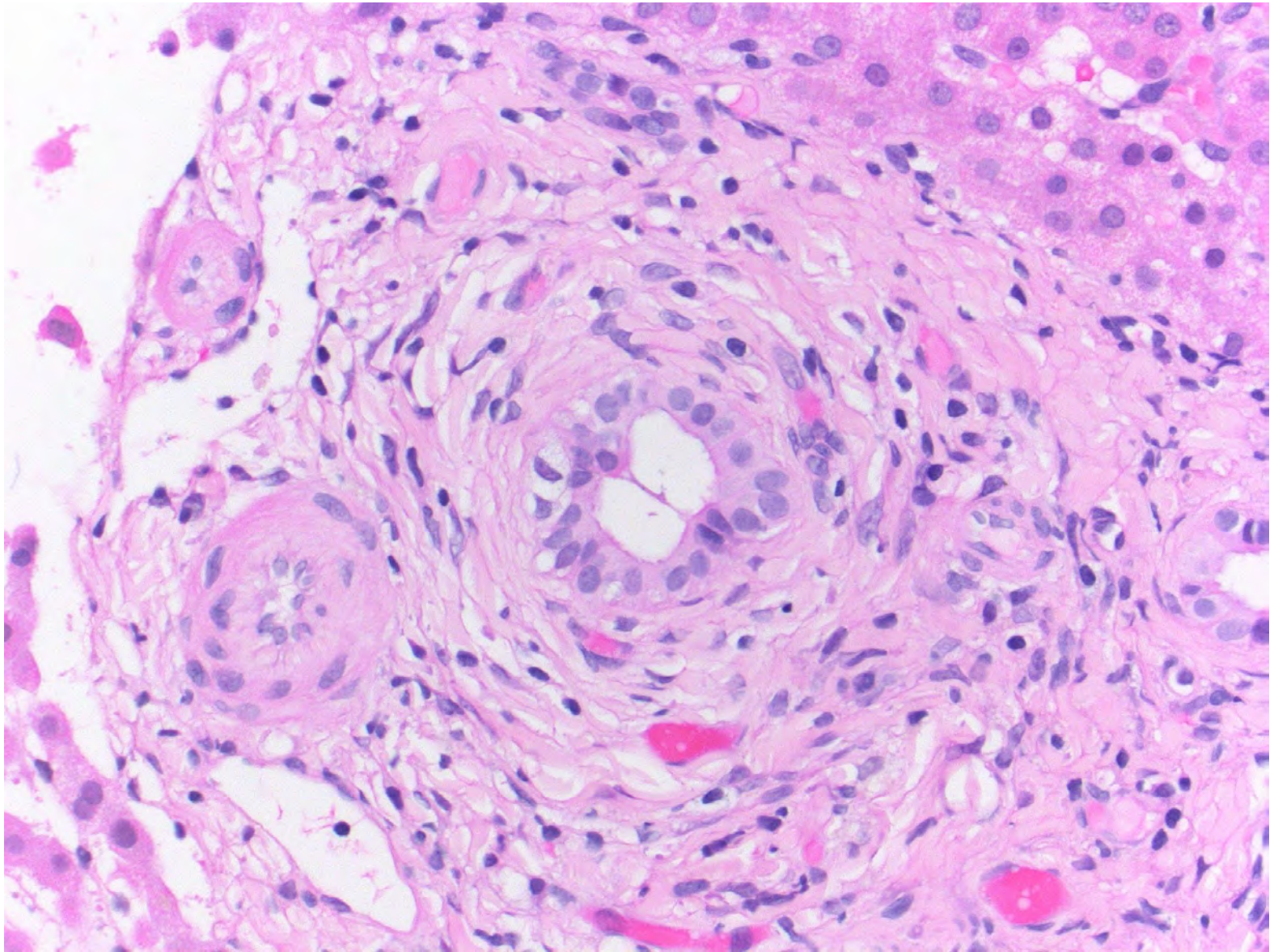


Bile duct injury

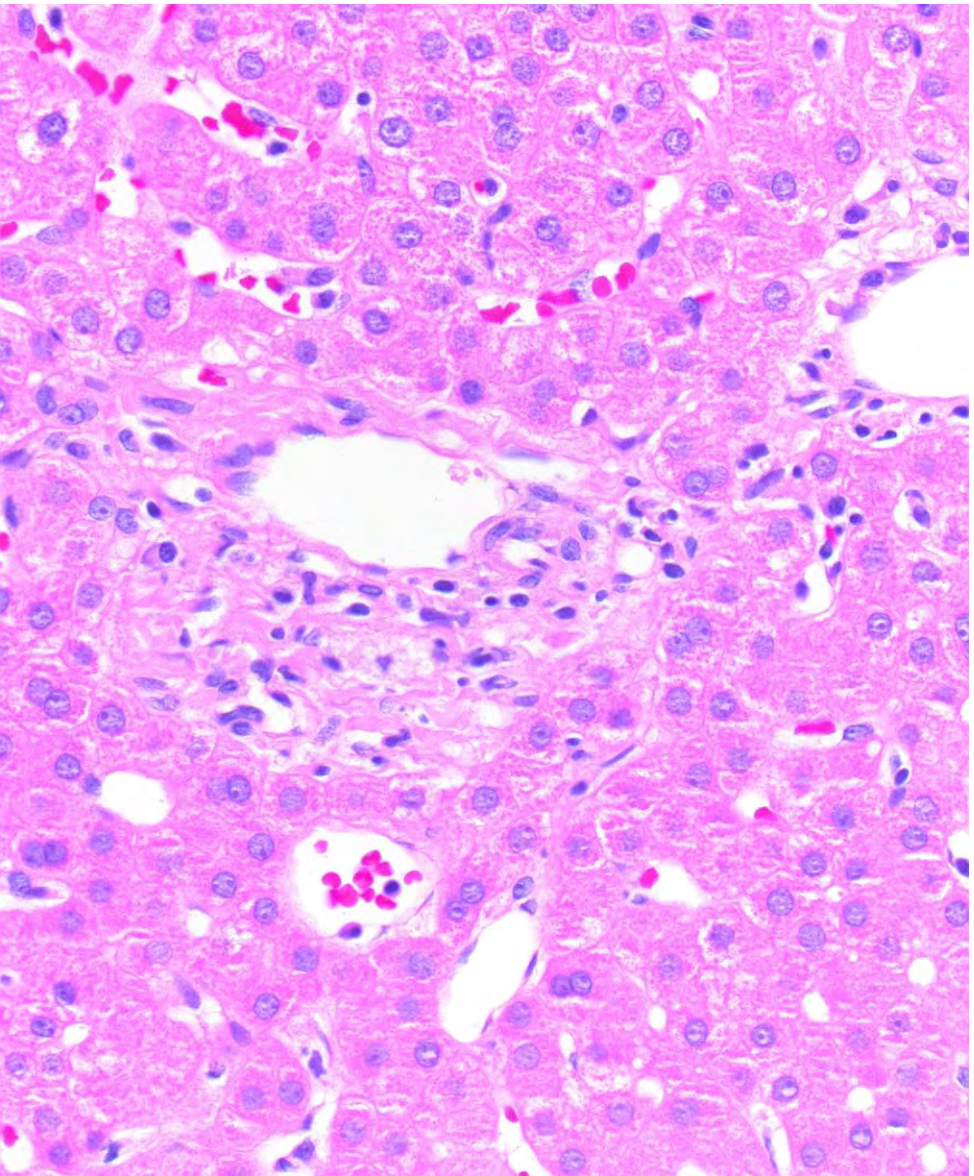


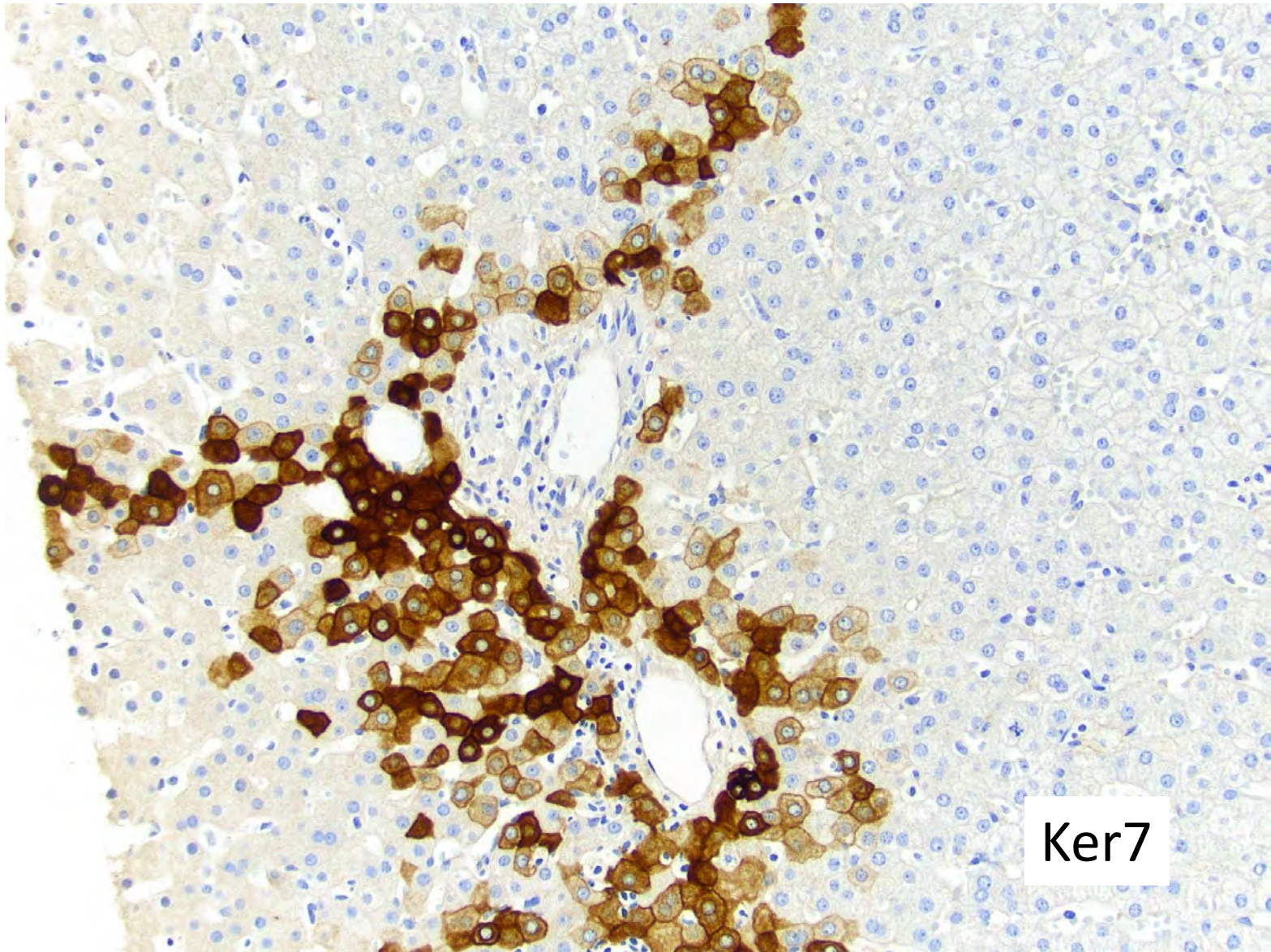


Focal onion-skinning fibrosis around bile duct



Focal duct loss - resolved with ursodeoxycholic acid UDCA





Case 2 – contraceptive cholestasis

- Audience response question: Which of the following is true?

1. the biopsy will show canalicular cholestasis – FALSE

   - *some cases show cholestasis, but not all.*

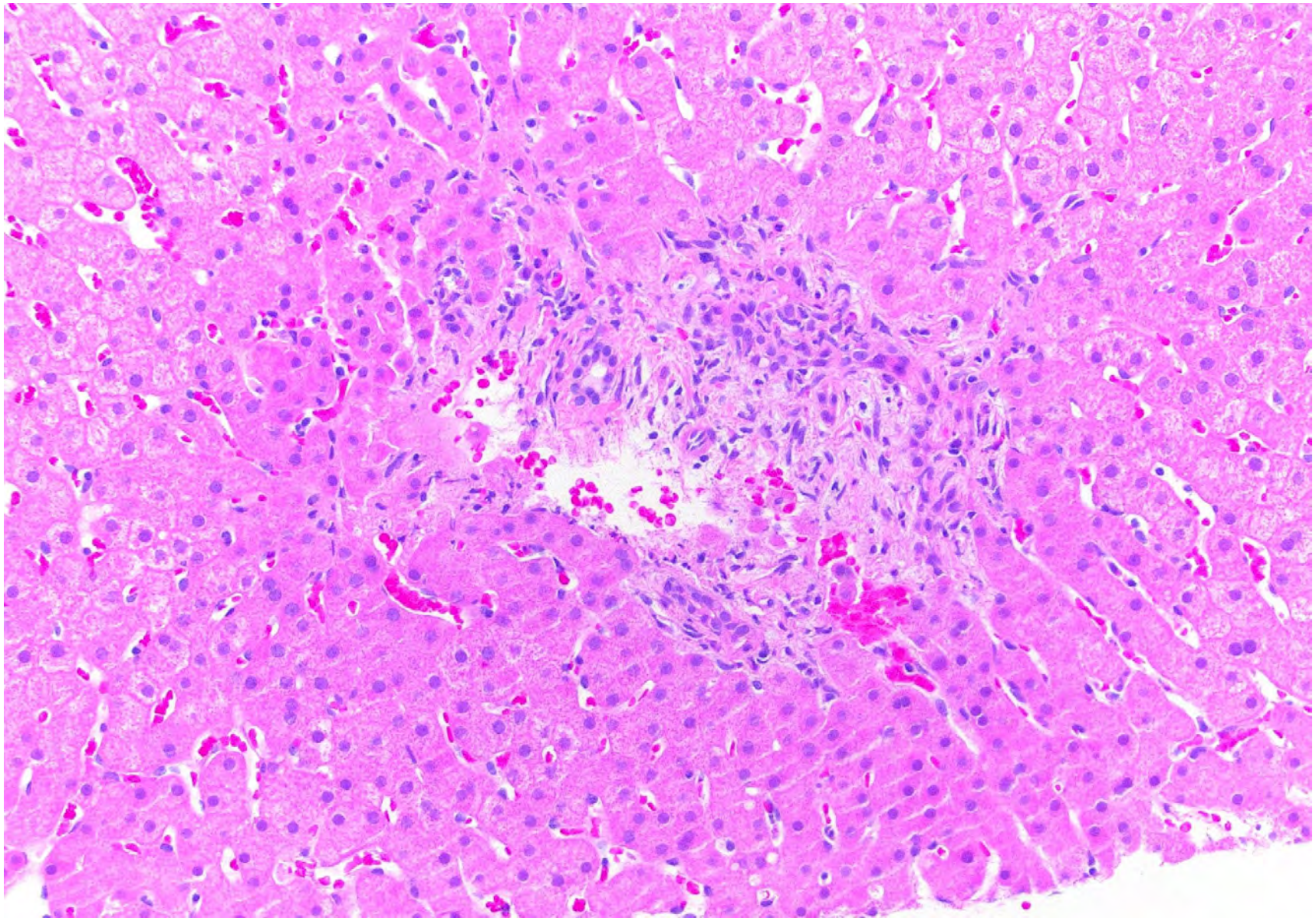
2. the maternal history of gallstones suggests *ABCB11* mutation (BSEP) is more likely than *ABCB4* (MDR3) mutation – FALSE

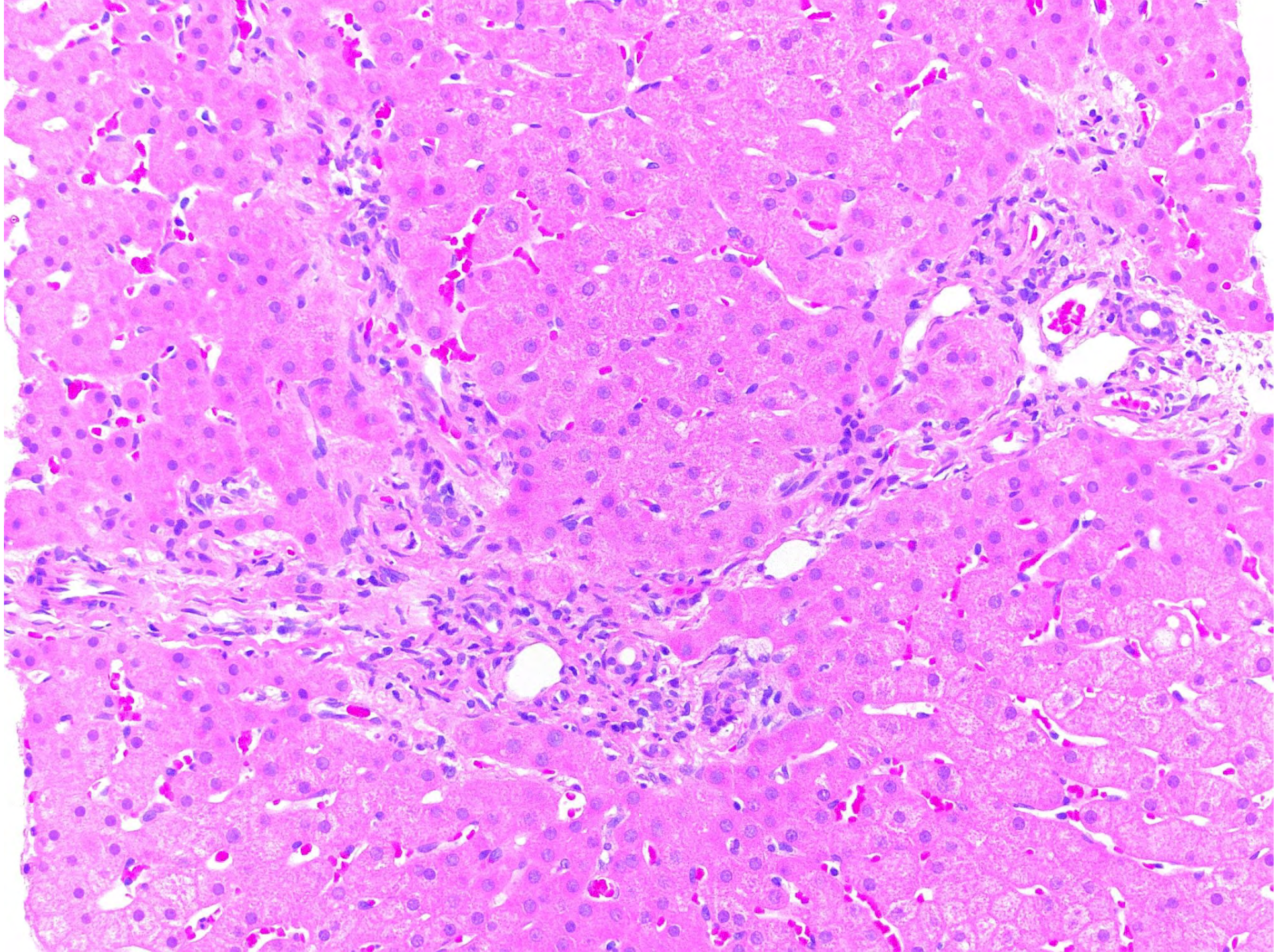
   - stones and duct injury are features of MDR3 deficiency - bile acids and cholesterol are not incorporated into micelles because phospholipid is deficient in the bile, thus injuring ducts and forming cholesterol stones

3. periductal onion-skinning fibrosis can be a rare histological feature - TRUE

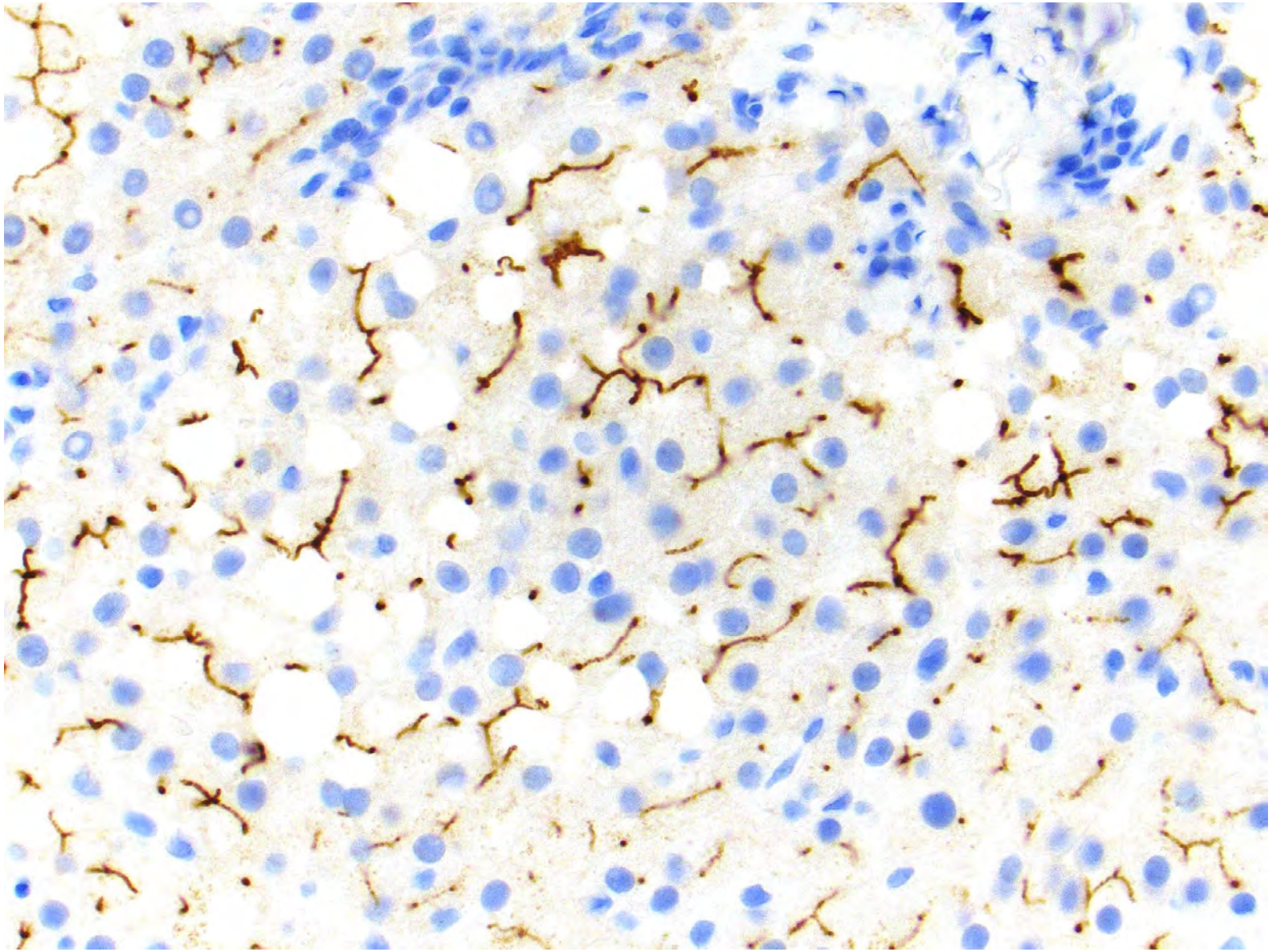
Wendum D et al. Virchows Arch 2012; 460:291-8
Poupon R et al. Hepatology 2013; 58:1105-1110

Case 3 – persisting cholestasis after pregnancy





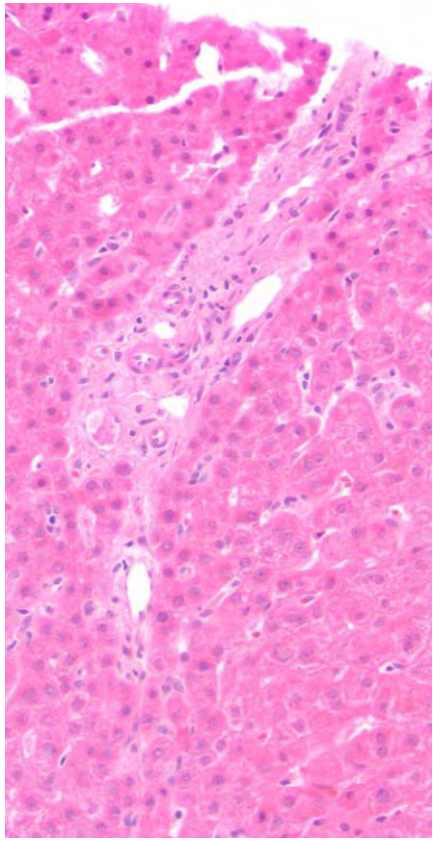
MDR3 IHC

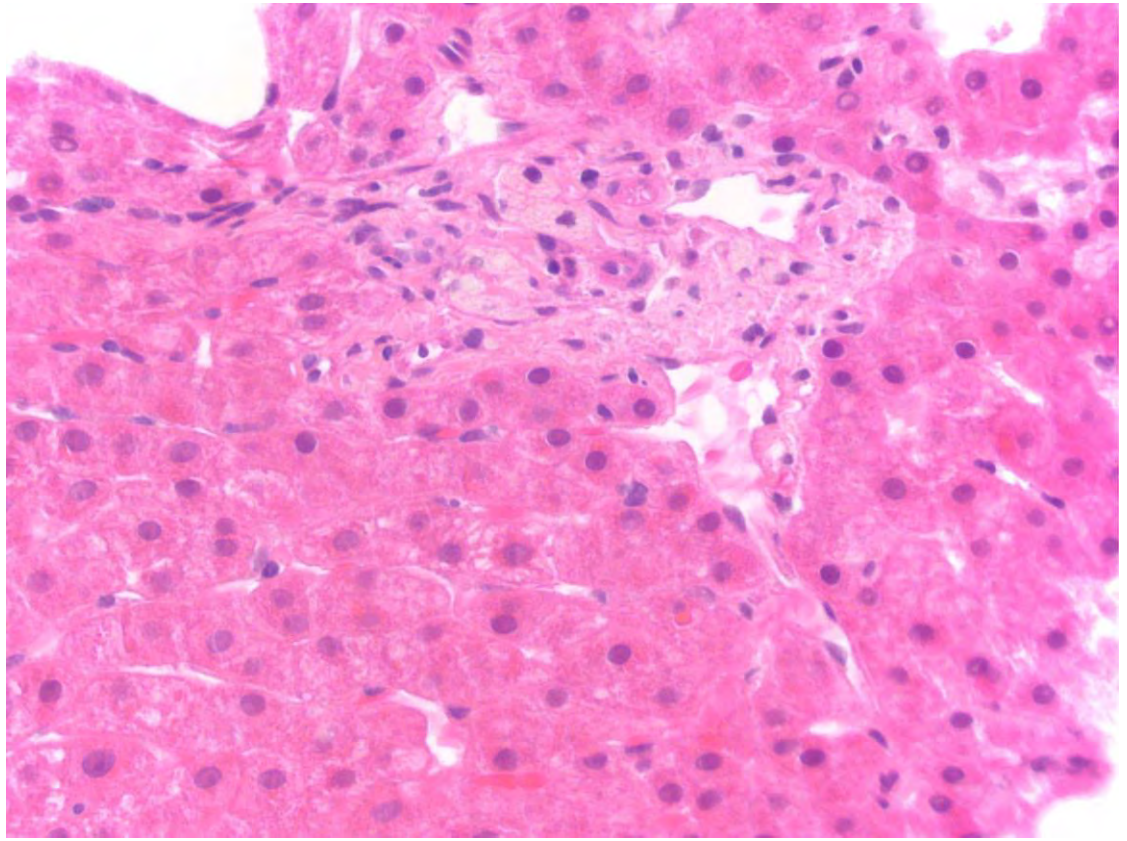


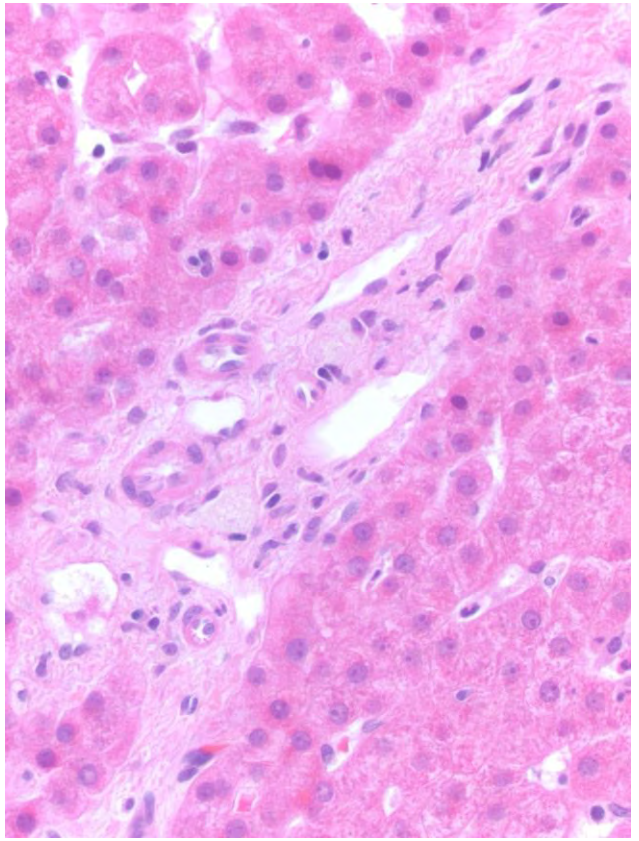
Sannier A et al. *Virchows Arch.* 2012; 460:535-537

Case 4 - idiopathic ductopenia

- F28, failed to resolve cholestasis of pregnancy (outside Dx “normal”)
- alk phos 300-400, partly resolved with urso, still elevated at 250 after 8 yrs

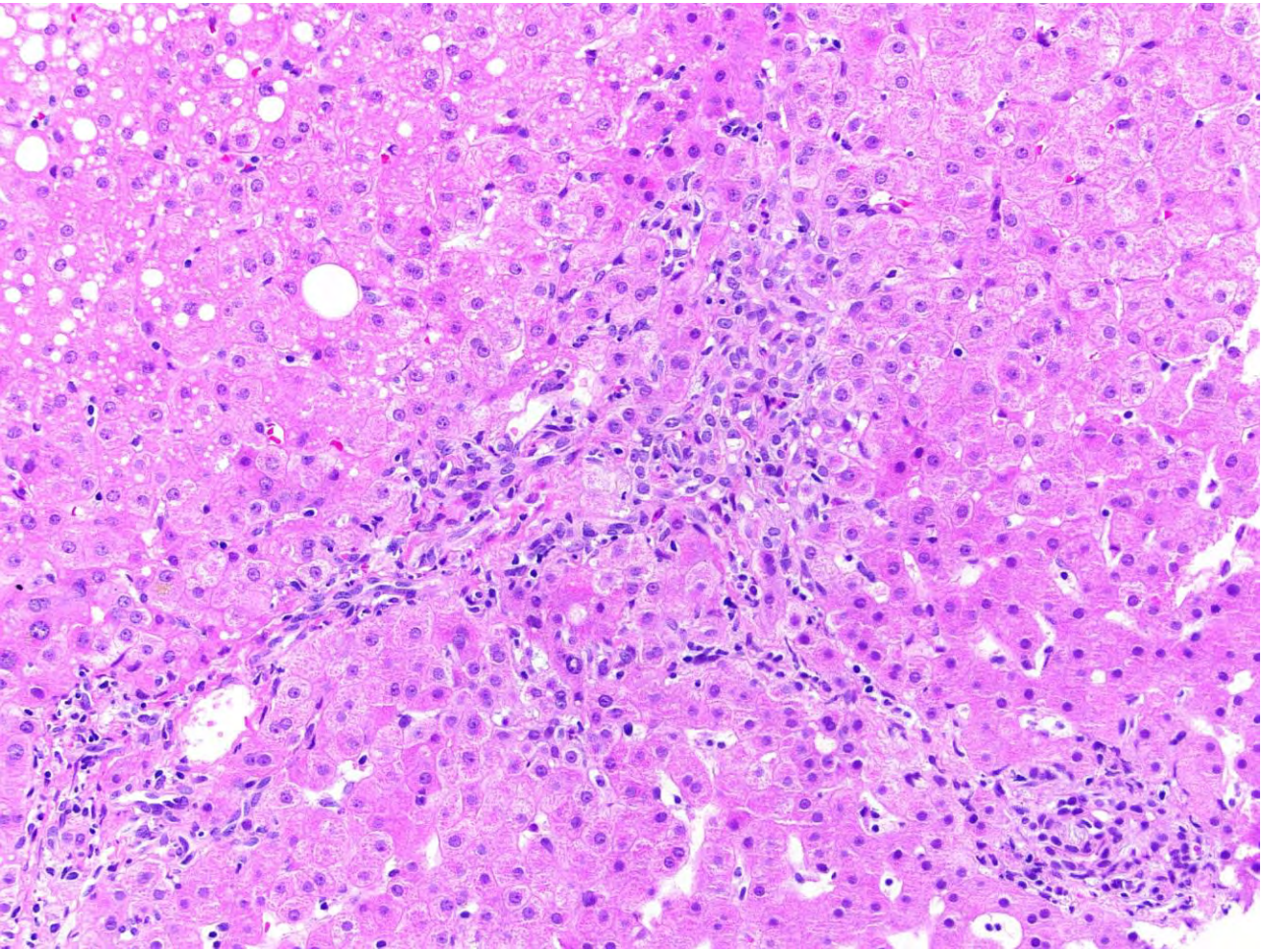


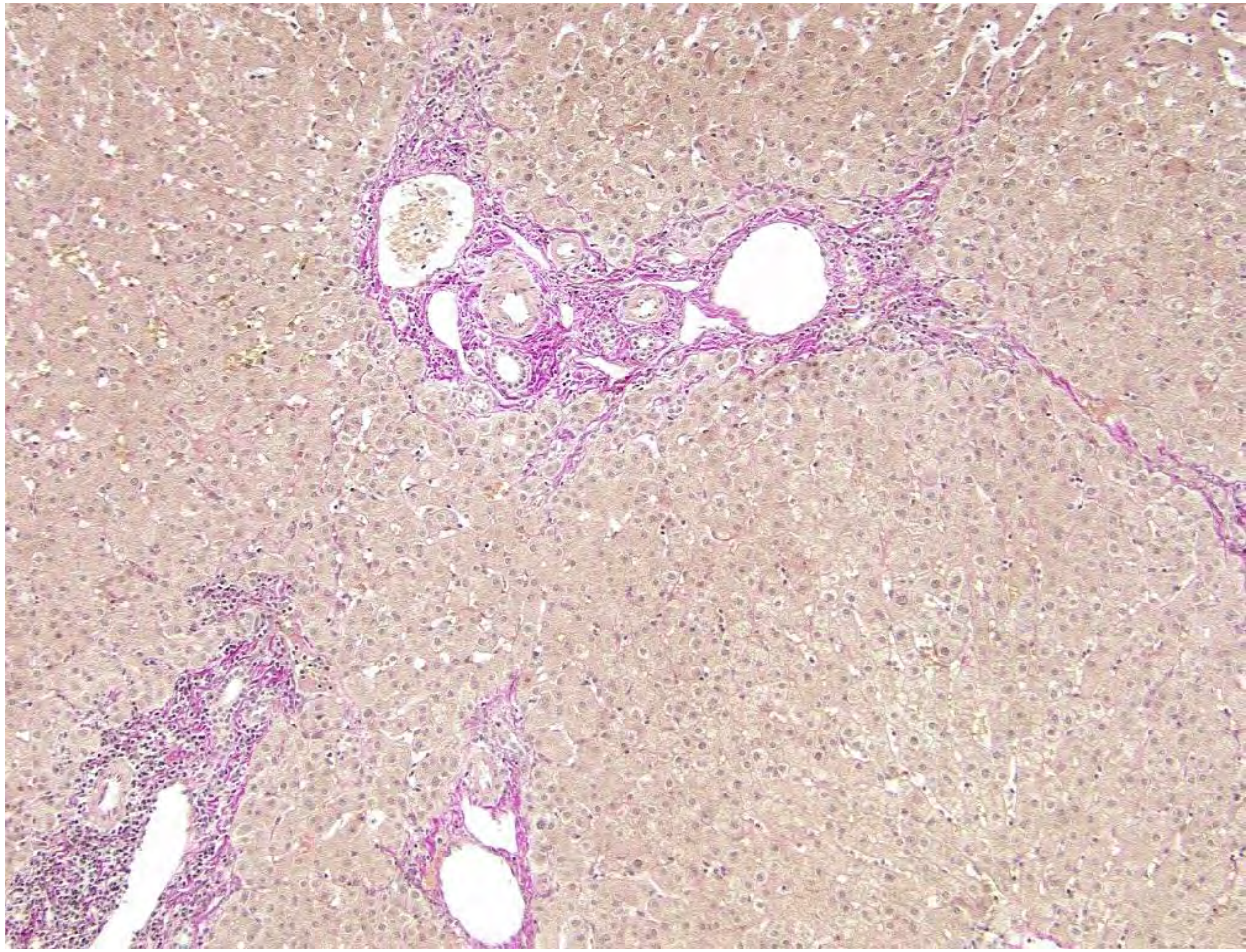




Courtesy Dr Joe Misdraji, Boston

Case – F48. 10 yrs cholestatic tests





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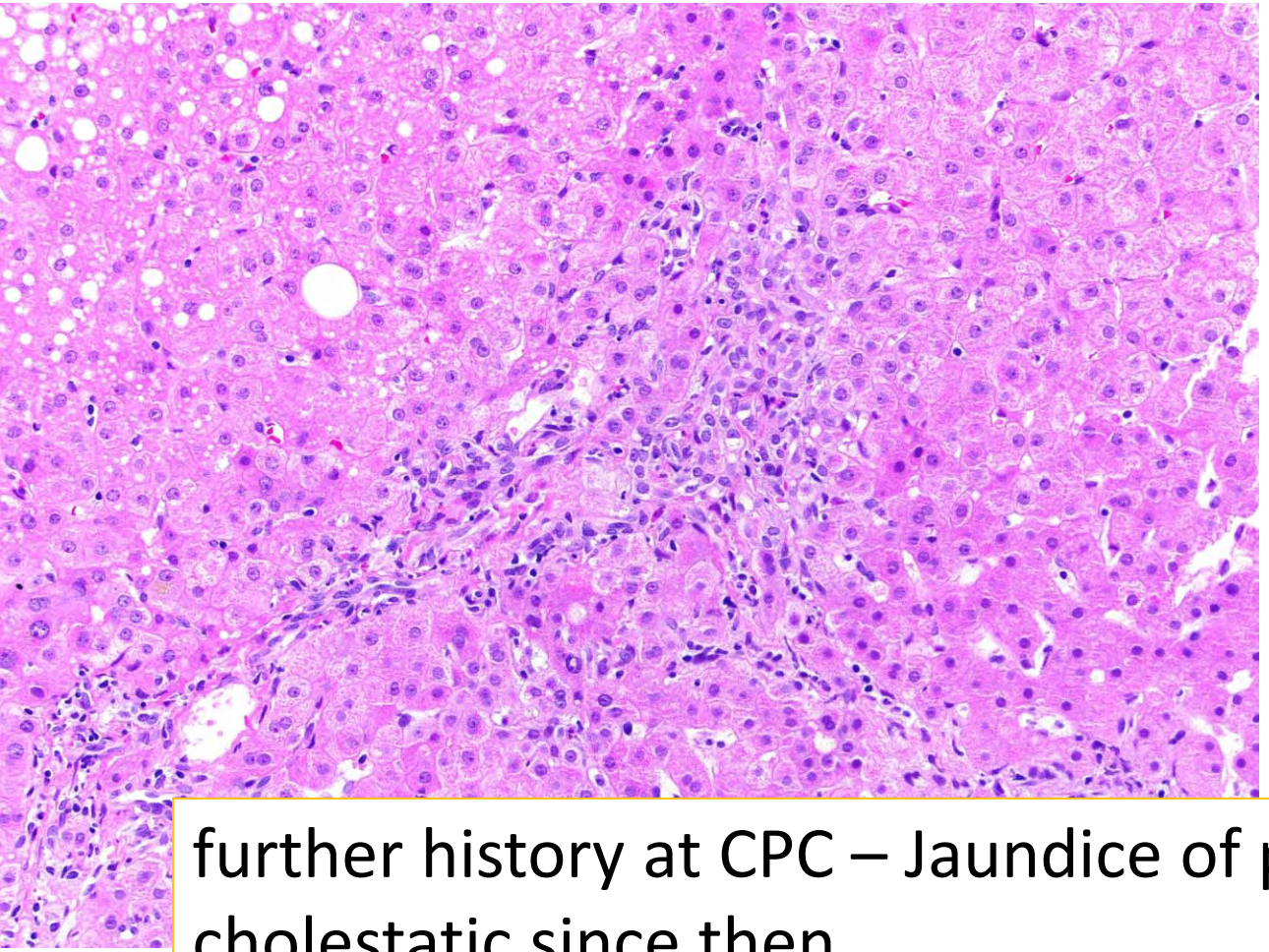
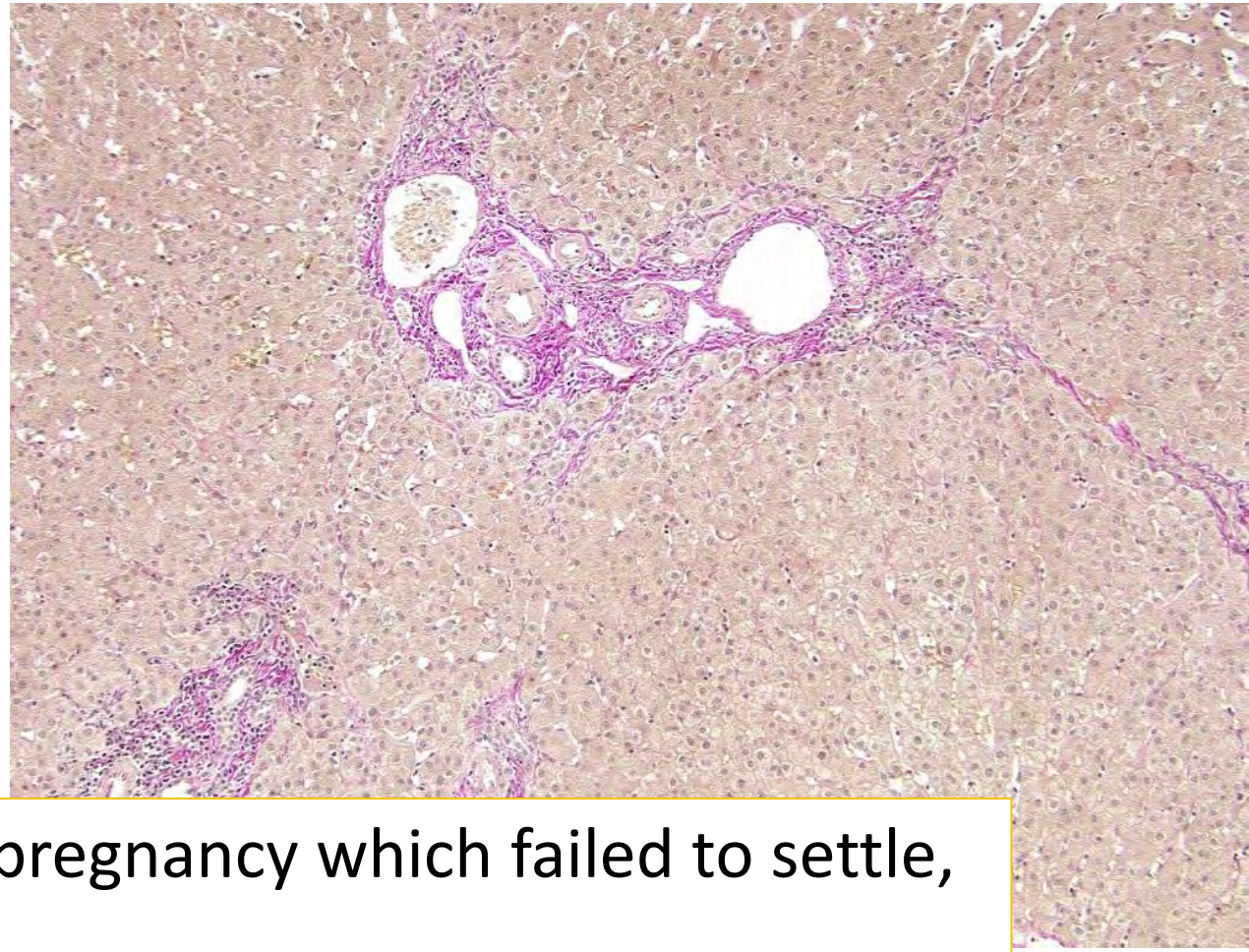
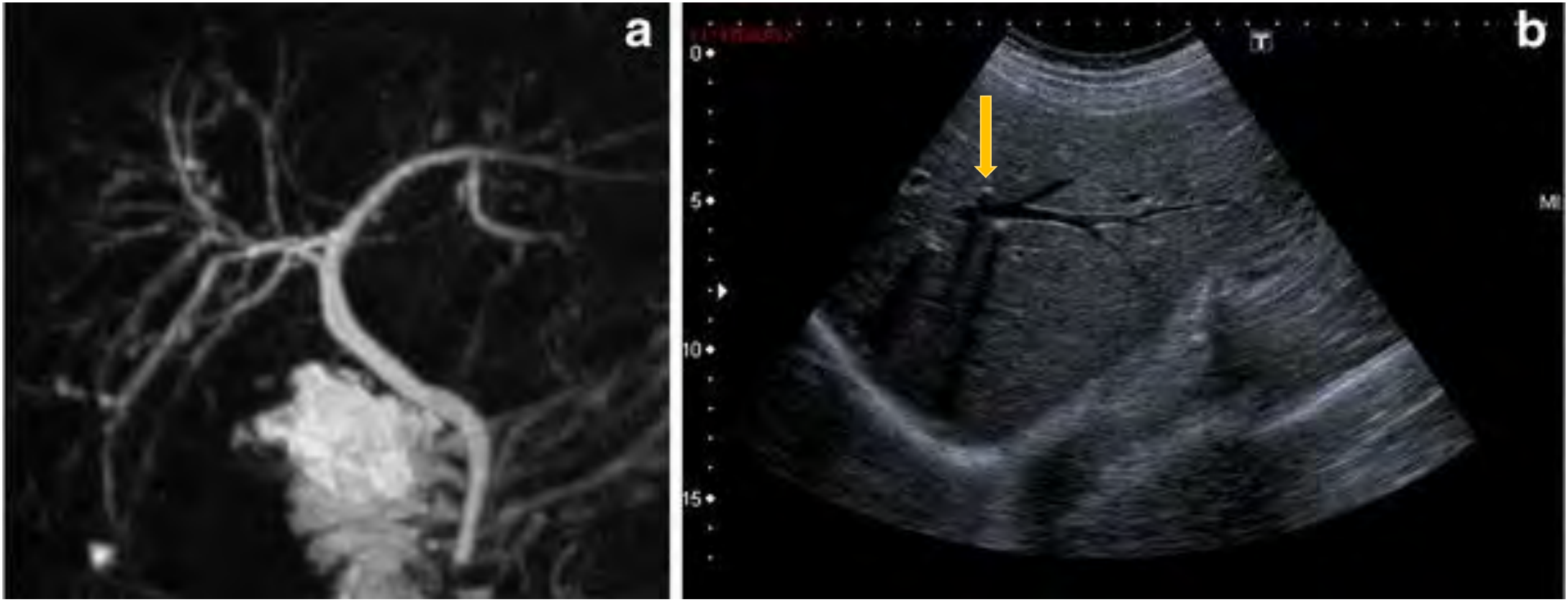




further history at CPC – Jaundice of pregnancy which failed to settle, cholestatic since then



Biliary irregularities in a 54-year-old man. Three-dimensional MRCP (**a**) and sagittal ultrasound of the right lobe (**b**) show right biliary abnormalities (**a**). These mild irregular calibre intrahepatic bile ducts were not demonstrated with ultrasound; on the other hand, small bile stones were easily depicted as hyperechoic formations with posterior attenuation

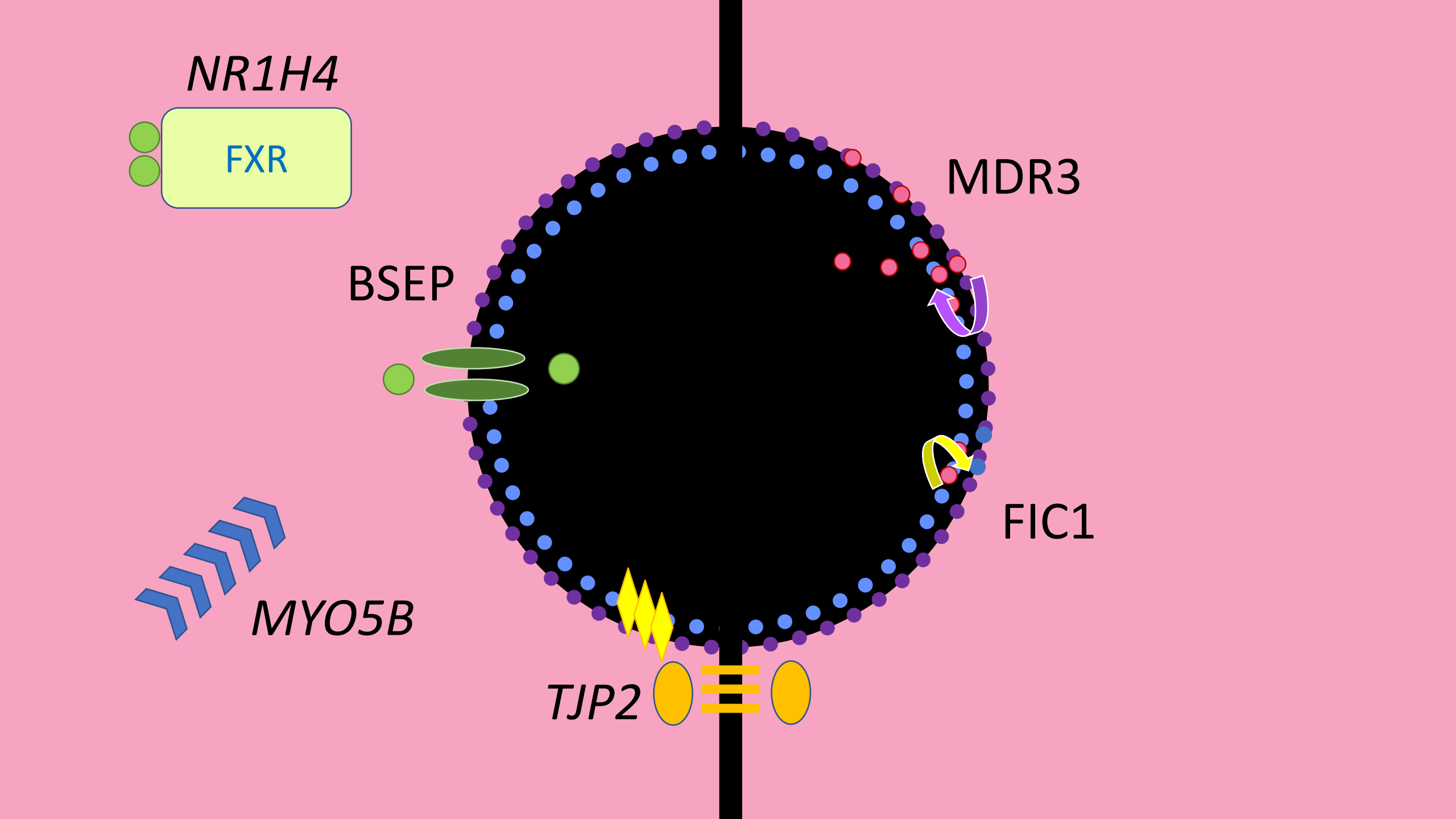
Benzimra J et al. Insights Imaging. 2013; 4:331–338

Extending PFIC - other genetic defects

Table 2
Phenotypic characteristics of PFIC types 1–6.

Type	1 (Byler syndrome)	2	3	4	5	6
Gene	***ATP8B1*** (FIC1)	***ABCB11*** (BSEP)	***ABCB4*** (MDR3)	***TJP2***	***NR1H4*** (FXR)	***MYO5B***
Reference	Klomp et al. *Hepatology* (2004)	Pawlikowska et al. *J Hepatol* (2010)	Smit et al. *Cell* (1993)	Sambrotta et al. *Nat Genet* (2014)	Gomez-Ospina et al. *Nat Comm* (2016)	Gonzalez et al. *Hepatology* (2017)
Transport	Phosphatidylserine flippase	Bile acid export	Phosphatidylcholine translocator	Tight junction protein 2	Nuclear bile acid receptor	Myosin 5b
Phenotypes	Biliary cirrhosis	Neonatal giant cell hepatitis	Biliary cirrhosis with neoductuli	Early onset chronic cholestasis	Neonatal cholestasis with rapid progression to end-stage liver disease	Giant-cell hepatocytes and persistent intralobular cholestasis
	BRIC type 1	Biliary cirrhosis	Gallstones (LPAC)	HCC	Vitamin K-independent coagulopathy	Transient or recurrent cholestasis
	Extrahepatic manifestations: Malabsoprtion, pancreatitis, deafness, pneumonia	BRIC type 2	ICP	Respiratory/CNS symptoms		Microvillus inclusion disease
		ICP	Cholangiocarcinoma HCC			
		Gallstones DILI HCC				
Labs	Low γ-GT	Low γ-GT	High γ-GT	Low γ-GT	Low γ-GT AFP increased	Low γ-GT
Therapy	OLT PBED	OLT	UDCA OLT	OLT	OLT	UDCA OLT

Reichert MC et al. Biochim Biophys Acta. 2018 Apr;1864(4 Pt B):1484-1490


NR1H4
FXR
MDR3
BSEP
FIC1
MYO5B
TJP2

Increasing heterozygosity found in chronic cholestasis

Author	Year	Patient number	Freq of mutation(s)	Clinical picture	Genes affected
DiGiorgio	2016	80	50%	ICP, JC	*ABCB4*
Gordo-Gilart	2016	67	13%	Chronic Chol	*ABCB4*
Dixon	2017	147	18%	ICP	*B4 > B11 > 8B1 > TJP2 > ABCC2*
Droge	2017	427	35%	Familial Chol	*B4 > B11 > 8B1*
Aamann	2018	33	27% definite + 27% possible	Chronic Chol	*B4 > B11 > ABCG5 > C2*
Vitale	2018	48	21% definite + 14% possible	Chronic Chol	*B11 > B4 > TJP2 > 8B1*

ICP: intrahepatic cholestasis of pregnancy; JC: juvenile cholelithiasis; Chronic Chol: chronic cholestasis; Familial Chol: familial cholestasis

Asmann et al. Scand J Gastroenterol 2018; 53:305 Dixon et al. Scientif Rep 2017; 7:11823 Droge et al. J Hepatol 2017; 67:1253
Gordo-Gilart et al. Liver Int 2016; 36:258 DiGiorgio et al. J Gastroenterol 2016; 51:271 Vitale et al. J Gastroenterol 2018; 53:945

Gene testing panels increasingly extensive

West Midlands Regional Genetics Laboratory

Our reference: D18.37110(1)

Basis of test:

Panel A genes tested: ABCB4 (NM_000443.3); ABCB11 (NM_003742.2); ATP8B1 (NM_005603.3); JAG1 (NM_000214.2); NOTCH2 (NM_024408.3); NPC1 (NM_000271.4); NPC2 (NM_006432.3); NR1H4 (NM_005123.3); SERPINA1 (NM_000295.4); SLC25A13 (NM_014251.2); TJP2 (NM_004817.3); VIPAS39 (LRG_1019); VPS33B (LRG_884).

Panel B genes tested: AKR1D1 (NM_005989.3); ALDOB (NM_000035.3); BAAT (NM_001701.3); CLDN1 (NM_021101.4); CYP27A1 (NM_000784.3); CYP7B1 (NM_004820.3); EPHX1 (NM_000120.3); HSD3B7 (NM_025193.3); PEX1 (NM_000466.2); PEX2 (NM_000318.2); UTP4 (NM_032830.2).

The Illumina HiSeq platform has been used to sequence coding regions and splicing sites (-5/+5) of the above genes captured by the TruSight One Panel target enrichment system (Illumina). Analysis was performed using an in-house pipeline; alignment BWA mem; variant calling Platypus; variant annotation Annovar; against human genome hg19 as a reference. Sanger sequencing used to complete screening of NPC1, NPC2, ATP8B1, ABCB11, ABCB4, SLC25A13, VPS33B, VIPAS39. Of the remaining genes, 98.12% of the target region has been covered to a minimum depth of 20X and regions below this minimum threshold have not been Sanger sequenced. NOTCH2 exons 1-4 have low mapping quality and there may be reduced sensitivity, however no pathogenic variants have been previously identified in these exons. Sequence nomenclature according to HGVS guidelines. Variants currently considered as benign polymorphisms and/or with minor allele frequency >1% are not reported. Variants currently considered likely to be benign have not been confirmed by Sanger sequencing and are recorded for information only in this report. Further details are available upon request. Please note that this method is not capable of detecting exonic or whole gene deletion/duplications. DNA has been stored. This result is dependent upon the information supplied being correct and complete.

For information only: The following variants identified in this patient are currently considered as likely to be benign:
ABCB11 (NM_003742.2): c.1331T>C; p.(Val444Ala), heterozygous.

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Panel A genes tested: ABCB4 (NM_000443.3); ABCB11 (NM_003742.2); ATP8B1 (NM_005603.3); JAG1 (NM_000214.2); NOTCH2 (NM_024408.3); NPC1 (NM_000271.4); NPC2 (NM_006432.3); NR1H4 (NM_005123.3); SERPINA1 (NM_000295.4); SLC25A13 (NM_014251.2); TJP2 (NM_004817.3); VIPAS39 (LRG_1019); VPS33B (LRG_884).

Panel B genes tested: AKR1D1 (NM_005989.3); ALDOB (NM_000035.3); BAAT (NM_001701.3); CLDN1 (NM_021101.4); CYP27A1 (NM_000784.3); CYP7B1 (NM_004820.3); EPHX1 (NM_000120.3); HSD3B7 (NM_025193.3); PEX1 (NM_000466.2); PEX2 (NM_000318.2); UTP4 (NM_032830.2).

The Illumina HiSeq platform has been used to sequence coding regions and splicing sites (-5/+5) of the above genes captured by the TruSight One Panel target enrichment system (Illumina). Analysis was performed using an in-house pipeline; alignment BWA mem; variant calling Platypus; variant annotation Annovar; against human genome hg19 as a reference. Sanger sequencing used to complete screening of NPC1, NPC2, ATP8B1, ABCB11, ABCB4, SLC25A13, VPS33B, VIPAS39. Of the remaining genes, 98.12% of the target region has been covered to a minimum depth of 20X and regions below this minimum threshold have not been Sanger sequenced. NOTCH2 exons 1-4 have low mapping quality and there may be reduced sensitivity, however no pathogenic variants have been previously identified in these exons. Sequence nomenclature according to HGVS guidelines. Variants currently considered as benign polymorphisms and/or with minor allele frequency >1% are not reported. Variants currently considered likely to be benign have not been confirmed by Sanger sequencing and are recorded for information only in this report. Further details are available upon request. Please note that this method is not capable of detecting exonic or whole gene deletion/duplications. DNA has been stored. This result is dependent upon the information supplied being correct and complete.

For information only: The following variants identified in this patient are currently considered as likely to be benign:
ABCB11 (NM_003742.2): c.1331T>C; p.(Val444Ala), heterozygous.

- genetic assessment not easy

16yo male, developing jaundice after infections (Rx antibiotics)

- ? BRIC
- ? DILI

gene studies favoured BRIC

Schreiner P et al. Liver Int 2019 (in press)

TABLE 1 Genetic analysis

rs number	g. (GRCh38)	c.	p.	AF (%) all/ NFE/ AFR
NR1H4 (FXR)				
only non-coding variants				
ATP8B1 (FIC1)				
rs319438 (ho)	57697620A>G[a]	696T>C	D232D	99.8/100.0/98.0
rs319443 (ho)	57695300T>G[a]	811A>C	R271R	99.7/100.0/97.2
rs222581 (ho)	57650444T>C	3454A>G	A1152T	gDNA vs. transcript
ABCB11 (BSEP)				
rs34313070	168973871del	1309-31del	-	0.2/0.01/1.9
rs4148777	169013391A>G	270T>C	F90F	4.6/3.6/6.8
rs2287622 (ho)	168973818A>G	1331T>C	V444A	56.9/59.7/56.6
rs138642043	168964291C>T	2093G>A	R698H	0.4/0.3/0.7
rs497692	168932506T>C	3084A>G	A1028A	54.5/53.6/26.6
ABCB4 (MDR3)				
rs2230028	87426860T>C	1954A>G	R652G	10.5/7.5/34.8
rs2109505 (ho)	g.87450090T>A	711A[b]		

Detected common intronic variants are listed in Table S1.
AF: allele frequency; taken from Genome Aggregation Database (gnomAD), Cambridge, MA (http://gnomad.broadinstitute.org/) [allele frequencies accessed November 2018]. AFs are given for the worldwide (all), Non-Finnish European (NFE) as well as the African population (AFR).
Variants are specified by rs number, exchange on genomic (g.) and coding (c.) DNA level and if applicable on protein level.
Reference sequences: NR1H4: NM_001206977.1; ATP8B1: NM_005603.4; ABCB11: NM_003742.2, ABCB4: NM_000443.3.
Abbreviation: ho: homozygous.
[a]In alternative reference sequence AF038007 C-alleles are designated as the reference allele.
[b]Reference allele A represents the risk allele.

Histology

- 13 patients – heterozygous

- ductular reaction
- mild portal inflammation
- fibrosis (variable)

MOST

- cholesterol crystals or spaces in ducts
- bile duct injury
- periductal onion-skinning fibrosis

SOME

Wendum *et al*. Virchows Arch 2012

Could small-duct PSC be *ABCB4 (*MDR3)?

Against:

1. PBC and PSC – no link to *ABCB4* mutation
2. 25% develop large-duct PSC
3. 50-88% have IBD
4. 10% recur in allograft (no mutation)

>> in those without IBD, consider *ABCB4* (MDR3)

Bjornsson E. Curr Gastroenterol Rep 2009; 11:37-41

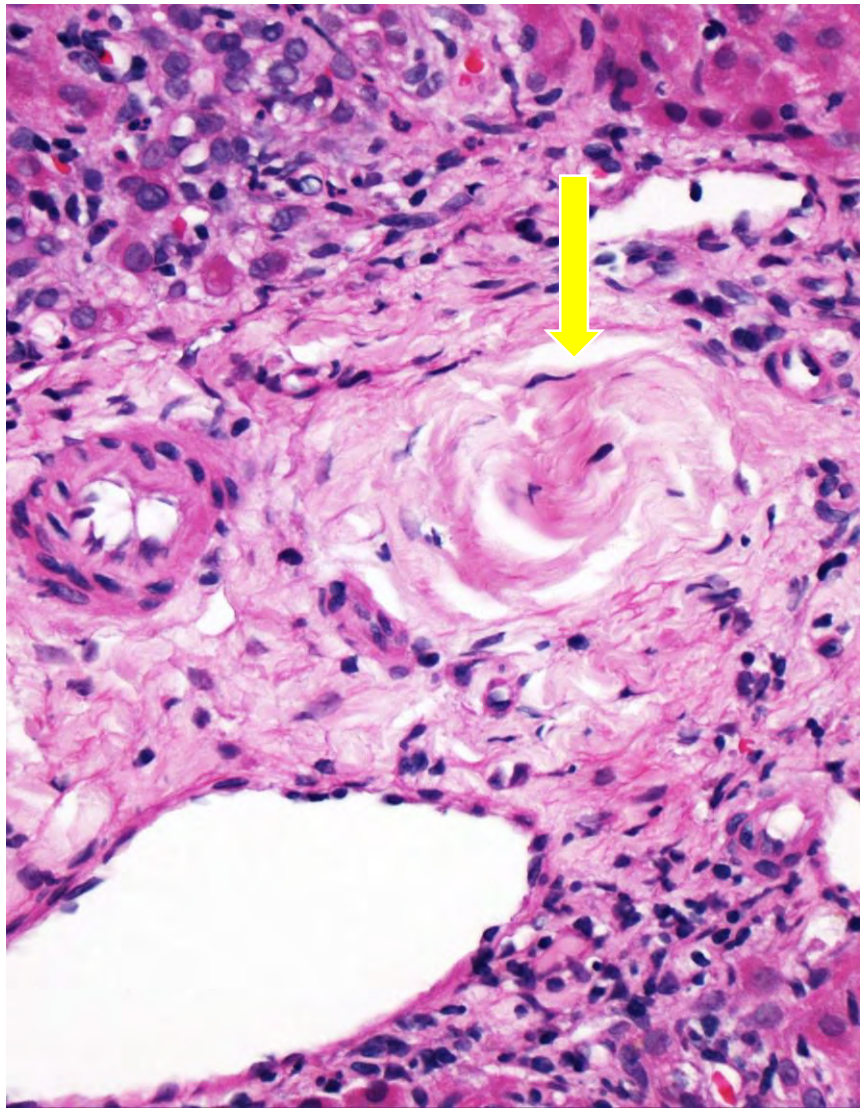
Small duct PSC

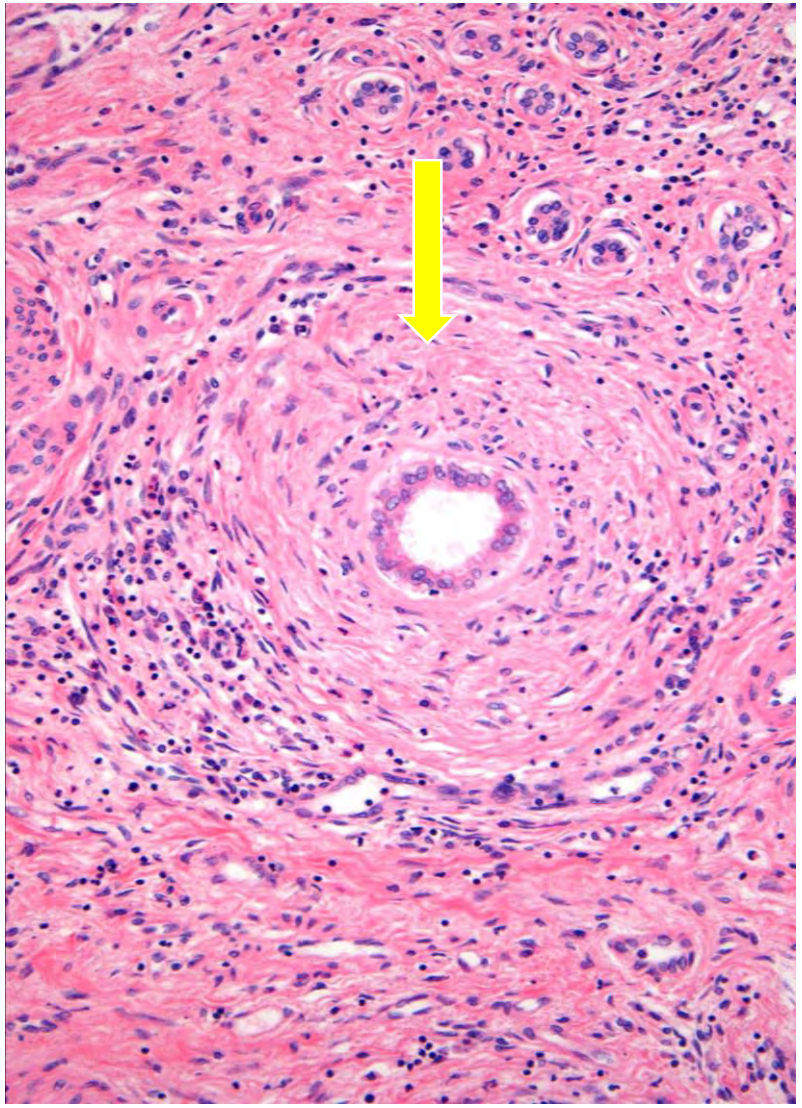
- originally called pericholangitis – patients with IBD but no large-duct PSC
- Ludwig recognized that small duct changes were same as PSC
  - "small-duct primary sclerosing cholangitis"

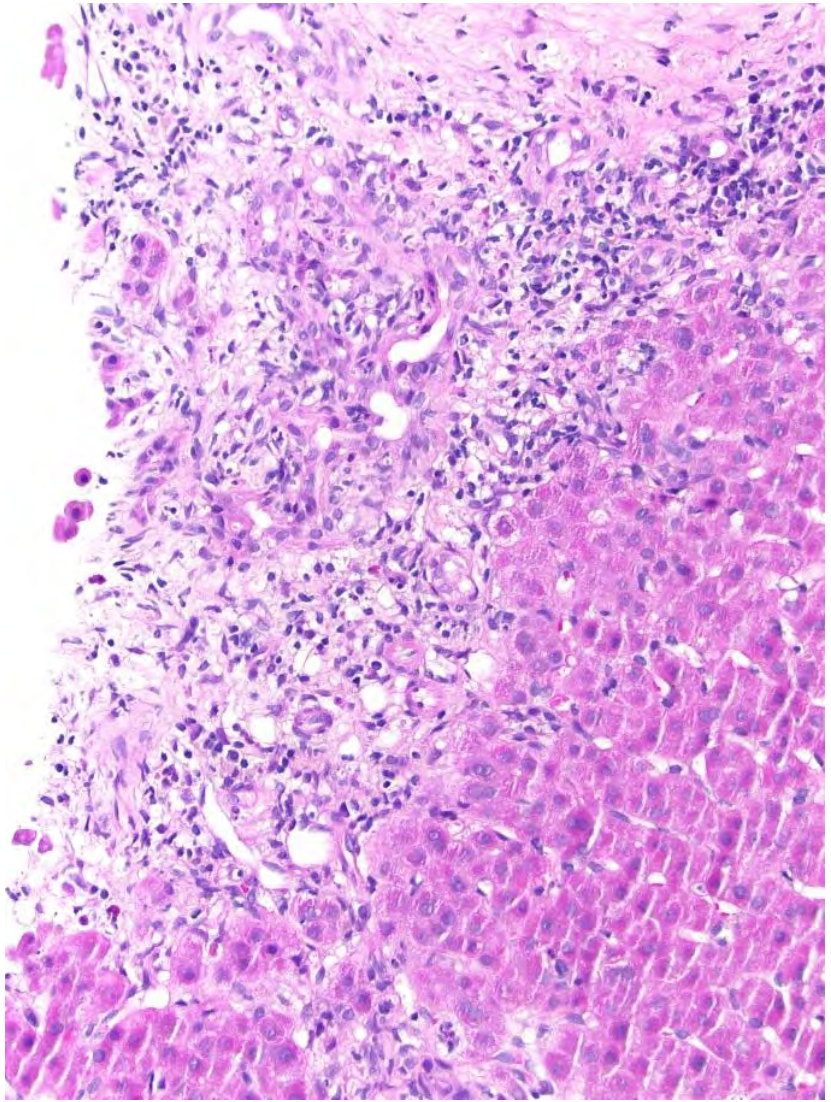
- ~15% of PSC - milder disease
- some evolve to LD-PSC - 25% progress by 8 years
- different IBD pattern - more commonly Crohn disease
- cholangiocarcinoma very rare - one case reported

Ludwig J et al. Hepatol 1981; 1:63240 Ludwig J. Semin Liver Dis 1991; 11:11-16
Bjornsson E at al. Gastroenterol 2008; 134:975-80 Lai J et al. Semin Liver Dis 2012; 32:360-6

Histology in small-duct PSC



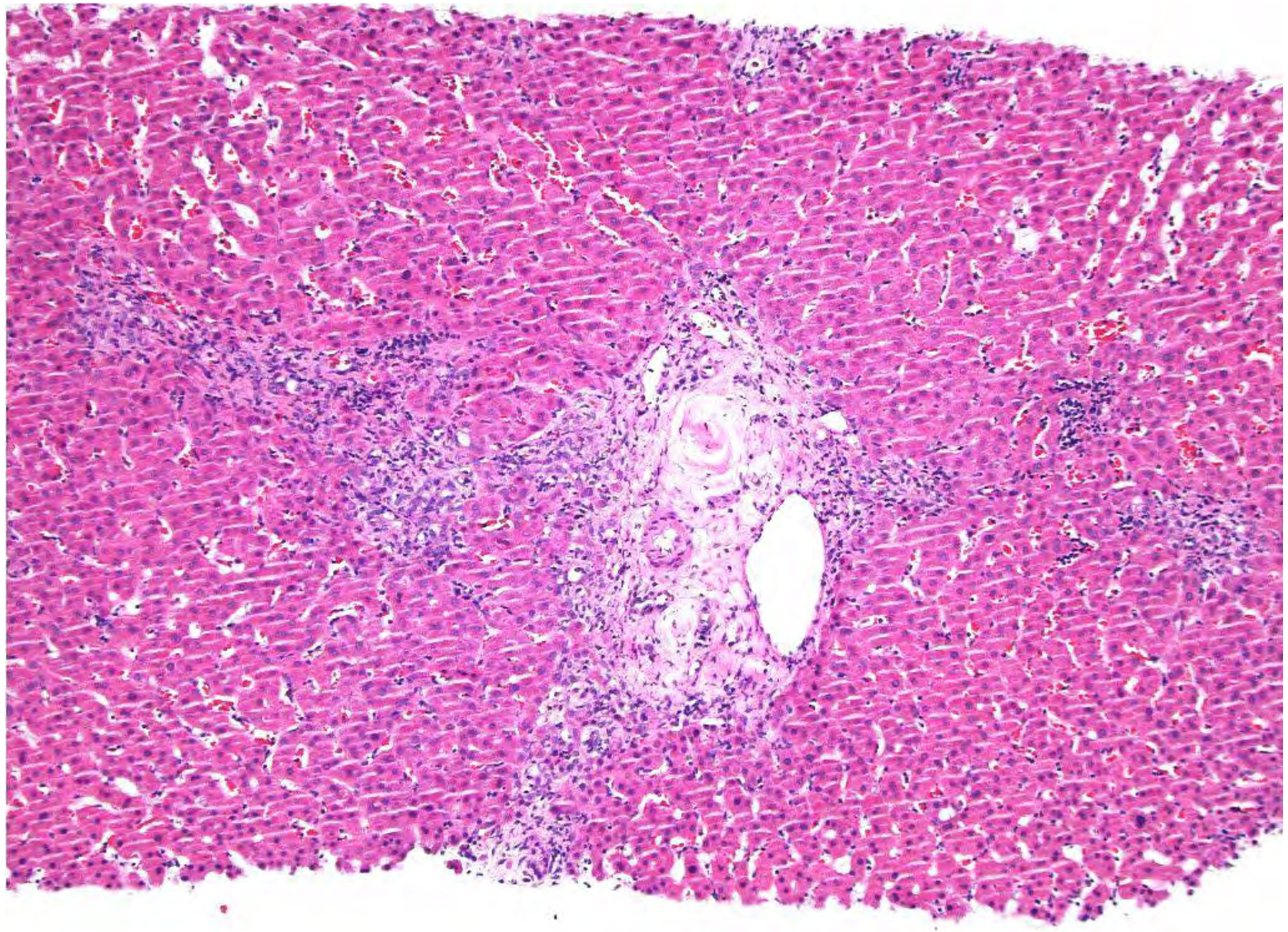




Histology in small-duct PSC

Ludwig:

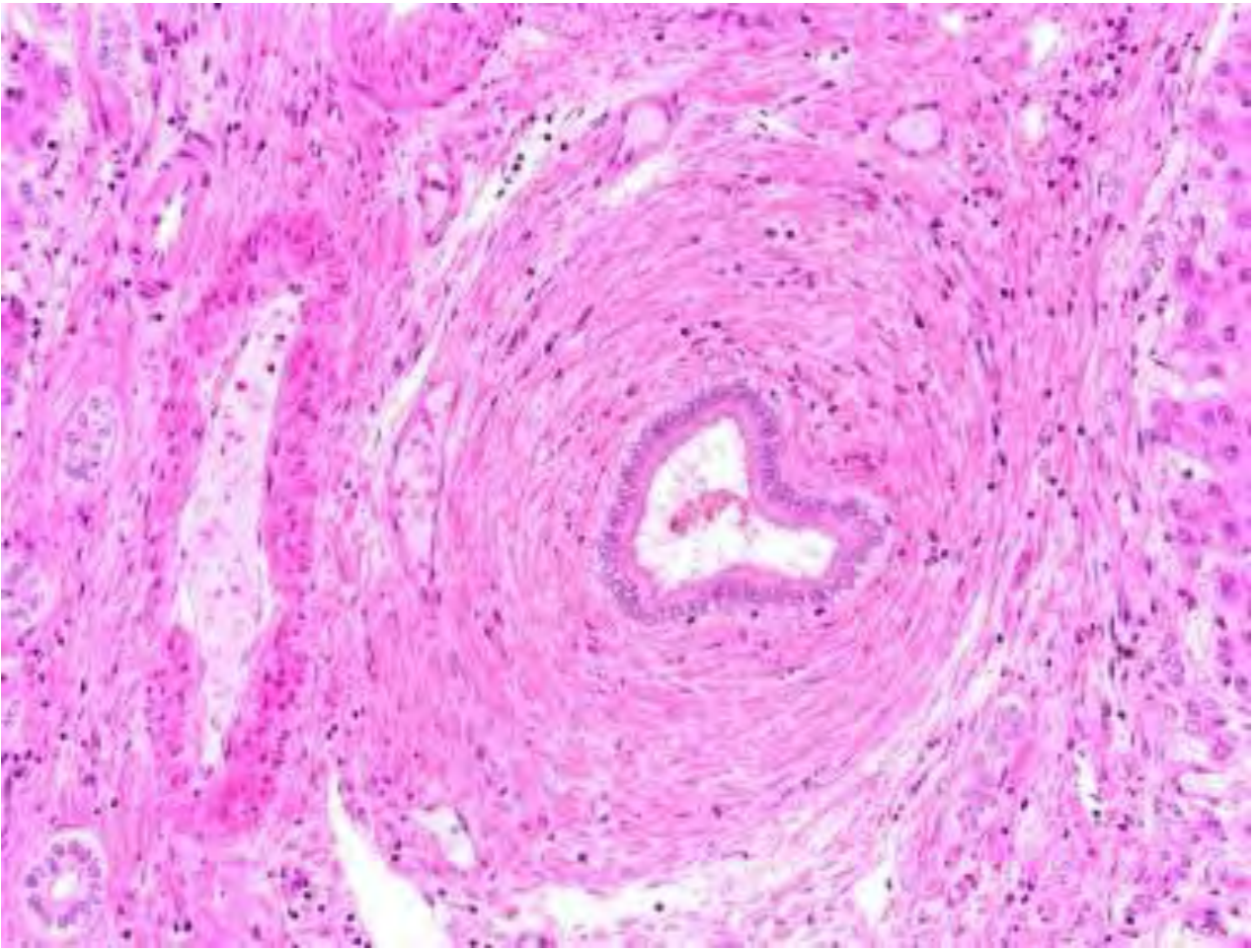
concurrent ductular reaction & duct loss



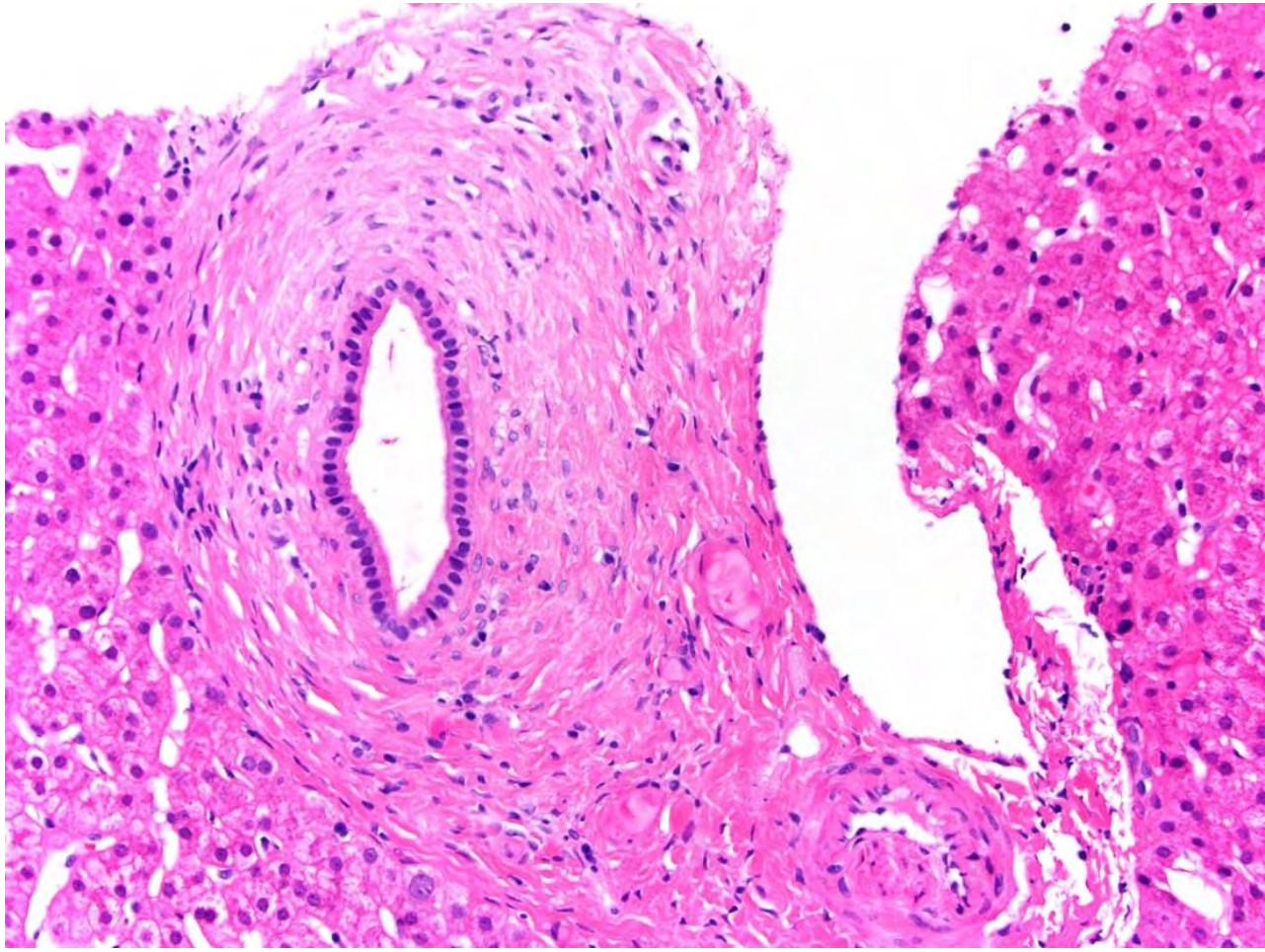
Ludwig J. Semin Liver Dis 1991; 11:11-16

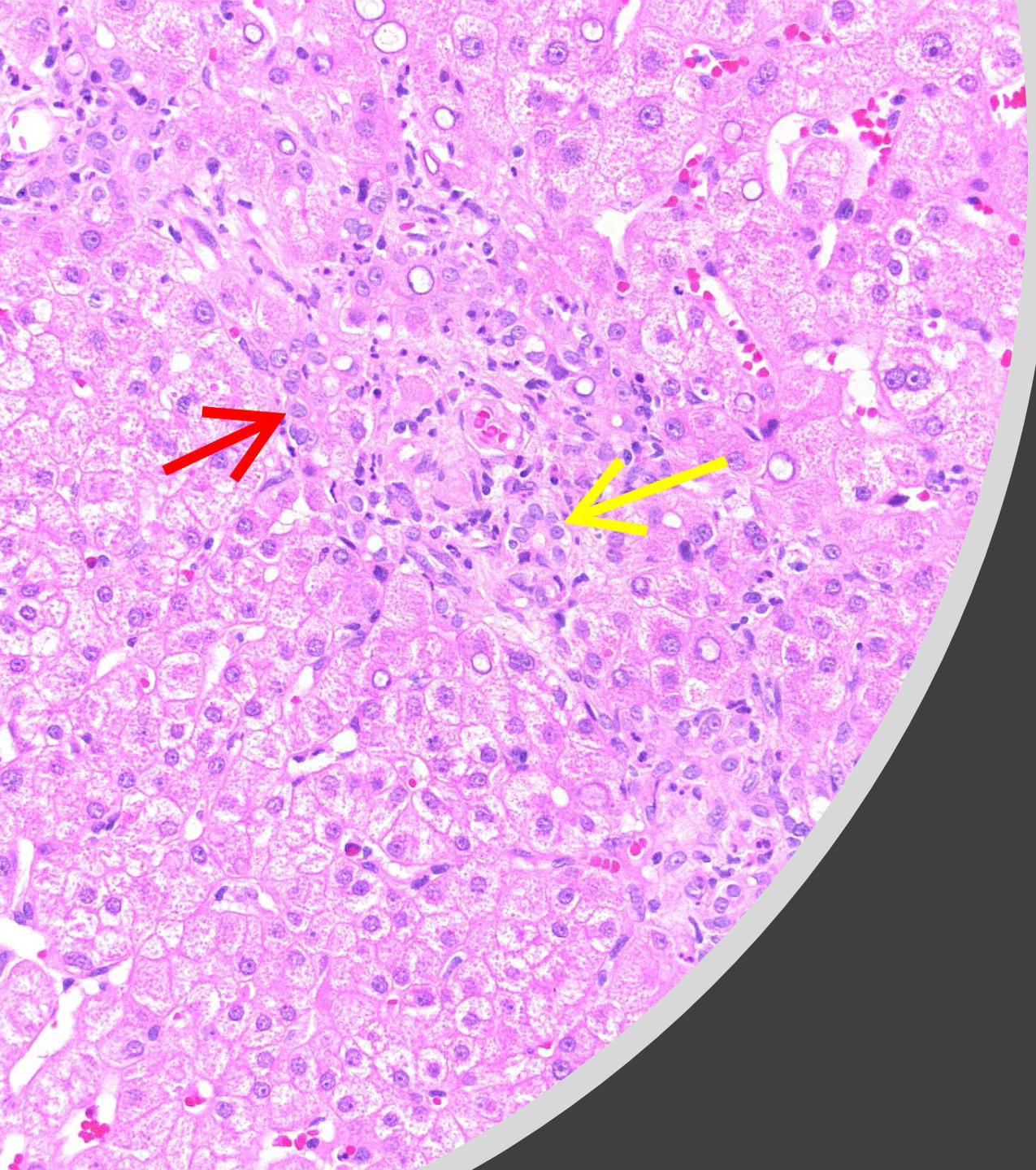
Transplant for SD-PSC – recurred as SD-PSC

explant



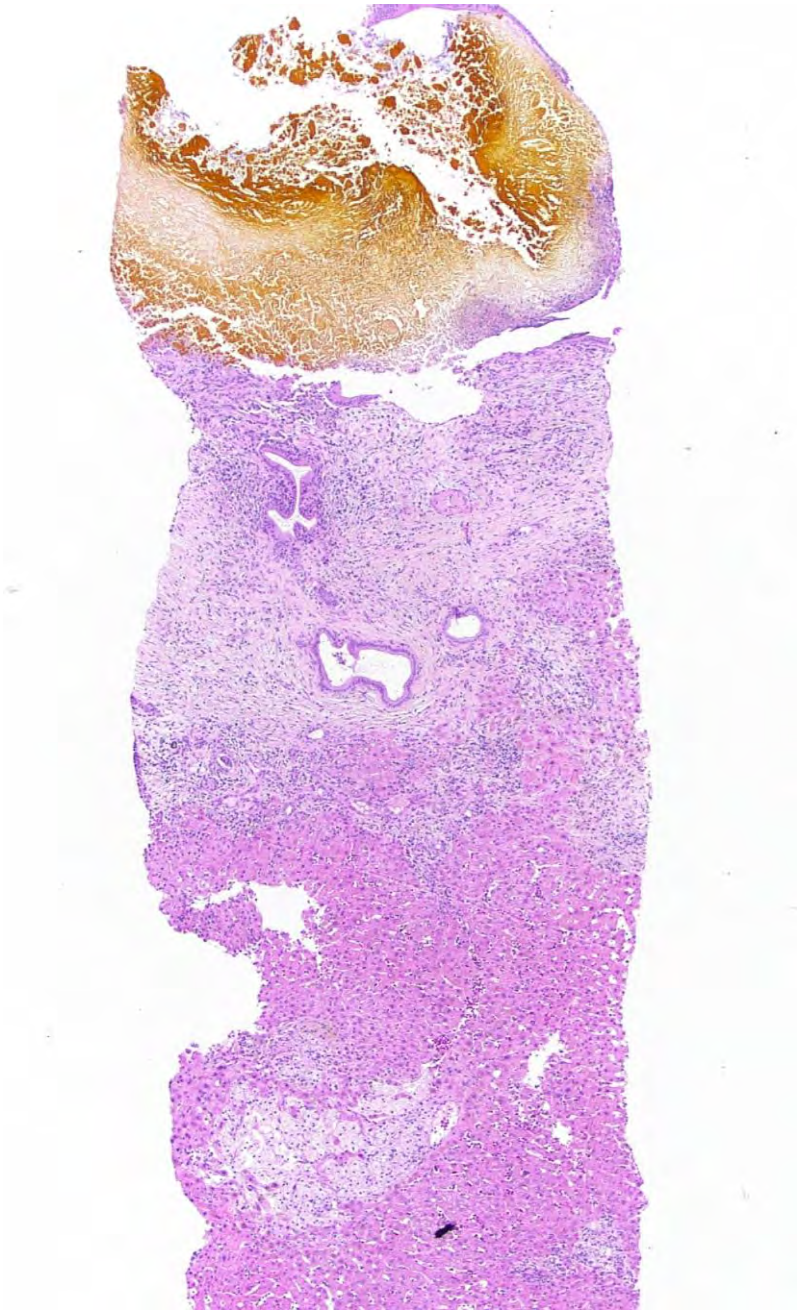
liver graft – 10 yrs

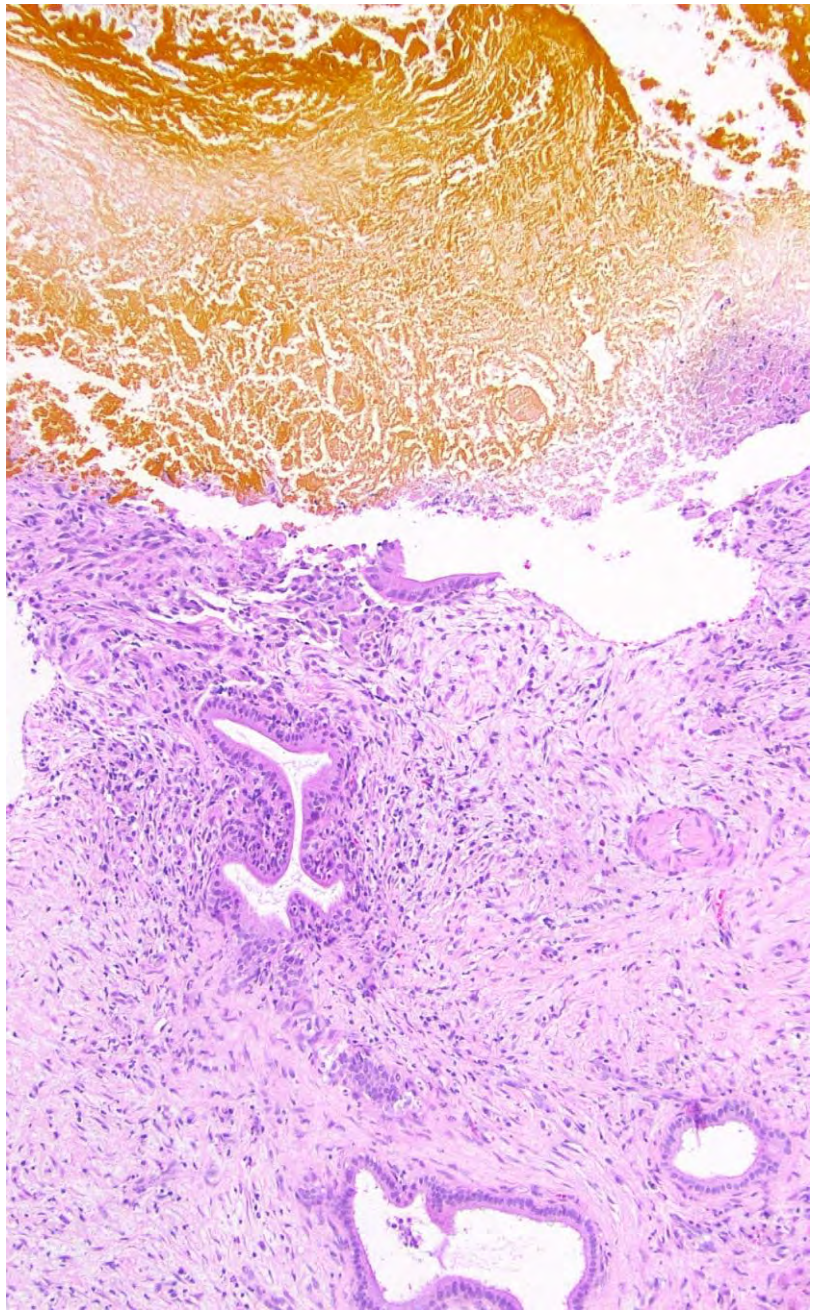


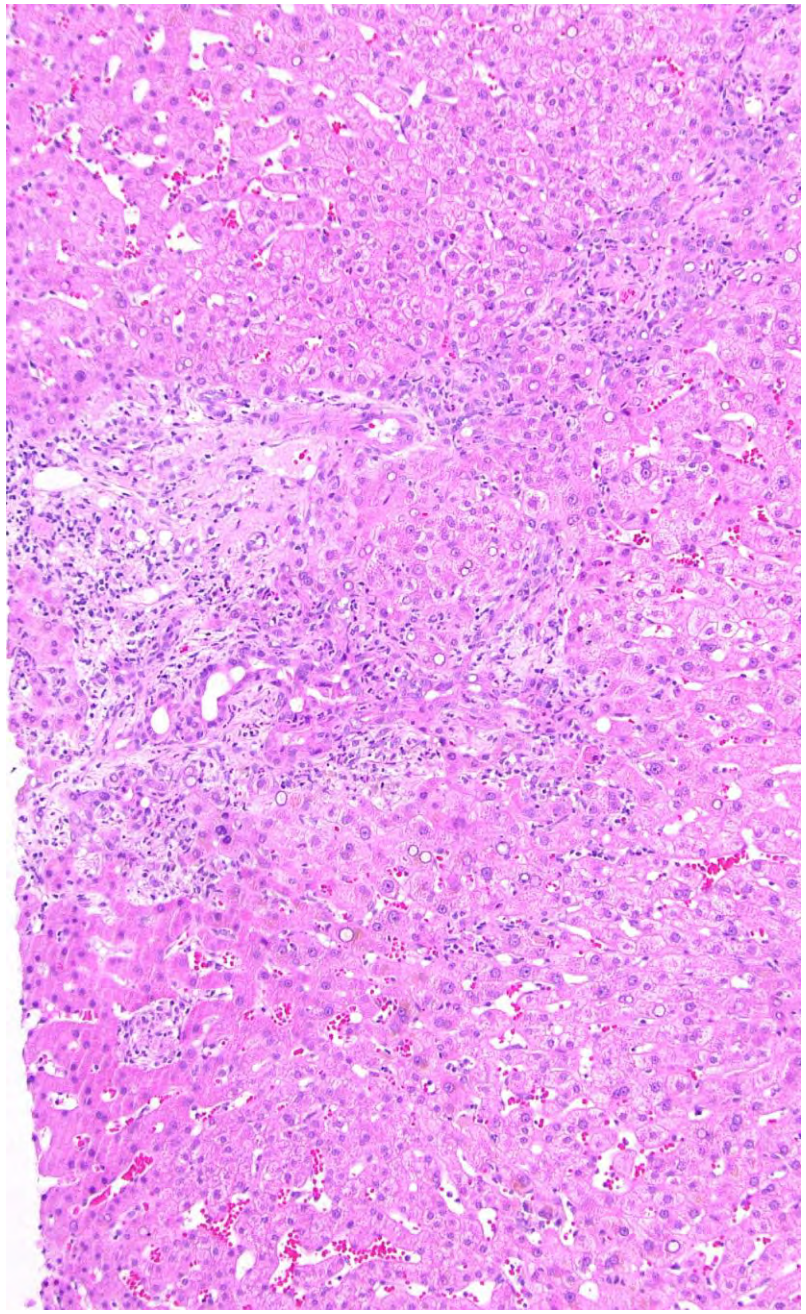


Checkpoint inhibitor sclerosing cholangitis

Zen Y et al. Histopathol 2019 doi: 10.1111/his.14000







Summary – mild canalicular transporter deficiency

- usually heterozygous mutation
- a range of mild cholestatic changes
  - chronic cholestatic LFTs
  - ductular reaction, mild portal fibrosis
  - some have bland cholestasis (contraceptives, pregnancy)
  - can mimic small-duct PSC (consider clinical scenario)

- family history useful
  - cholestasis of pregnancy, Pill-induced cholestasis
  - early gallstones, unexplained cholestatic liver disease
- gene testing available
- treatment is with ursodeoxycholic acid

newyorkermag

"Bile exits the gallbladder, passes through the cystic duct, gets released into the intestines, and, ultimately, winds up on the Internet."

3,086 likes

newyorkermag A cartoon by Benjamin Schwartz.
#TNYcartoons