

# Hepatocellular lesions: Molecular classification and diagnostic pearls

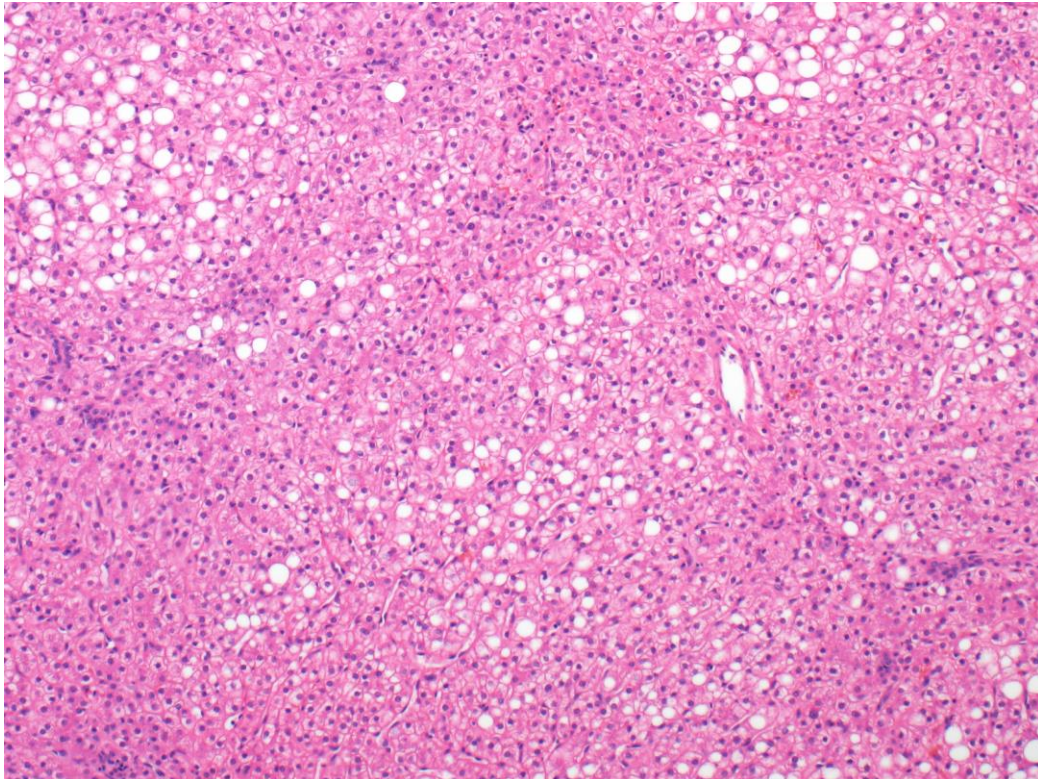
Kim Evason, MD, PhD

University of Utah

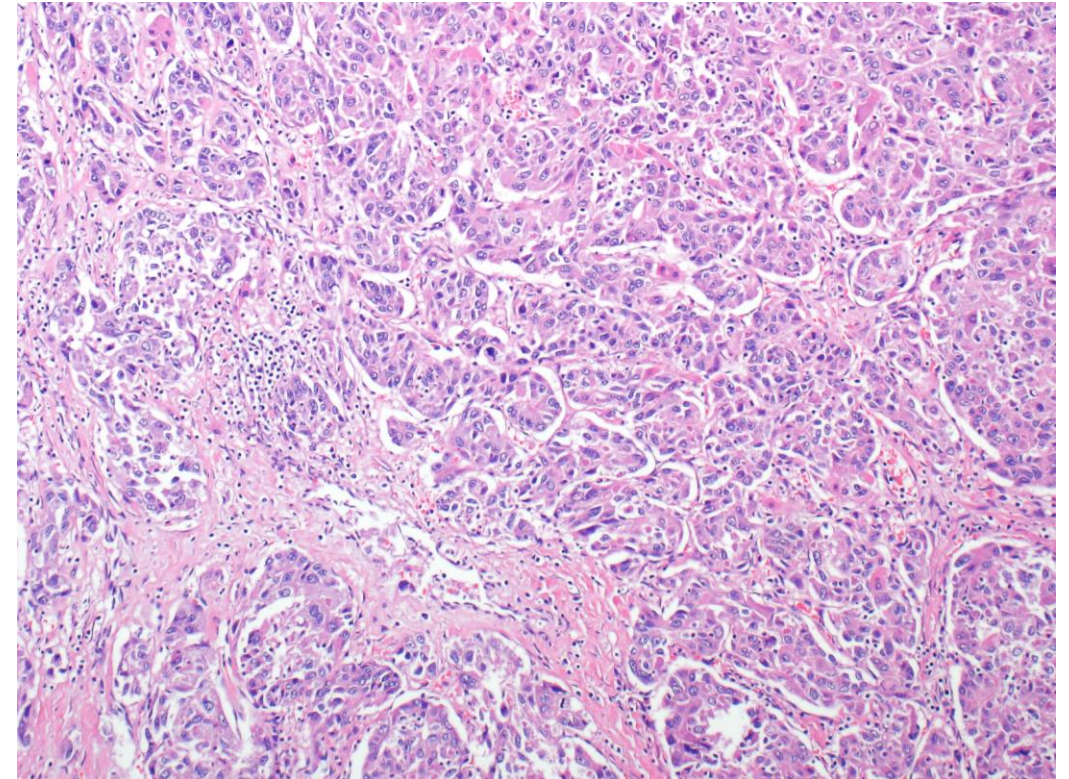
February 10, 2022

# Question determines the approach

1. Is this malignant or not?



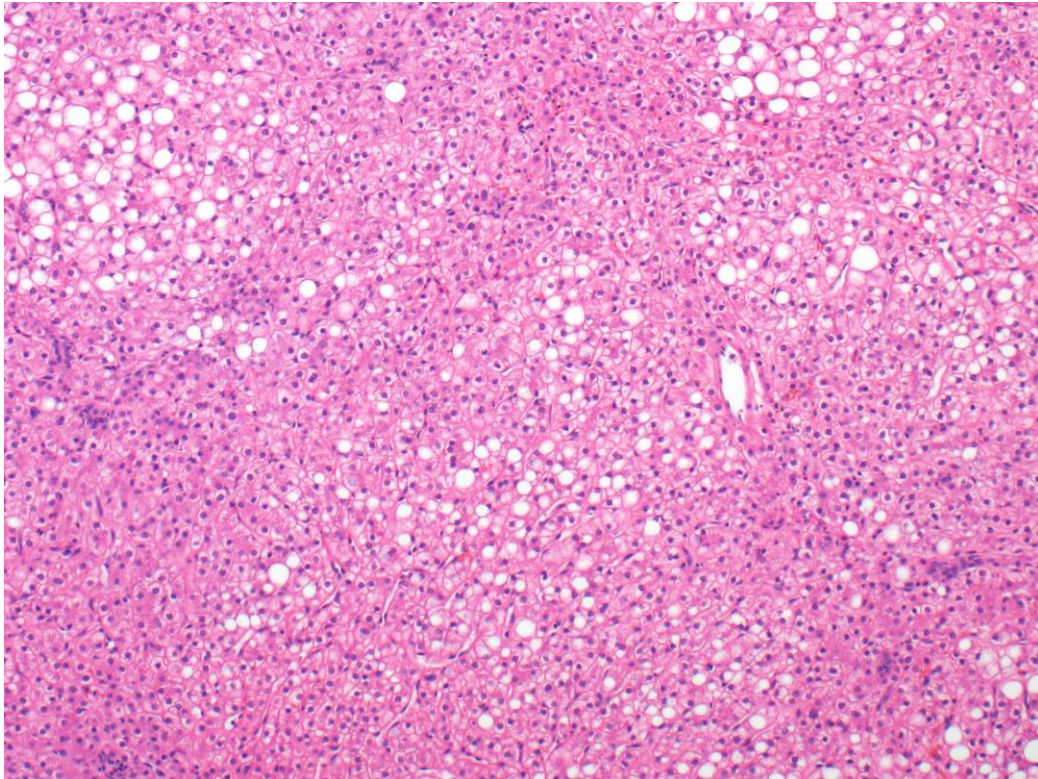
2. Is this hepatocellular or not?



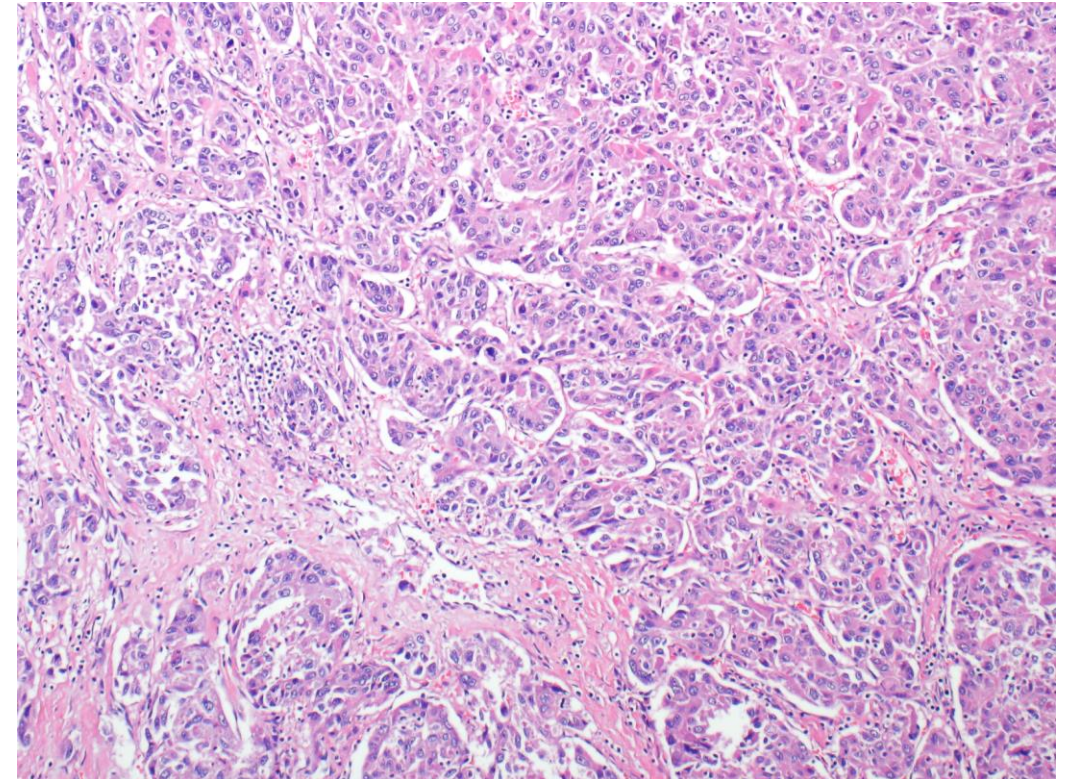


# Question determines the approach

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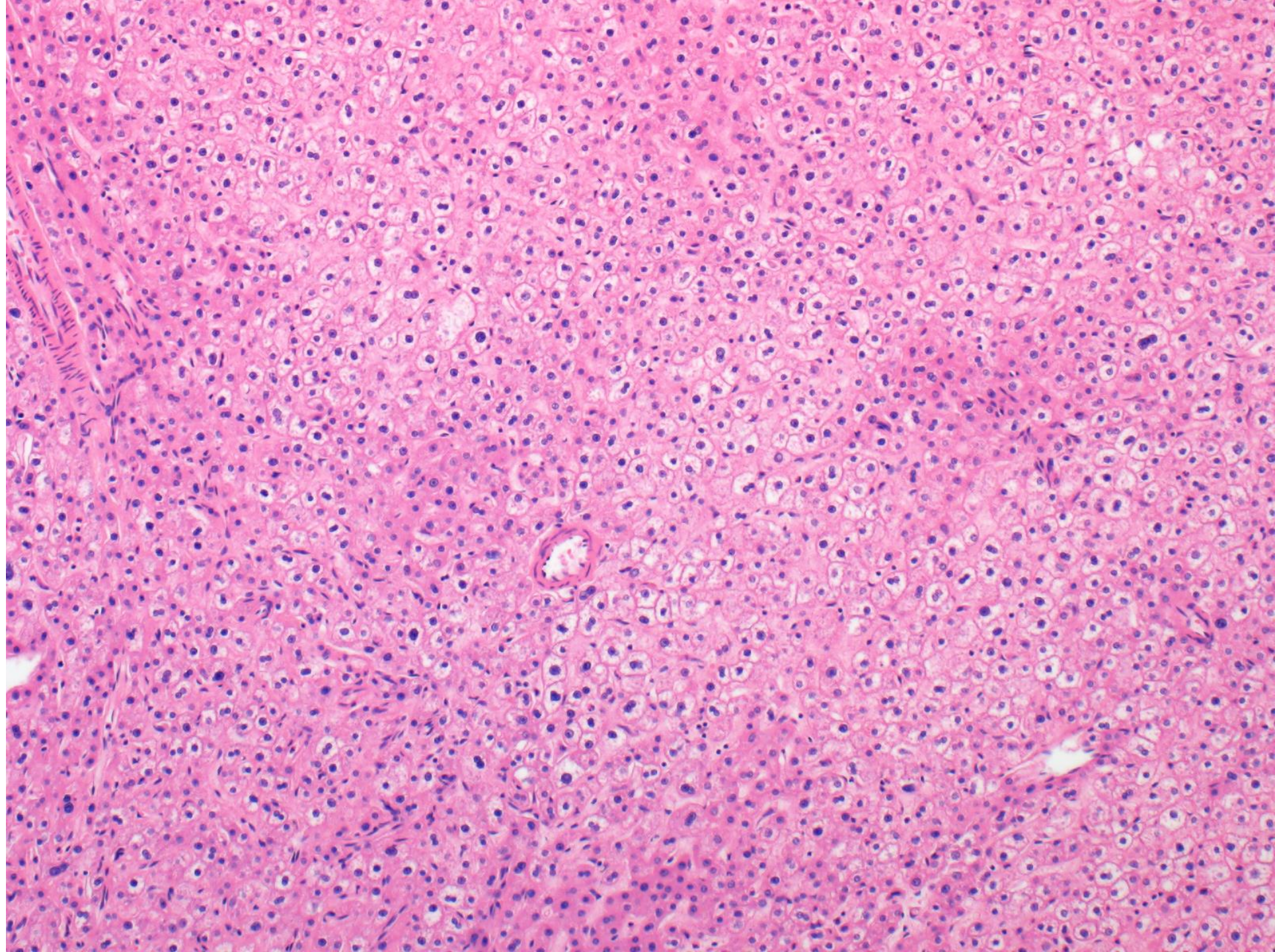
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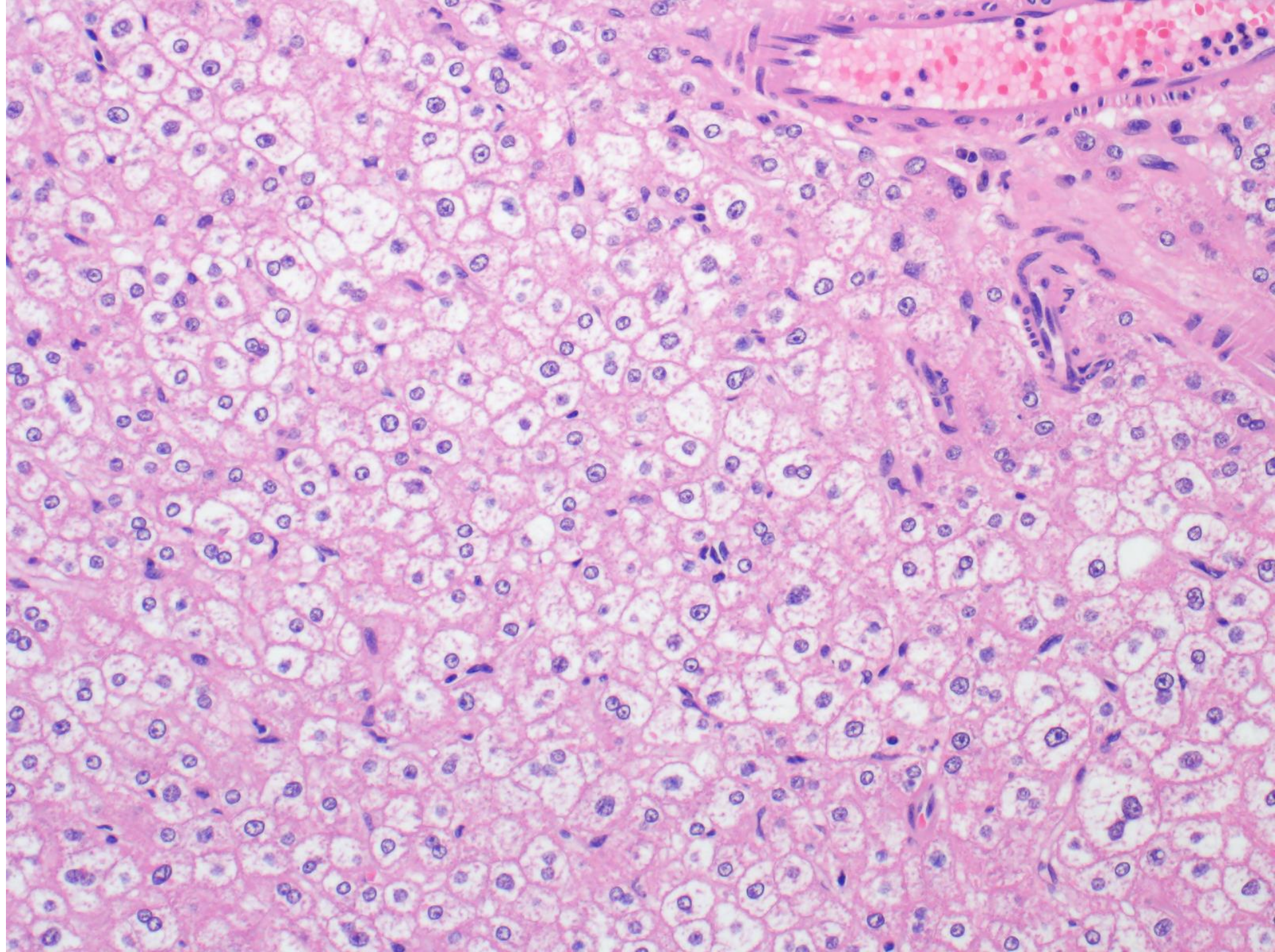
# Case #1

- 55-year-old woman
- History of epigastric and right lower quadrant pain
- MRI showed multiple bilateral hypoechoic liver lesions up to 5.6 cm
- No history of chronic liver disease
- Background liver showed active steatohepatitis with no fibrosis

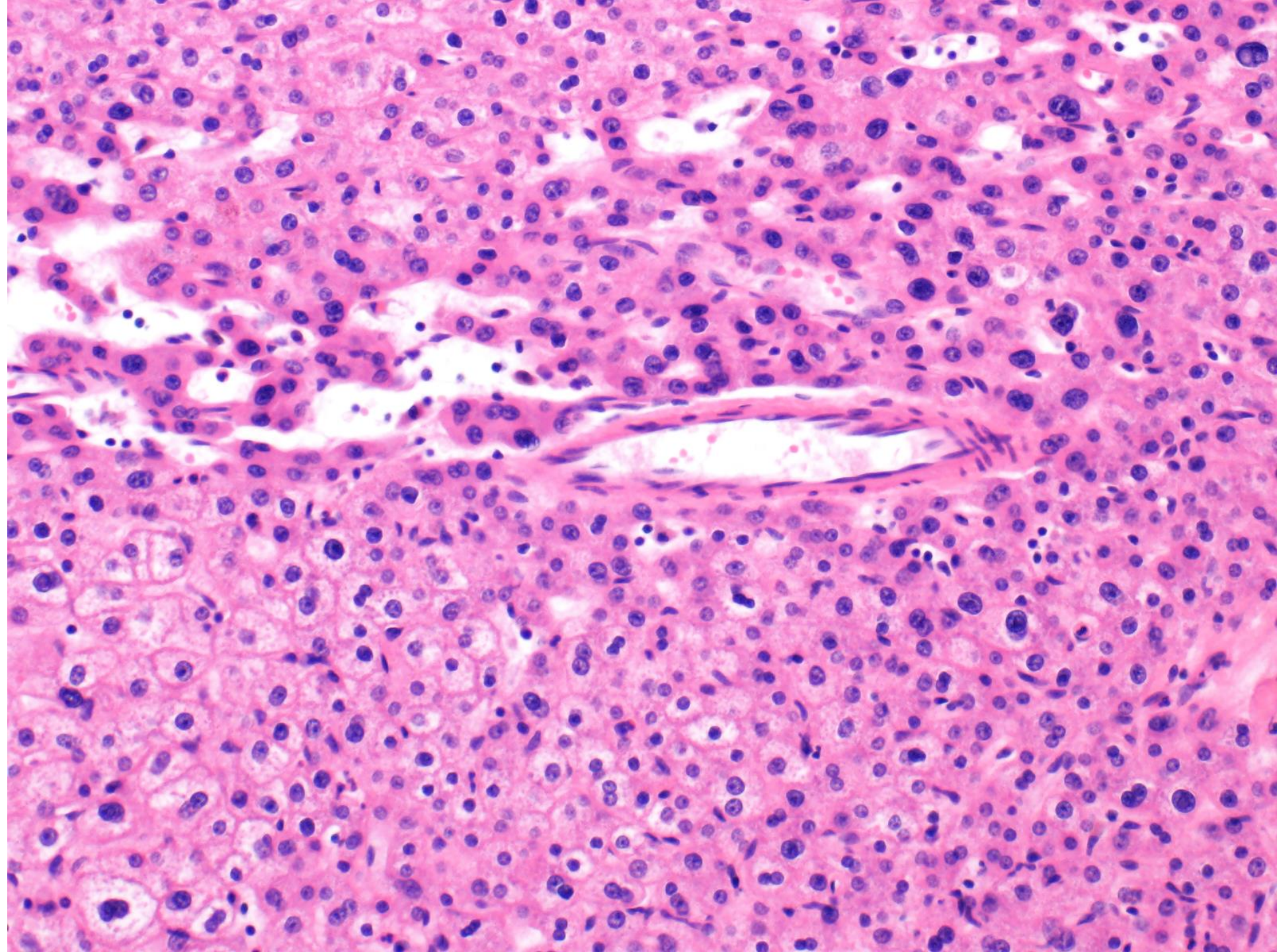




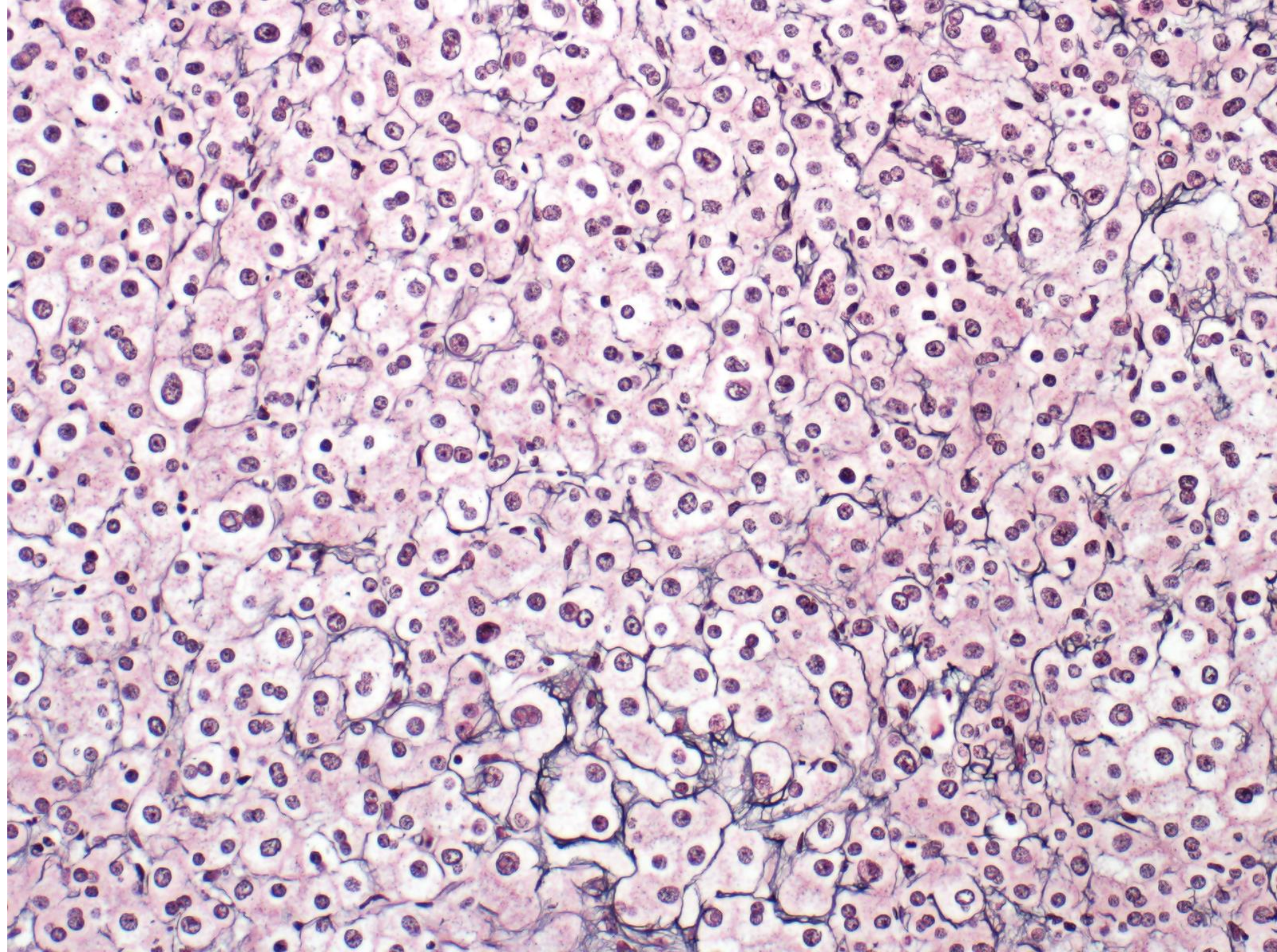










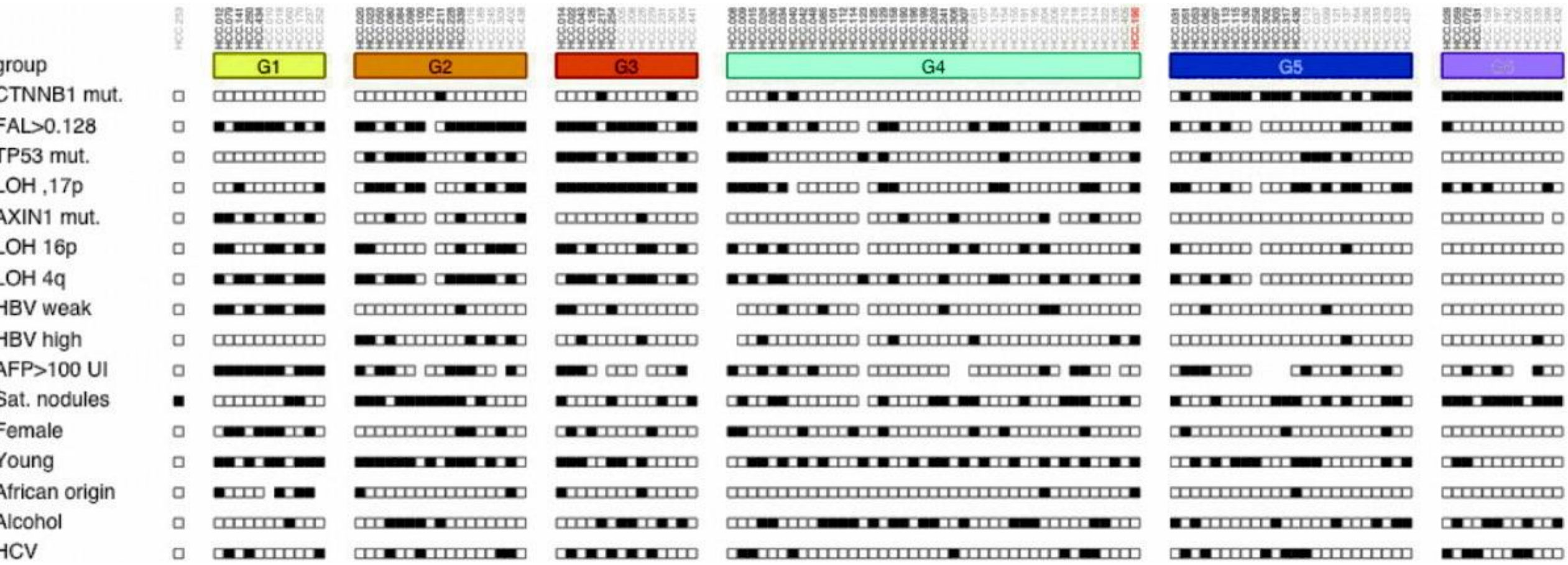




# Case #1: Well-differentiated hepatocellular carcinoma

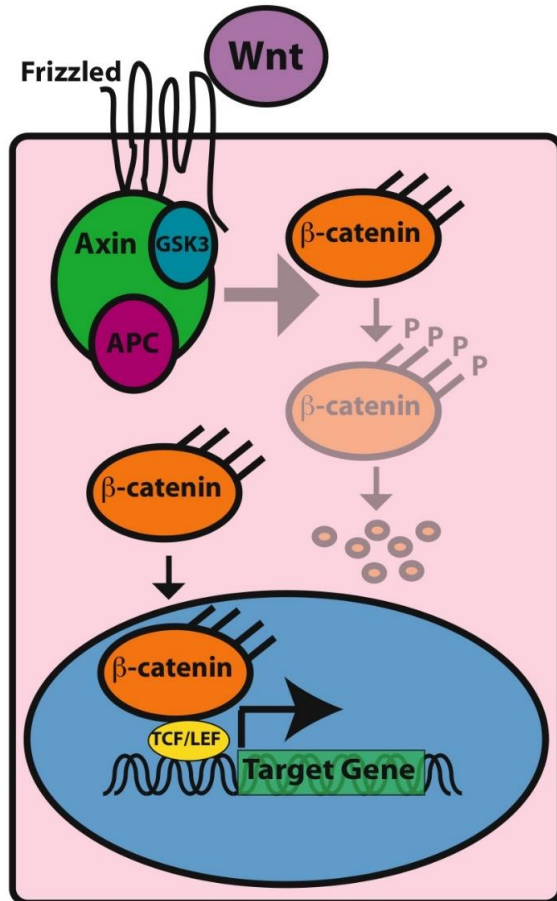
- Hepatocellular differentiation and loss of normal portal structures
- Cytologic atypia and reticulin loss

# Molecular basis of hepatocellular carcinoma



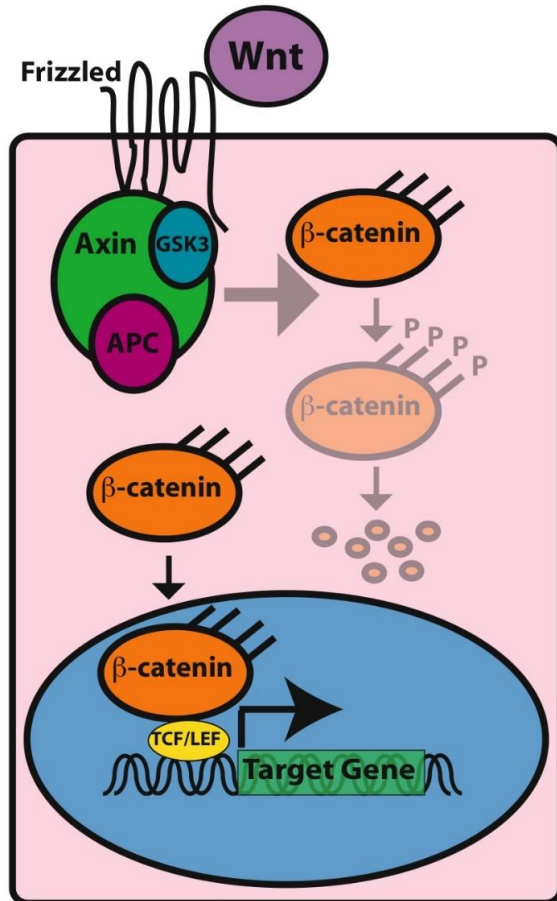


# Wnt/ $\beta$ -catenin signaling pathway

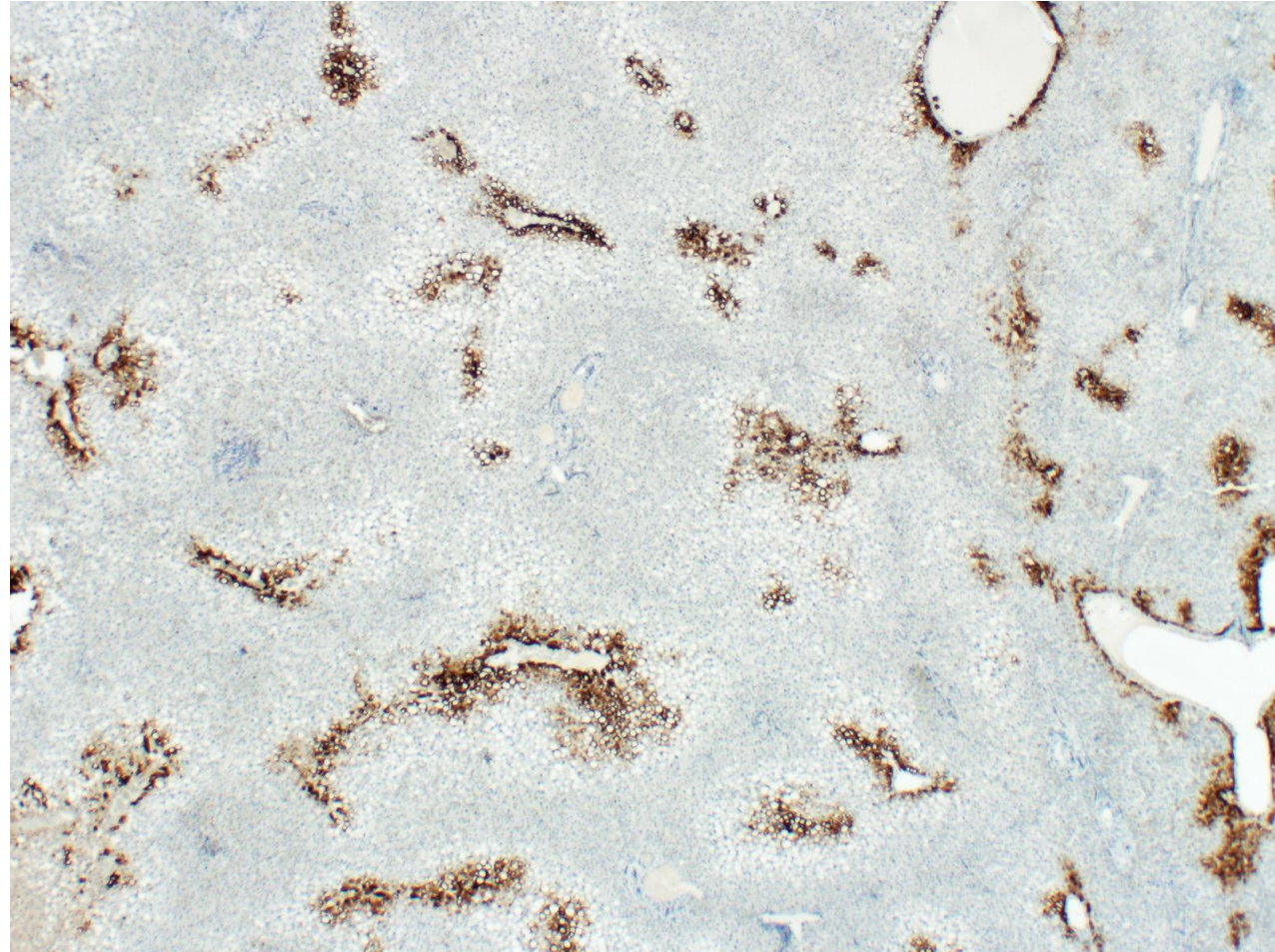


Liver development

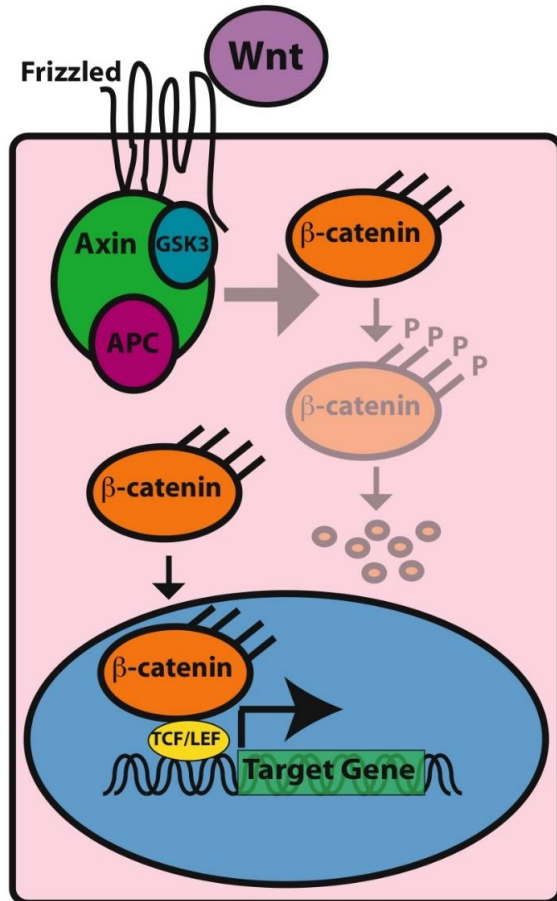
# Wnt/ $\beta$ -catenin signaling pathway



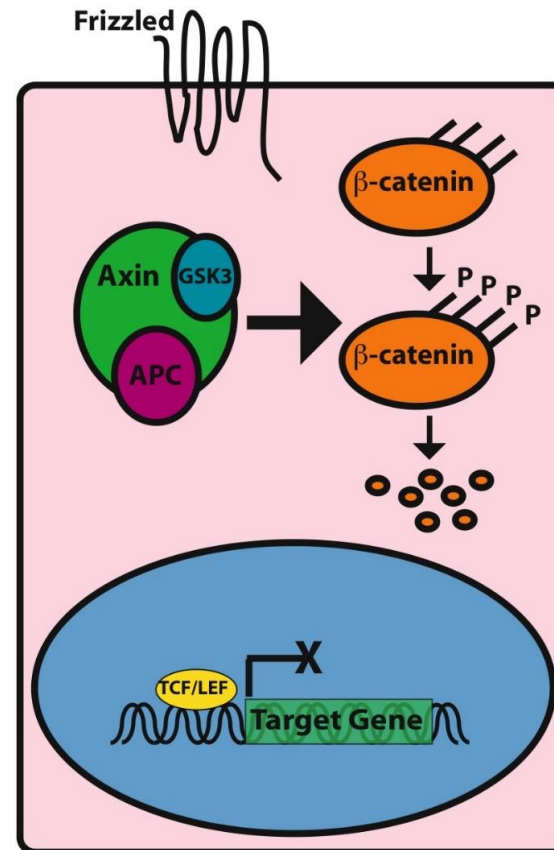
Liver development



# Wnt/ $\beta$ -catenin signaling pathway



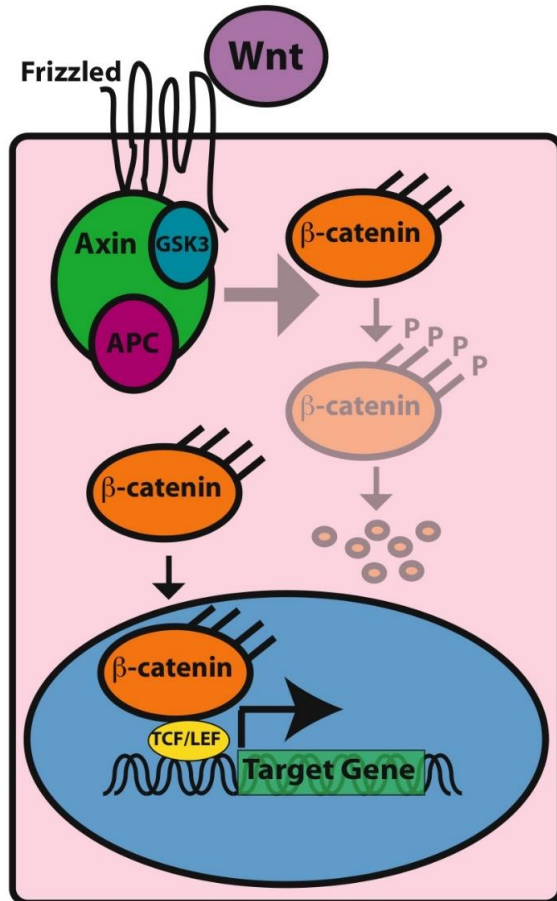
Liver development



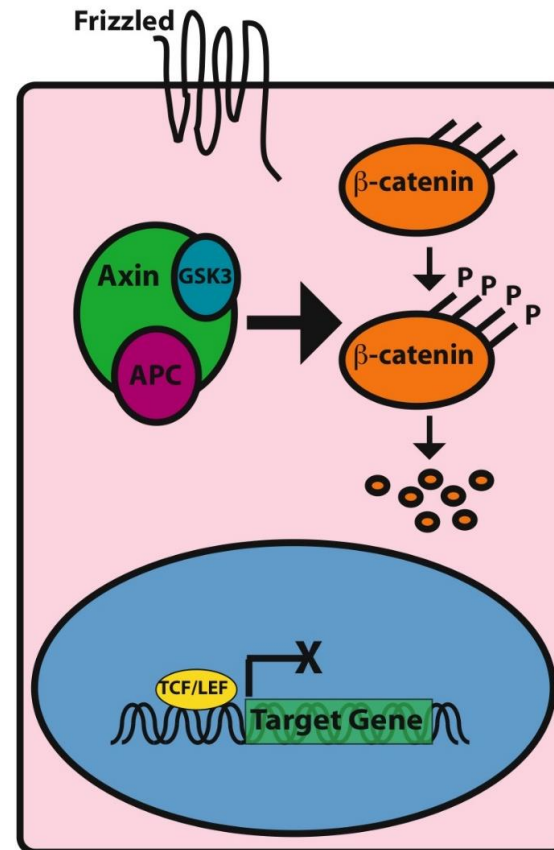
Normal liver cell



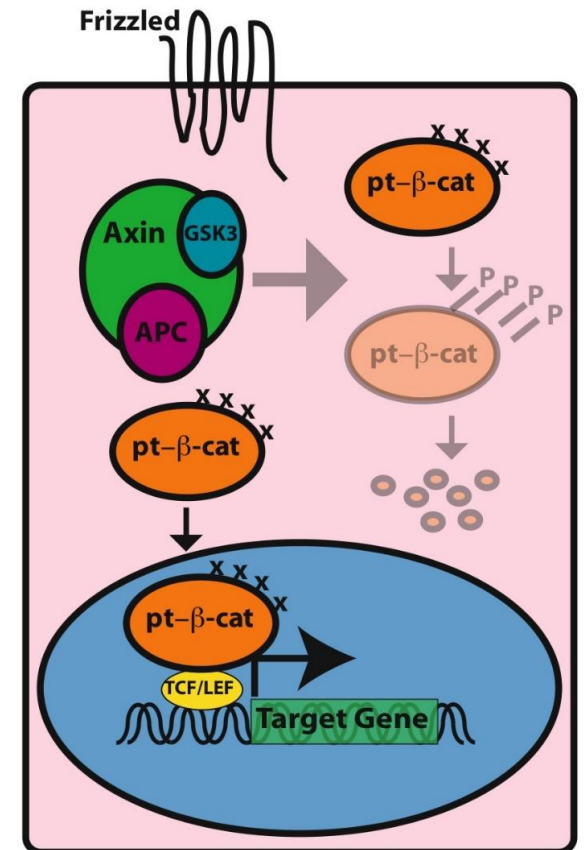
# Wnt/ $\beta$ -catenin signaling pathway



Liver development

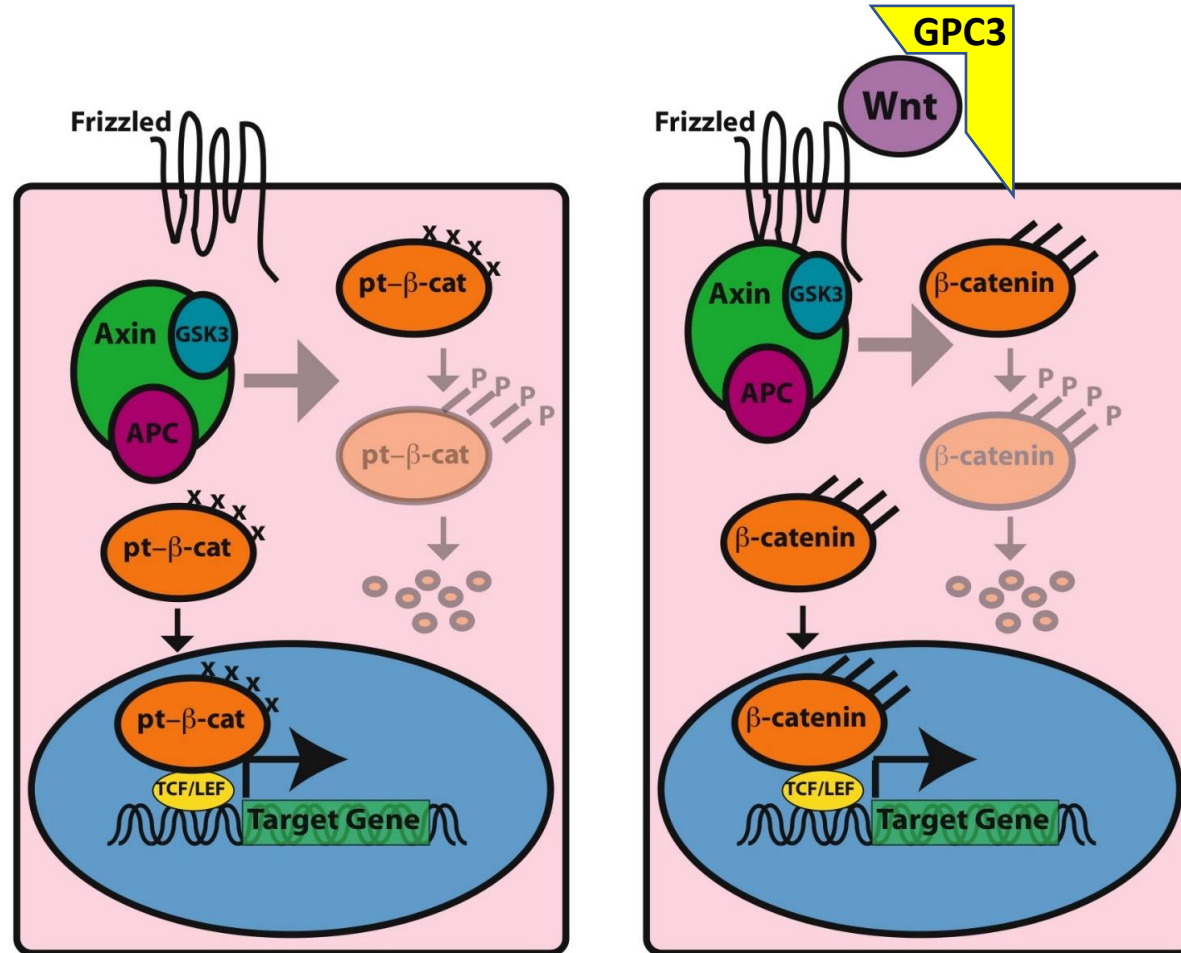


Normal liver cell



Liver cancer

# Wnt/ $\beta$ -catenin signaling pathway in HCC



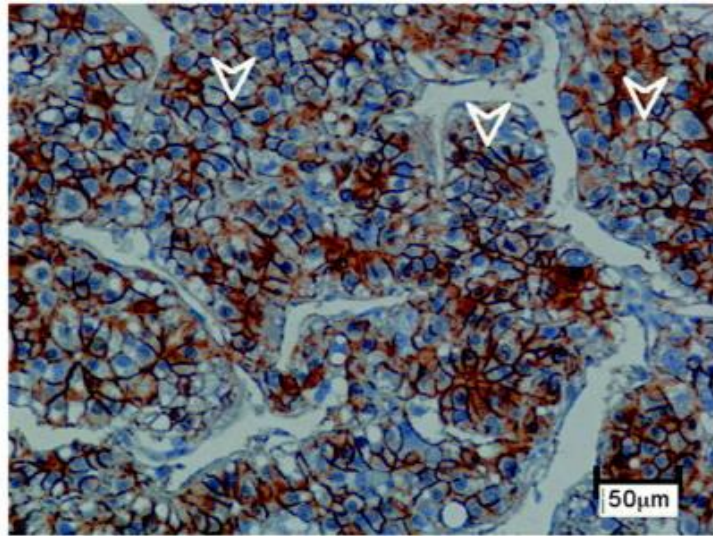
Chien *et al.*, 2009  
Nejak-Bowen and Monga, 2011  
Li *et al.*, 2019



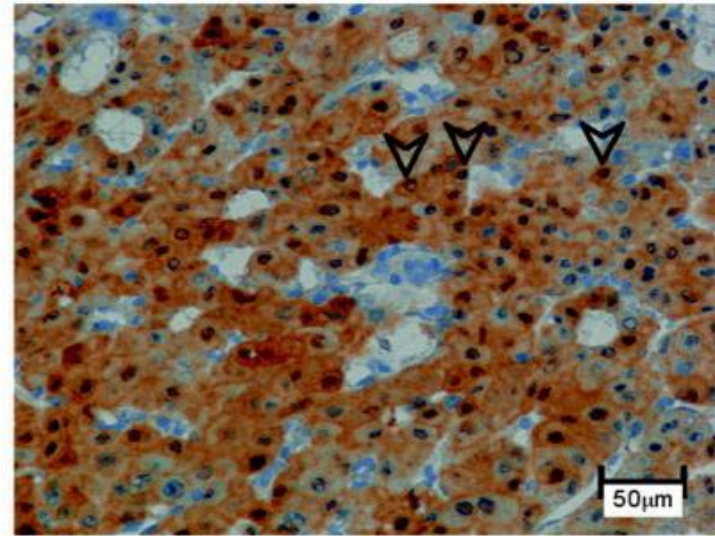


# G5/G6 subgroups

- *CTNNB1* mutations in 70%/100%
- Overexpression of glutamine synthetase (GS)
- Nuclear  $\beta$ -catenin staining in G6

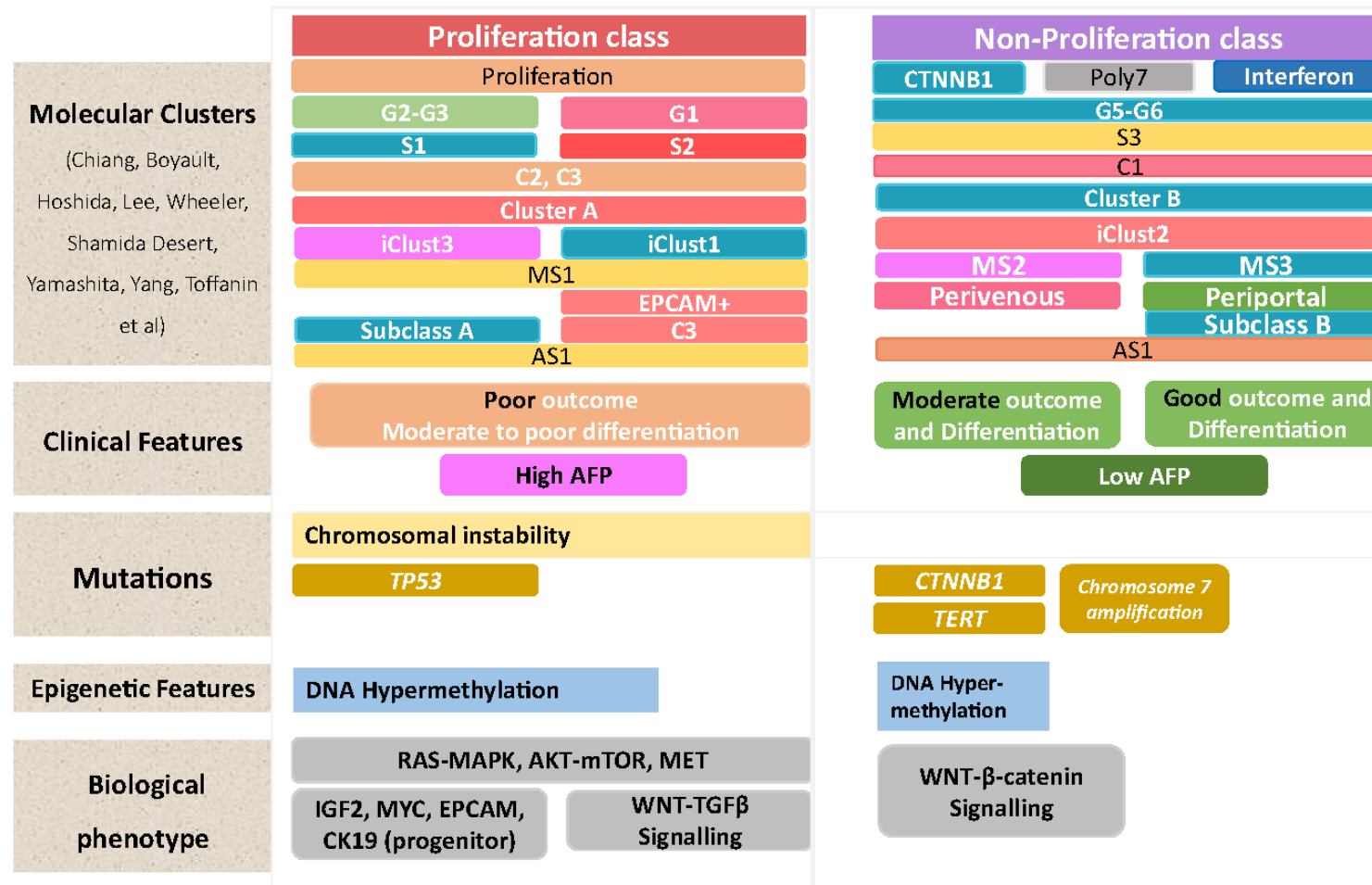


HCC 303 G5



HCC 305 G6

# Molecular basis of hepatocellular carcinoma



- None are widely used clinically



(Potentially) helpful molecular tests:  
Glutamine synthetase, glypican-3, and HSP70

- In cirrhotic liver, 2/3 markers positive → 72% sensitivity, 100% specificity for HCC
- GPC3 less helpful in non-cirrhotic liver
- Positive staining of GS and/or HSP70
  - 78-100% of well-diff HCC and pathologically atypical HCA
  - 0-43% of typical and clinically atypical HCA

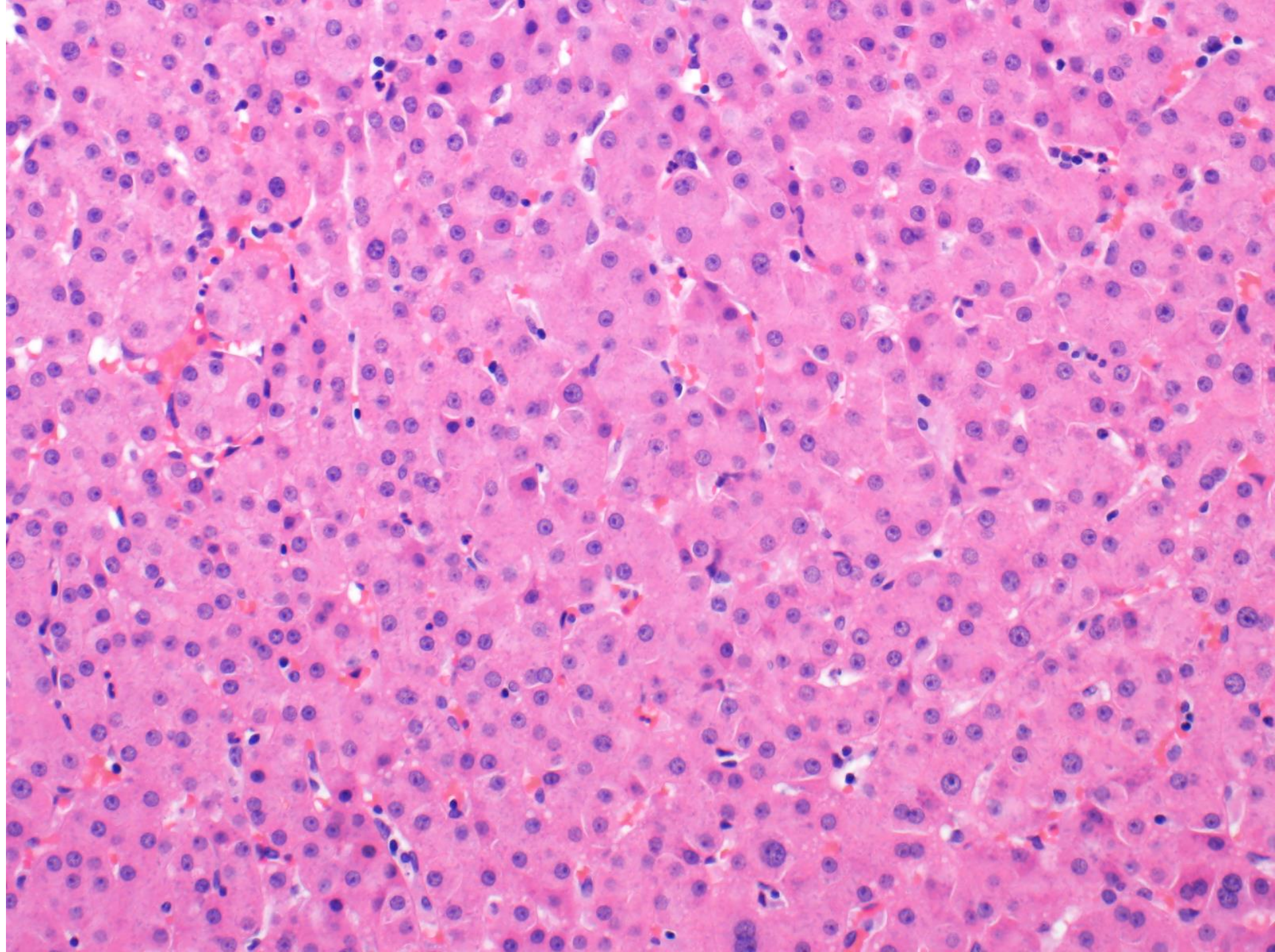
## (Potentially) helpful molecular tests: Ki67

- Ki67 index increases during progression from cirrhotic macroregenerative nodules → dysplastic nodules → HCC
- Ki67 index >4%
  - 7.5% of HCA
  - 51% of HCC

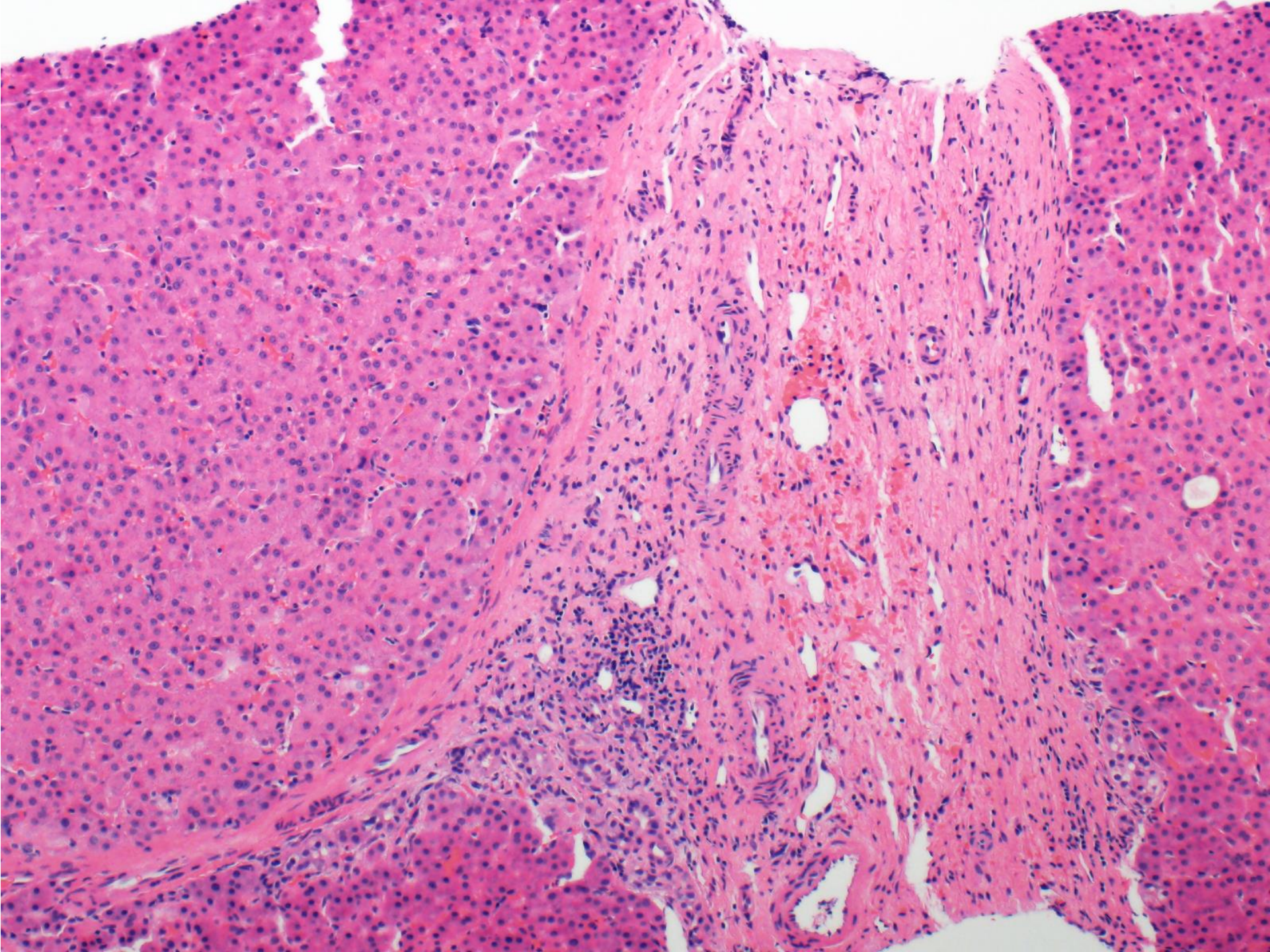


## Case #2

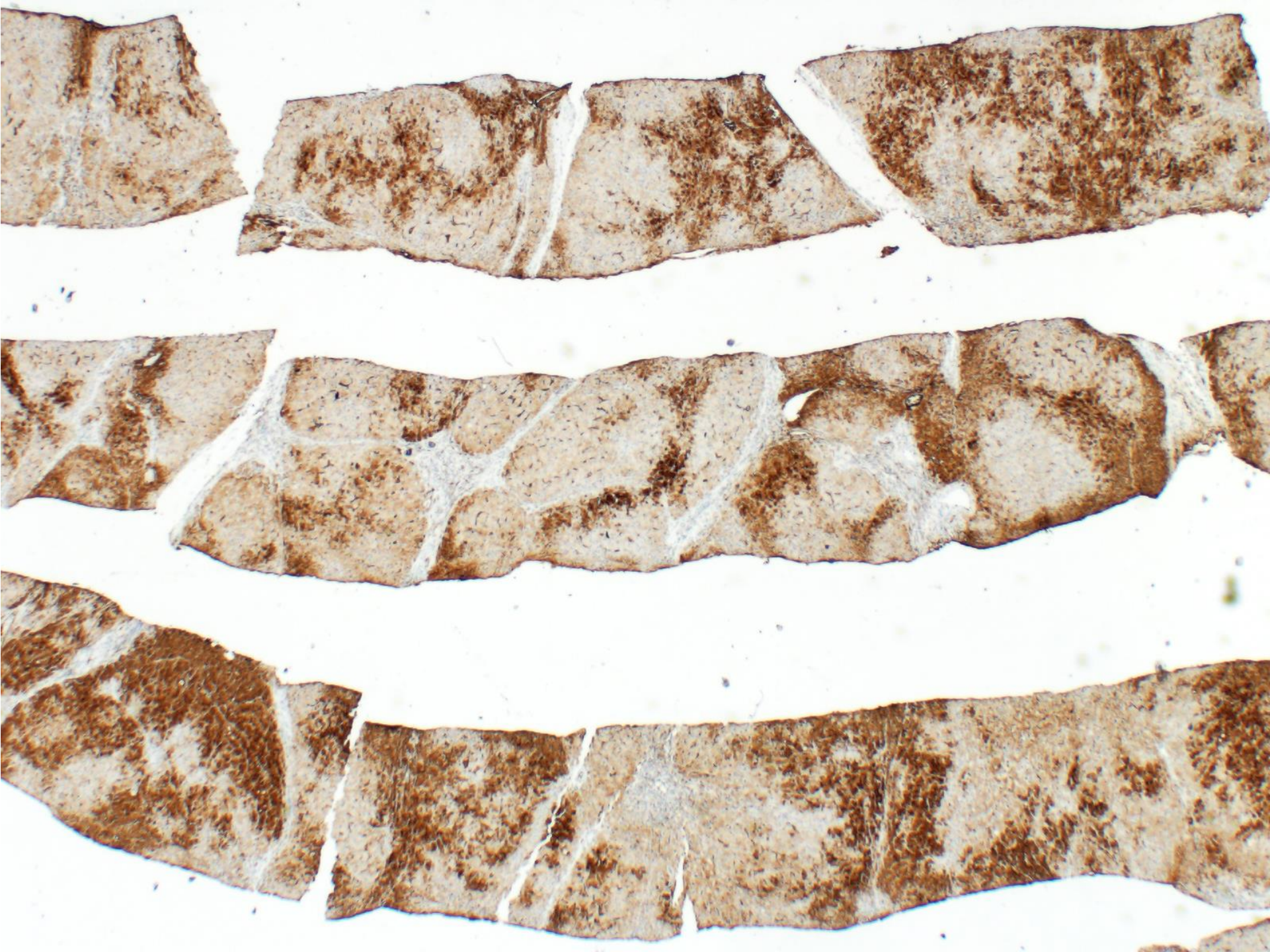
- 27-year-old woman with right adrenal mass (adrenal cortical carcinoma)
- 2 to 3 cm liver lesion with appearance concerning for metastasis





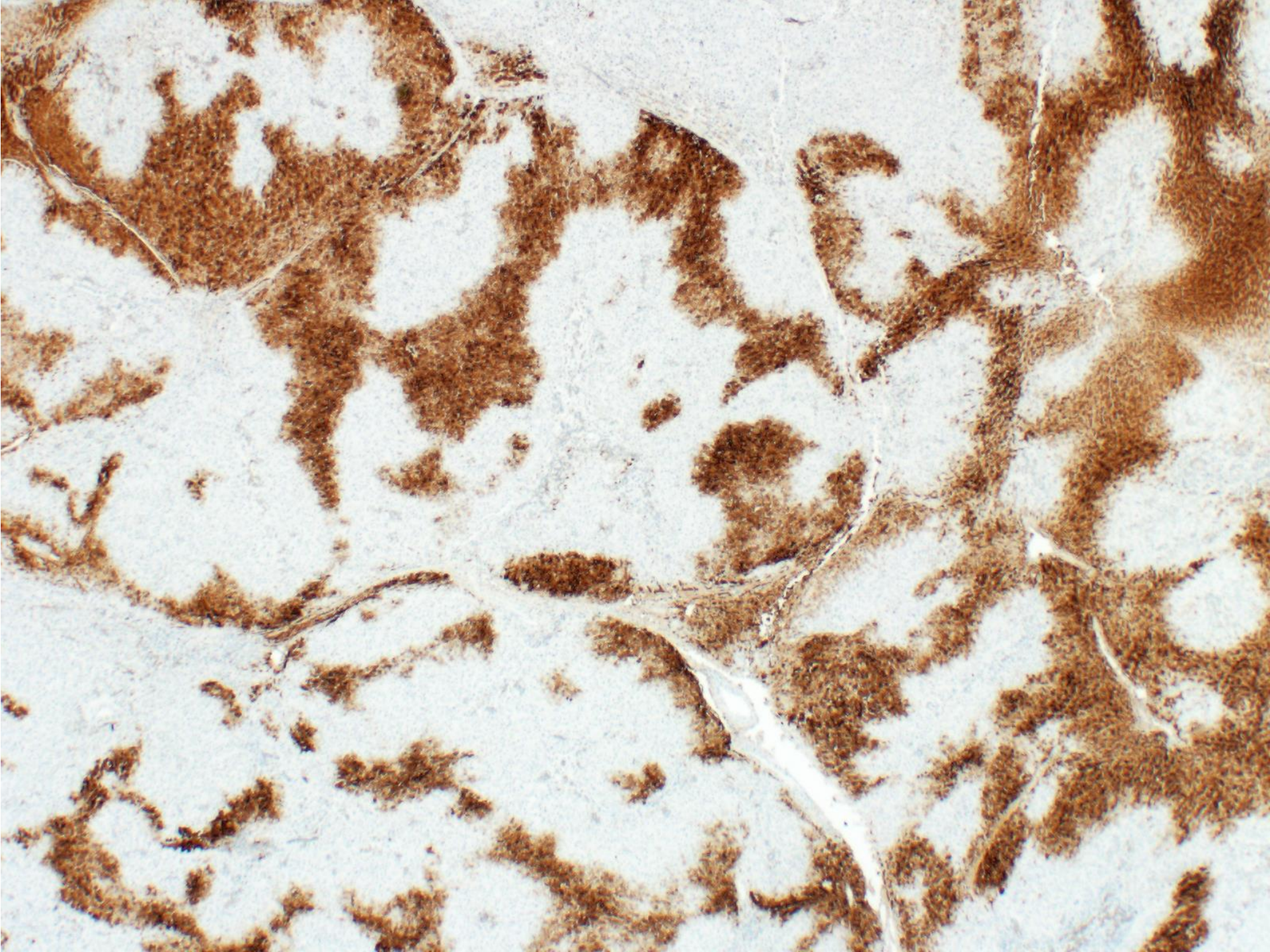






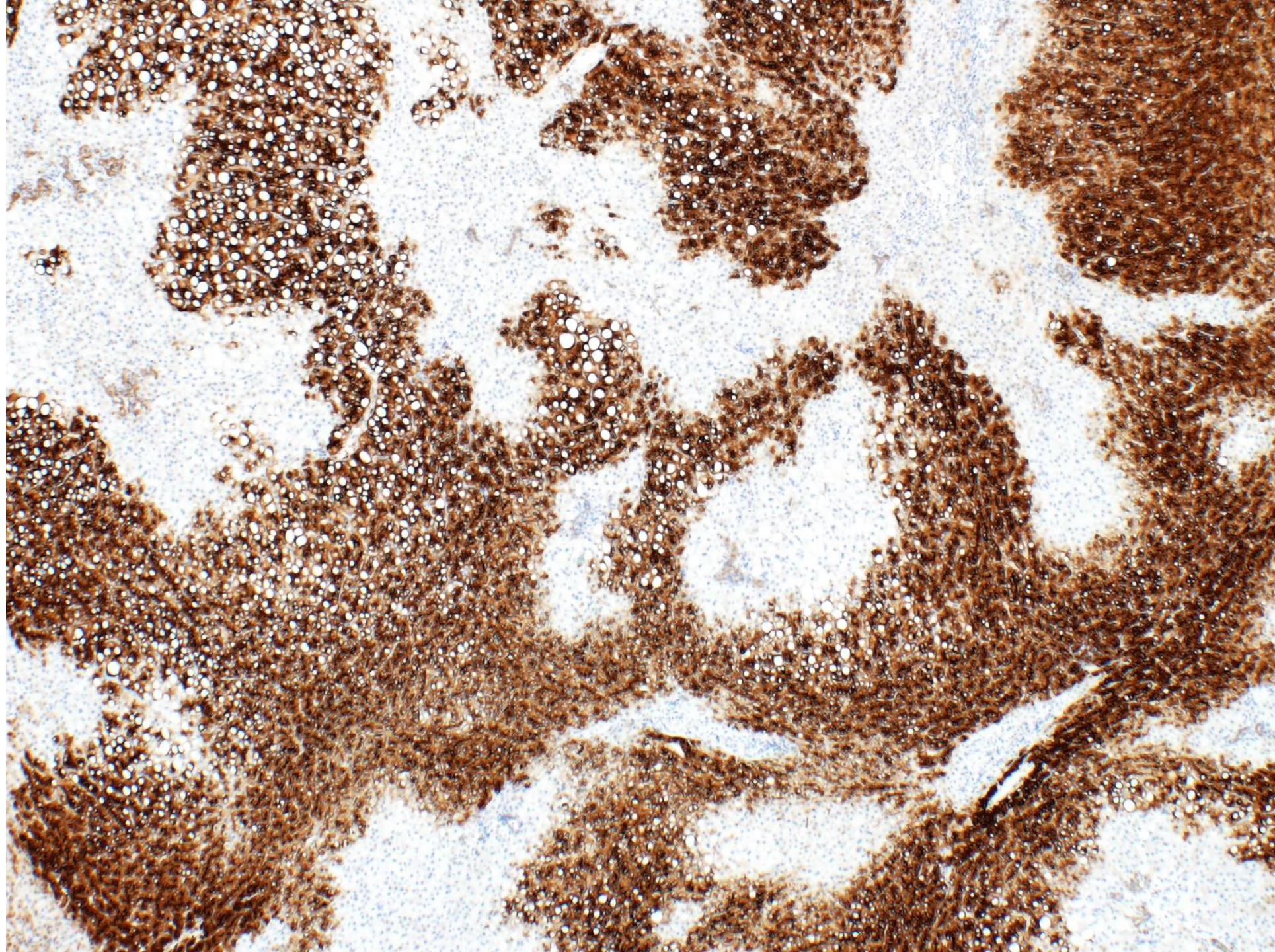
Glutamine synthetase





Glutamine  
synthetase





Glutamine  
synthetase



## Case #2: Focal nodular hyperplasia

- Hepatocellular differentiation and loss of normal portal structures
- No cytologic atypia, architectural atypia, or reticulin loss
- Fibrous scar, ductular reaction
- Map-like glutamine synthetase staining
- Localized response to vascular flow abnormalities
- Not clonal (generally)

Bioulac-Sage *et al.*, 2009

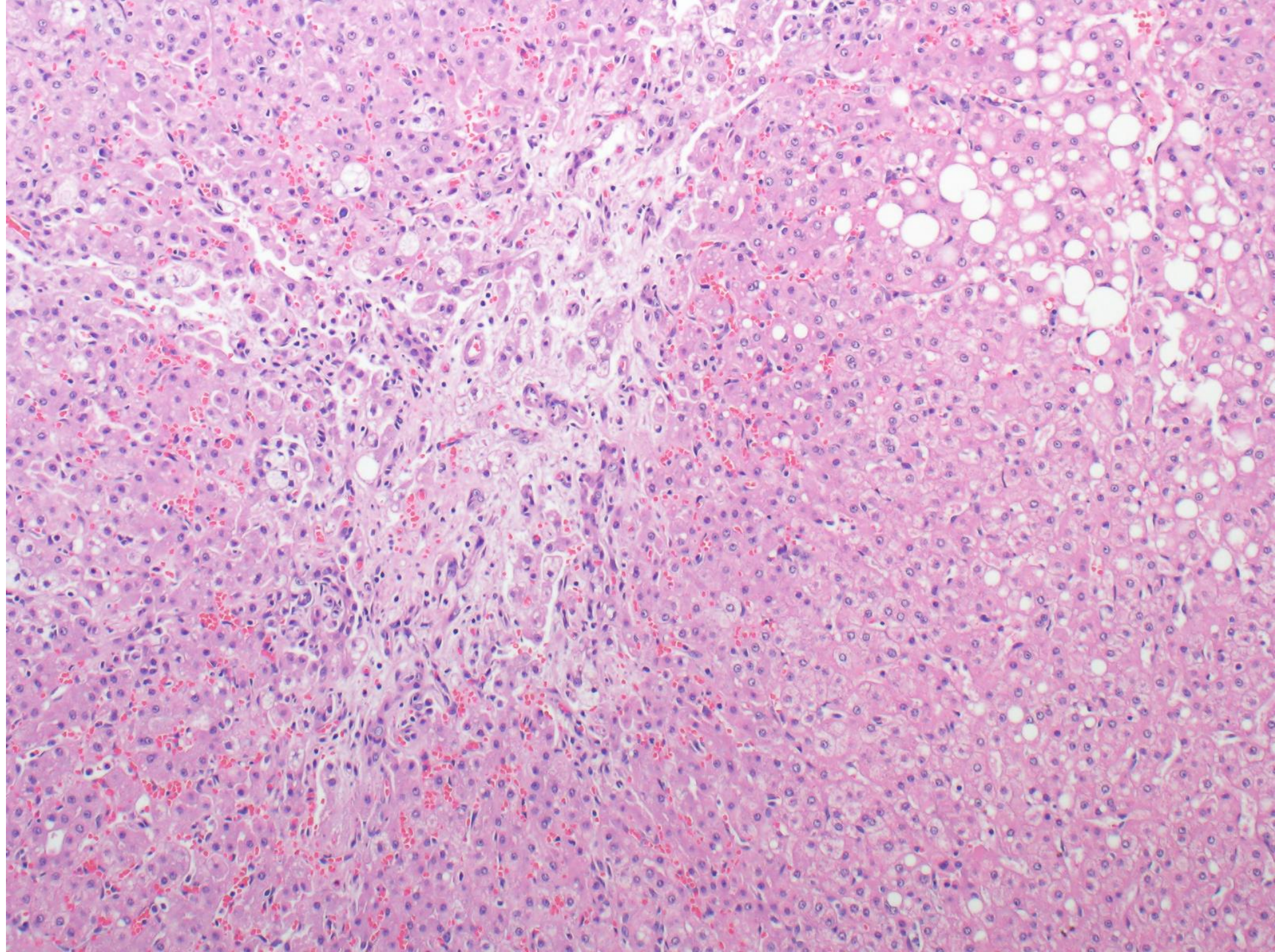
Cai *et al.*, 2009

Rowan *et al.*, 2021

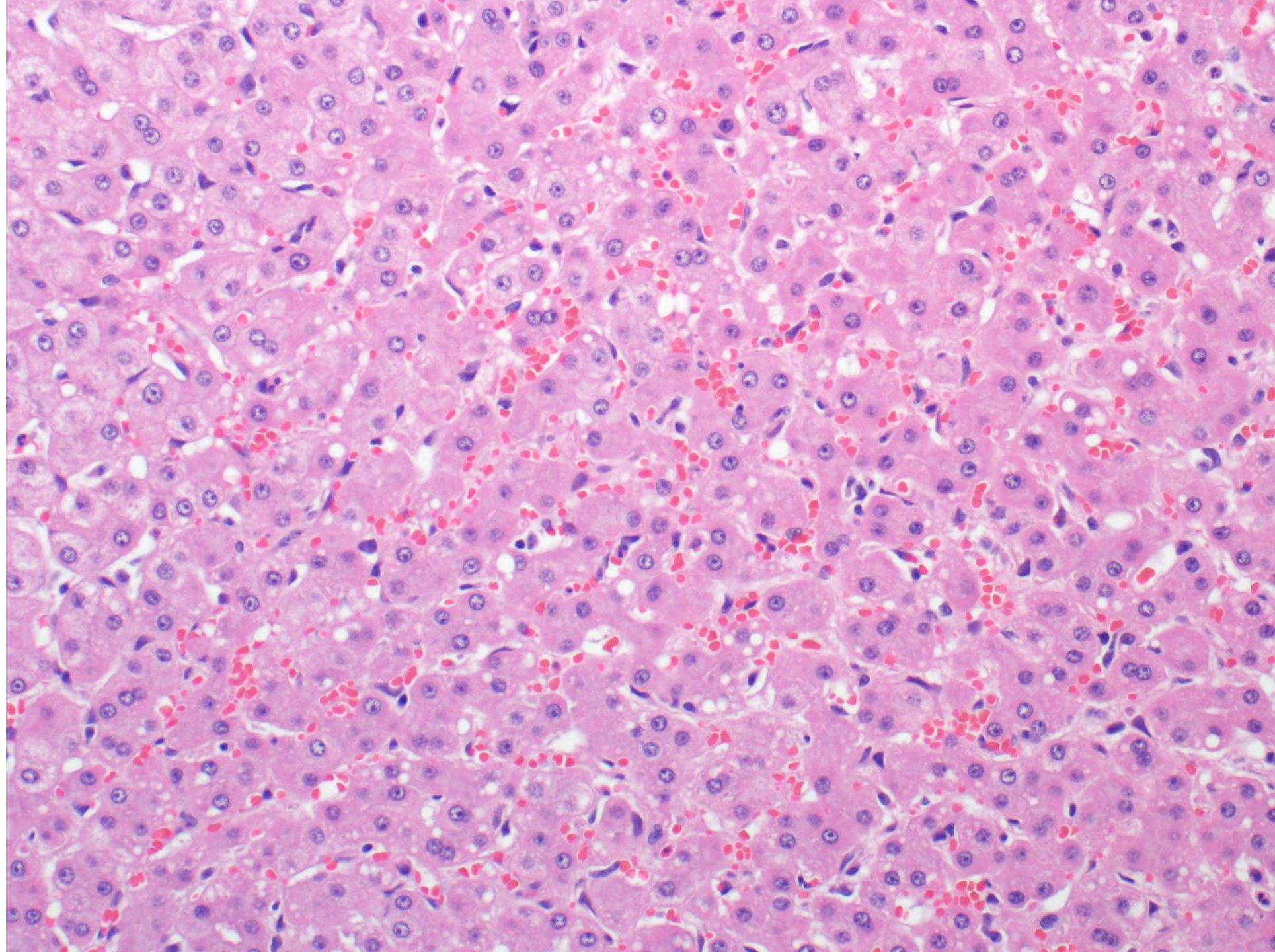
## Case #3

- 37-year-old woman with no major medical problems
- Presented with abdominal pain
- CT showed 2.1 cm liver mass

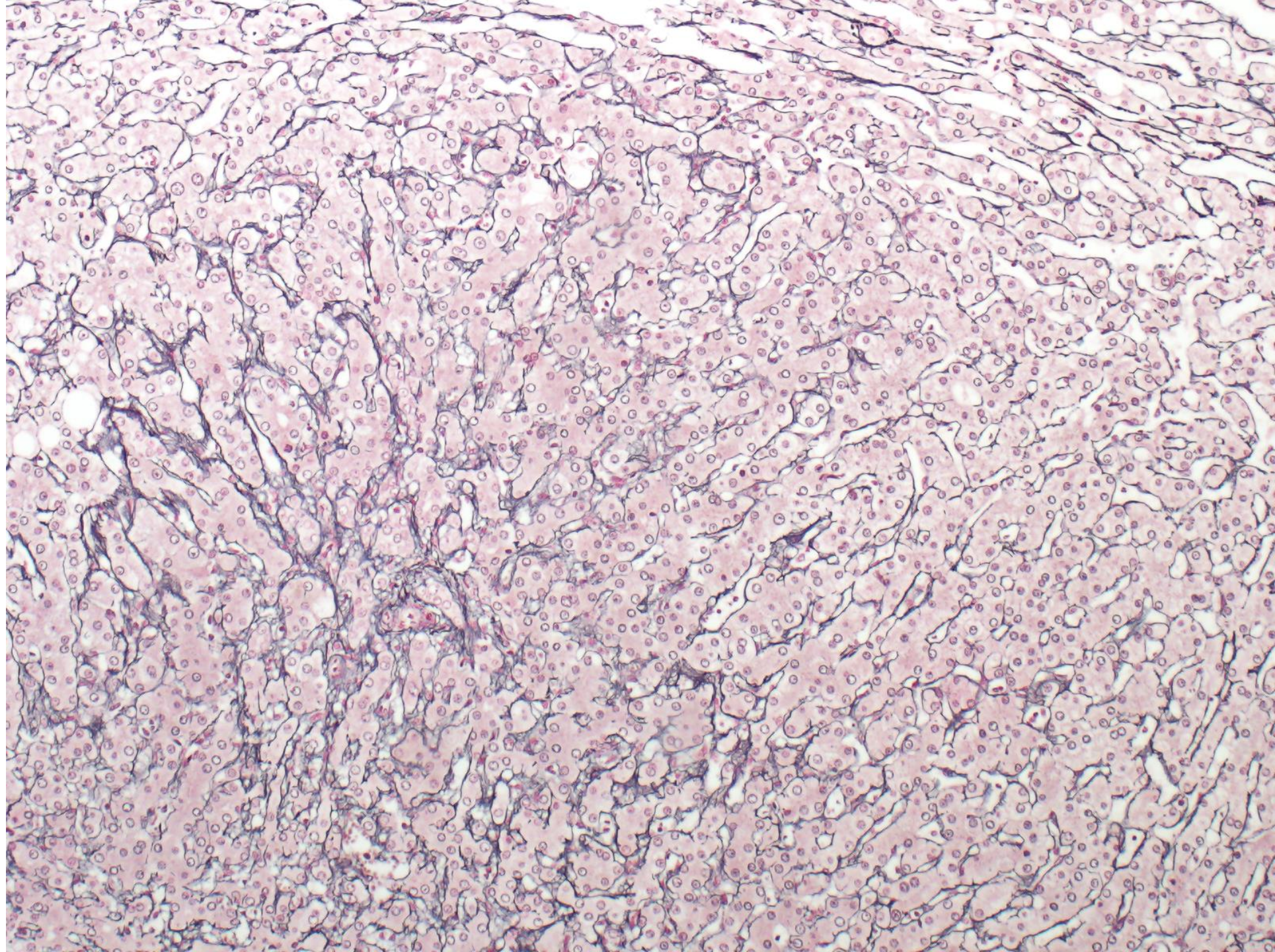




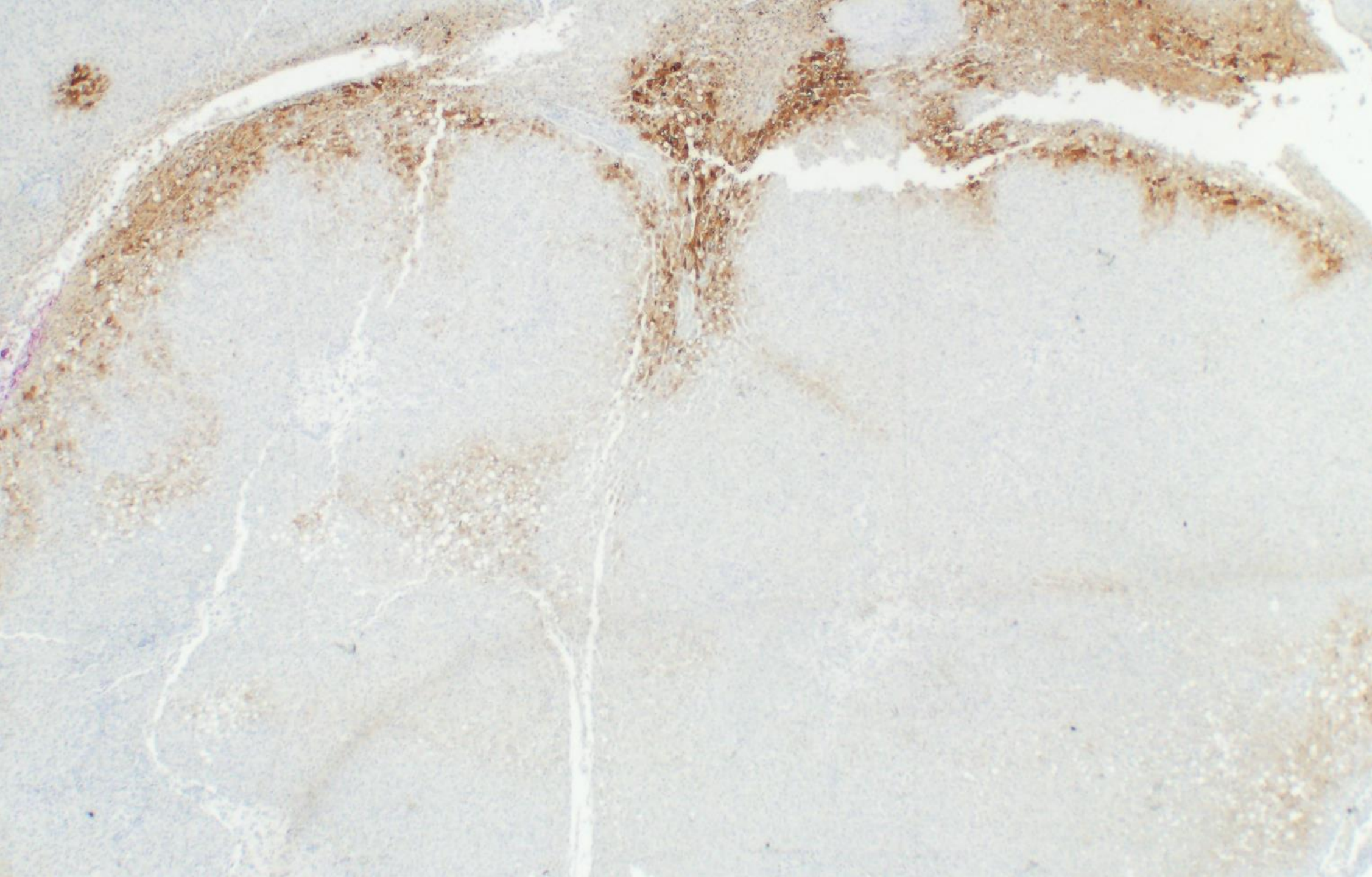






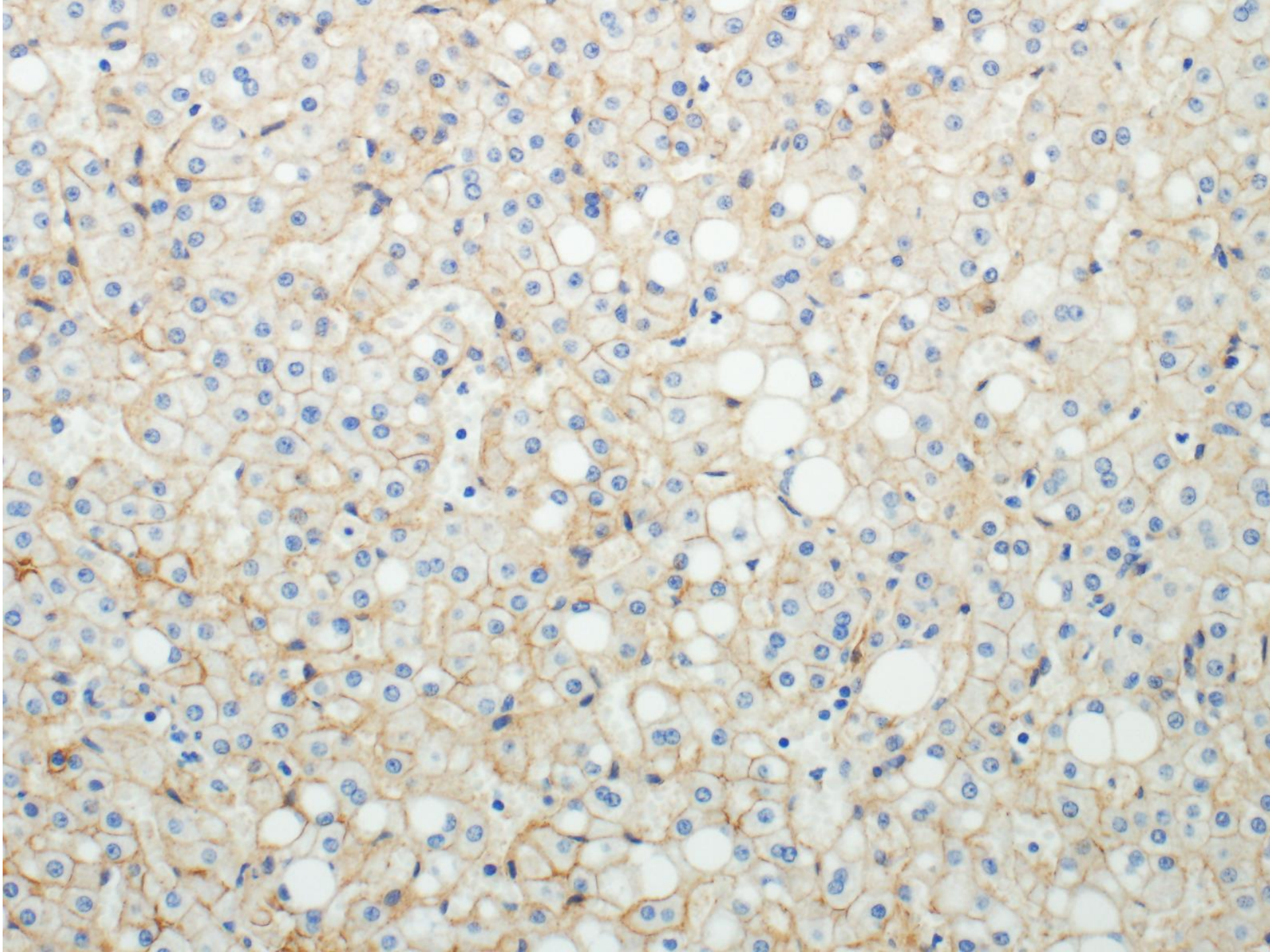






Glutamine  
synthetase





$\beta$ -catenin

## Case #3: Hepatocellular adenoma

- Hepatocellular differentiation and loss of normal portal structures
- No cytologic atypia, architectural atypia, or reticulin loss
- What kind of adenoma is it? Does it matter?



# Hepatocellular Adenoma: WHO 2019 and beyond

- $\beta$ -catenin-activated HCA
- Inflammatory HCA
- *HNF1A*-inactivated HCA
- $\beta$ -catenin-activated inflammatory HCA
- Unclassified
  - Sonic hedgehog HCA
  - $\beta$ -catenin exon 7/8 HCA

Zucman-Rossi *et al.*, 2006  
Bioulac-Sage *et al.*, 2007  
Nault *et al.*, 2017

# $\beta$ -catenin-activated hepatocellular adenoma

- Somatic mutations of *CTNNB1* exon 3 and/or
- Strong overexpression of glutamine synthetase and LGR5/GPR49
- More commonly associated with:
  - Atypical clinical settings
  - Atypical morphology
  - Cytogenetic abnormalities

Zucman-Rossi *et al.*, 2006

Bioulac-Sage *et al.*, 2007

Evason *et al.*, 2013

Nault *et al.*, 2017



# Inflammatory hepatocellular adenoma

- Activating mutations in *IL6ST*, *FRK*, *JAK1*, *STAT3*, *GNAS* and/or
- Serum amyloid A (SAA) and C-reactive protein (CRP) overexpression
  
- Can have coexistent *CTNNB1* mutations
  - $\beta$ -catenin-activated IHCA ( $b^{\text{ex3}}$ IHCA) or  $b^{\text{ex7,8}}$ IHCA
- More commonly associated with:
  - Inflammation
  - Telangiectasia

# $\beta$ -catenin exon 7/8 hepatocellular adenoma

- Somatic mutations of *CTNNB1* in exon 7 or 8
- Exclusive of activating mutations in  $\beta$ -catenin (exon 3)
- Associated with weak activation of Wnt signaling pathway



# *HNF1A*-inactivated hepatocellular adenoma

- *HNF1A* bi-allelic mutations and/or
- Loss of LFABP and UGTB7
  
- Exclusive of all other subtypes
- Steatotic
- Germline *HNF1A* mutation associated with familial liver adenomatosis

# Sonic hedgehog-activated hepatocellular adenoma

- Overexpression of *PTGDS*, *HHIP*, *FRCLA*, *TPCH1*, *GPR97*, *TNNC1*
- Exclusive of all other subtypes



# Risk of malignant transformation and bleeding

	HHCA	b <sup>ex7,8</sup> HCA	b <sup>ex7,8</sup> IHCA	IHCA	b <sup>ex3</sup> IHCA	b <sup>ex3</sup> HCA	shHCA	UHCA
Number of cases	133	15	21	116	30	30	16	28
HCC on HCA or borderline HCC/HCA	7 (5%)	0 (0%)	1 (5%)	7 (6%)	8 (27%)	14 (47%)	1 (6%)	4 (14%)
Symptomatic bleeding	20 (18%)	4 (33%)	1 (5%)	13 (13%)	1 (4%)	2 (7%)	10 (71%)	3 (14%)

# Risk of malignant transformation and bleeding

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# High-risk features in HCA

- *CTNNB1* exon 3 mutations
- Nuclear  $\beta$ -catenin expression (IHC)
- Glutamine synthetase expression (IHC)
- *TERT* promoter mutations
- Male sex
- Unique nodule at imaging
- High alcohol intake
- Fibrosis in non-tumor liver
- Type 2 diabetes mellitus

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- Glutamine synthetase expression (IHC)
- *TERT* promoter mutations
- **Male sex**
- **Unique nodule at imaging**
- **High alcohol intake**
- **Fibrosis in non-tumor liver**
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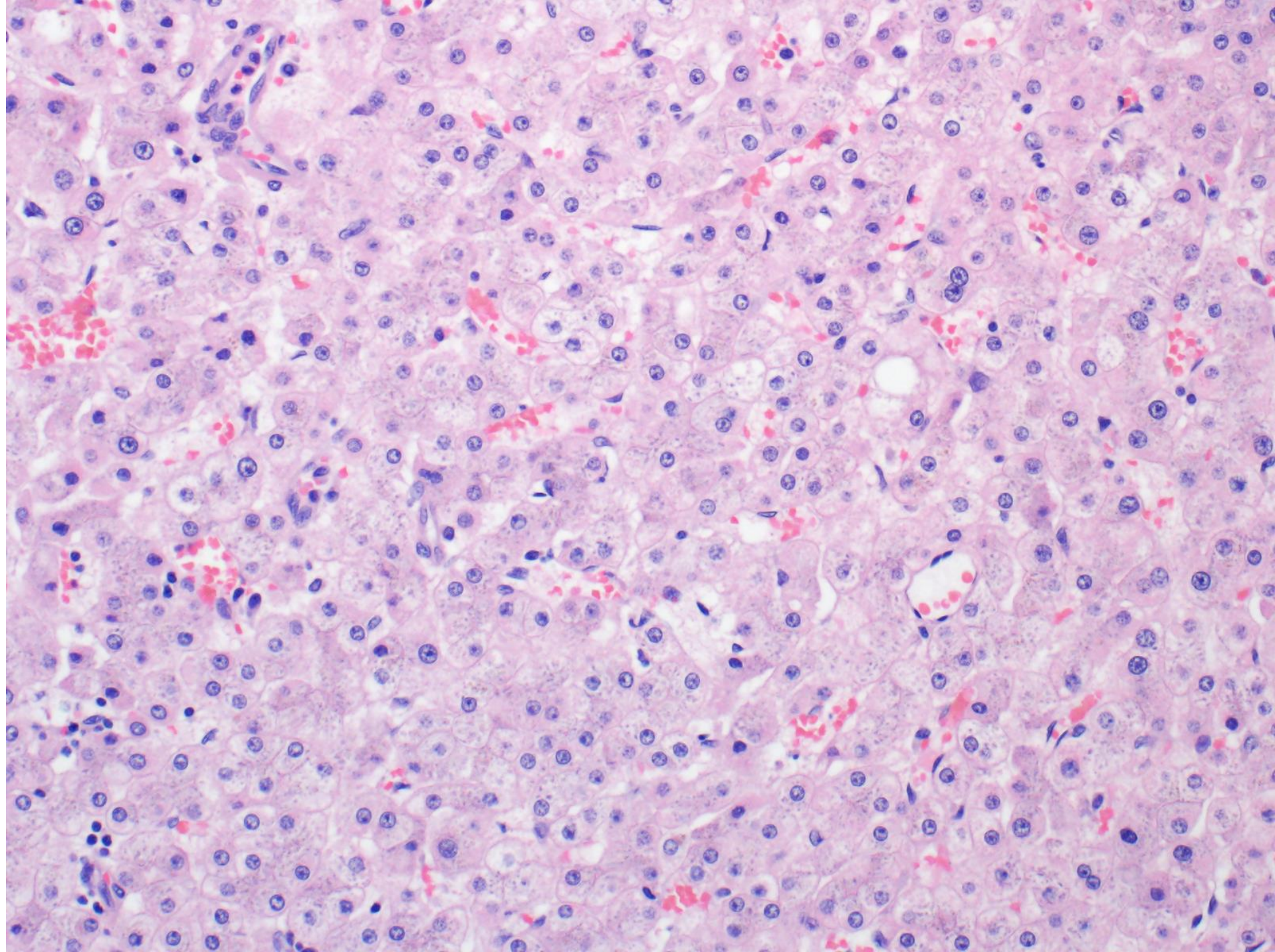
## Case #3: Hepatocellular adenoma

- Hepatocellular differentiation and loss of normal portal structures
- No cytologic atypia, architectural atypia, or reticulin loss
- What kind of adenoma is it? Does it matter?
  - Not  $\beta$ -catenin activated
  - Low risk of malignant transformation

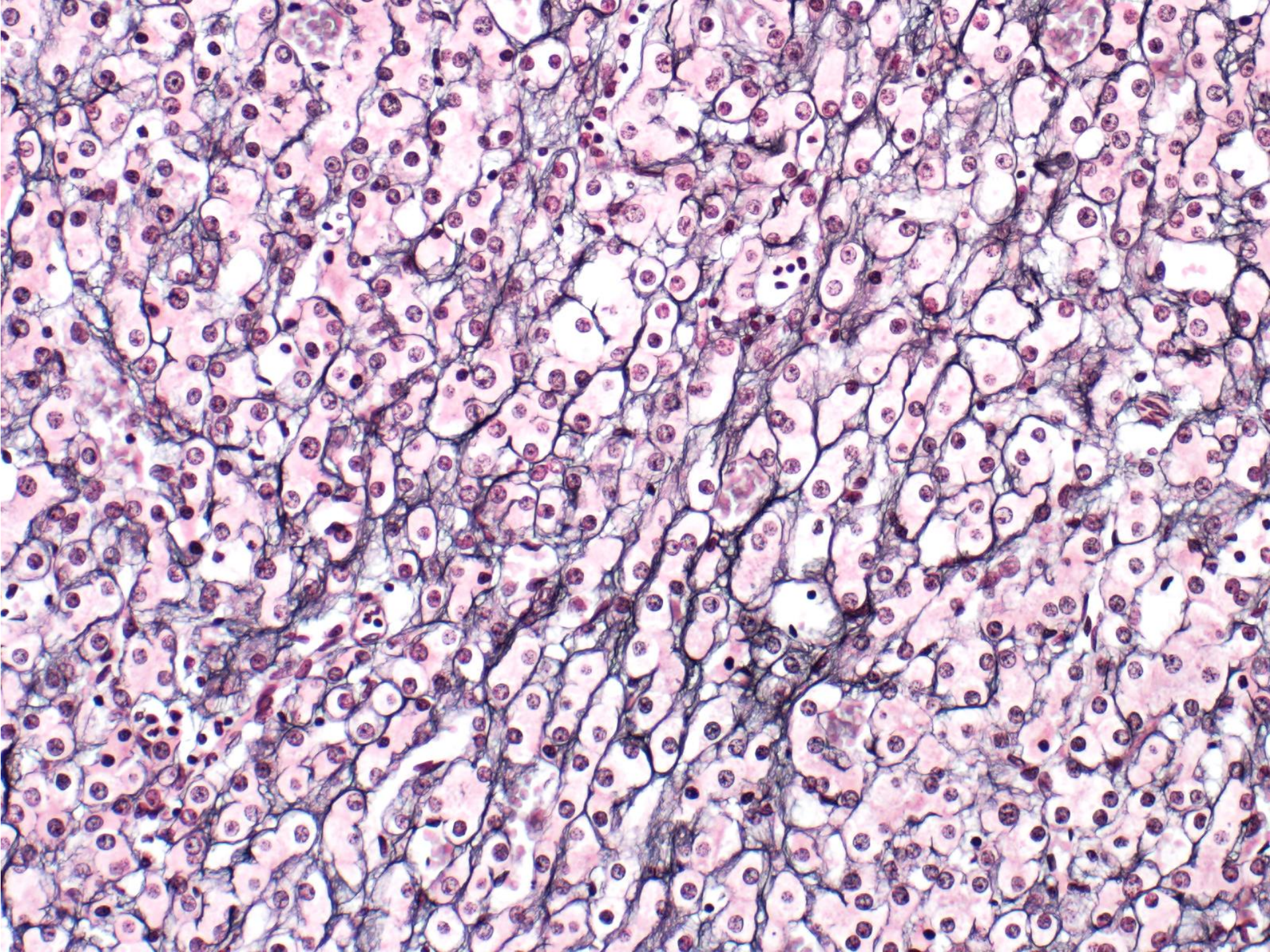
# Case #4

- 41-year-old with history of diabetes and morbid obesity
- 6.7 cm tumor in segment 6/7 of liver, found during evaluation for UTI

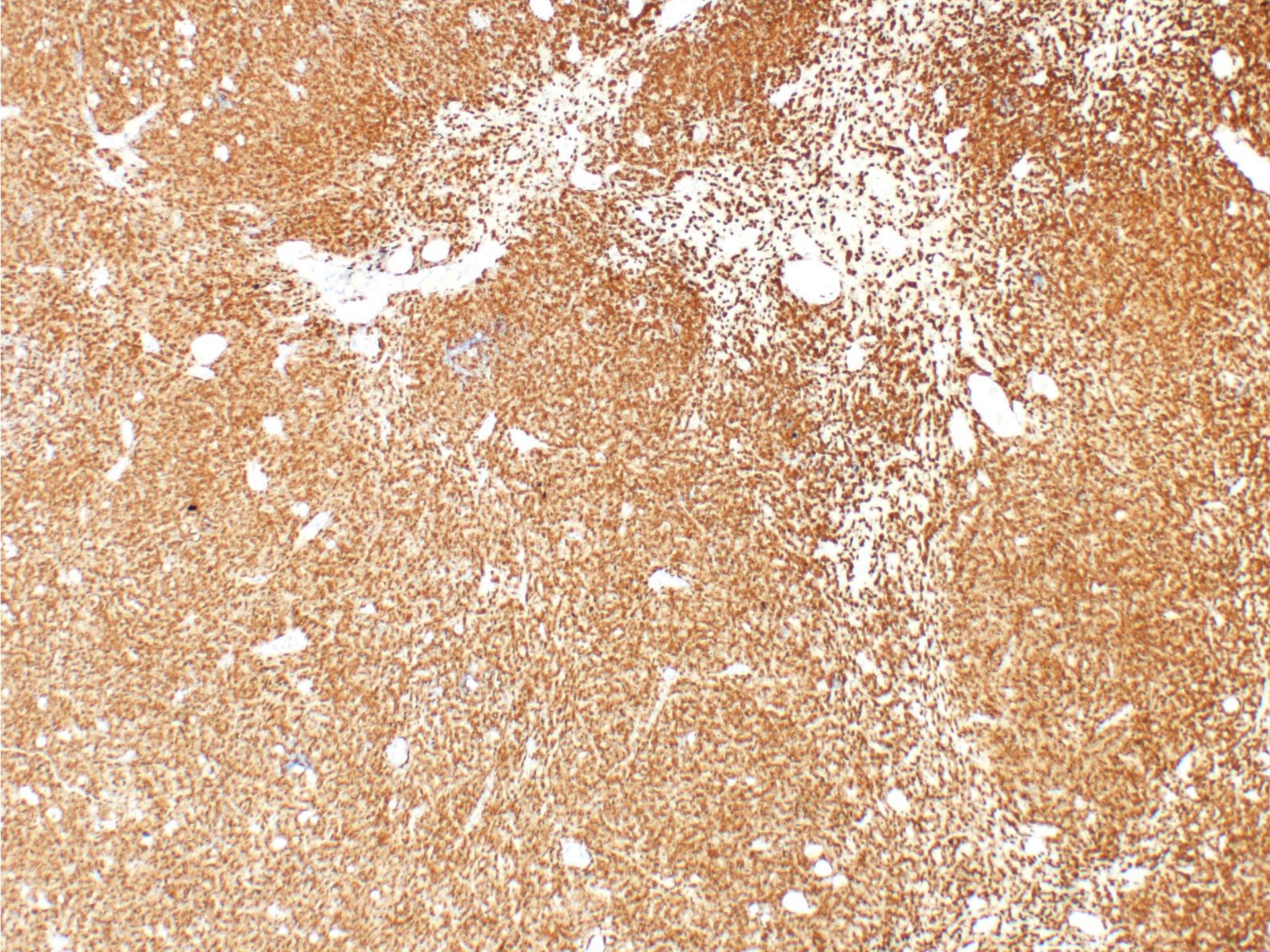






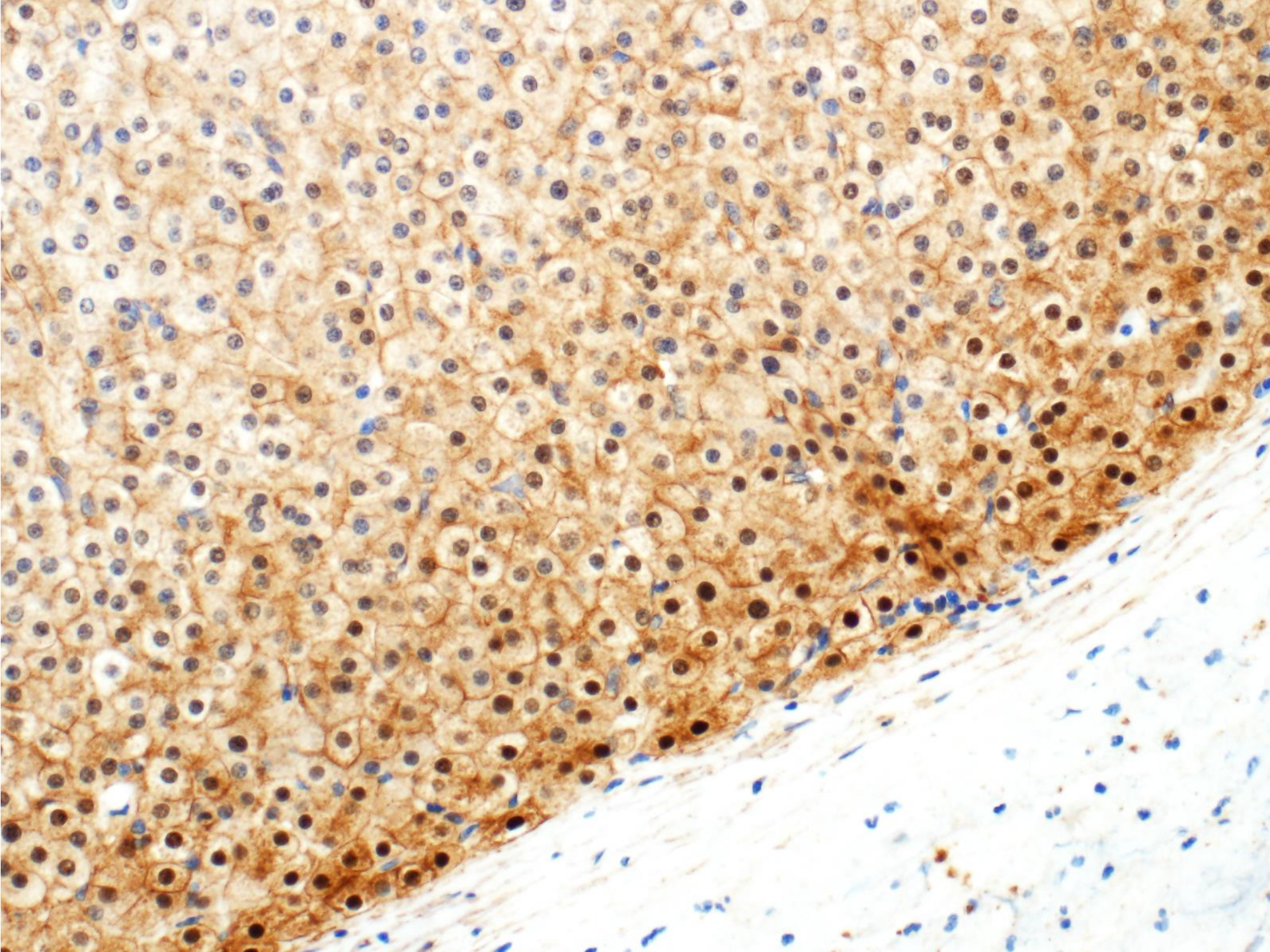






Glutamine  
synthetase





$\beta$ -catenin



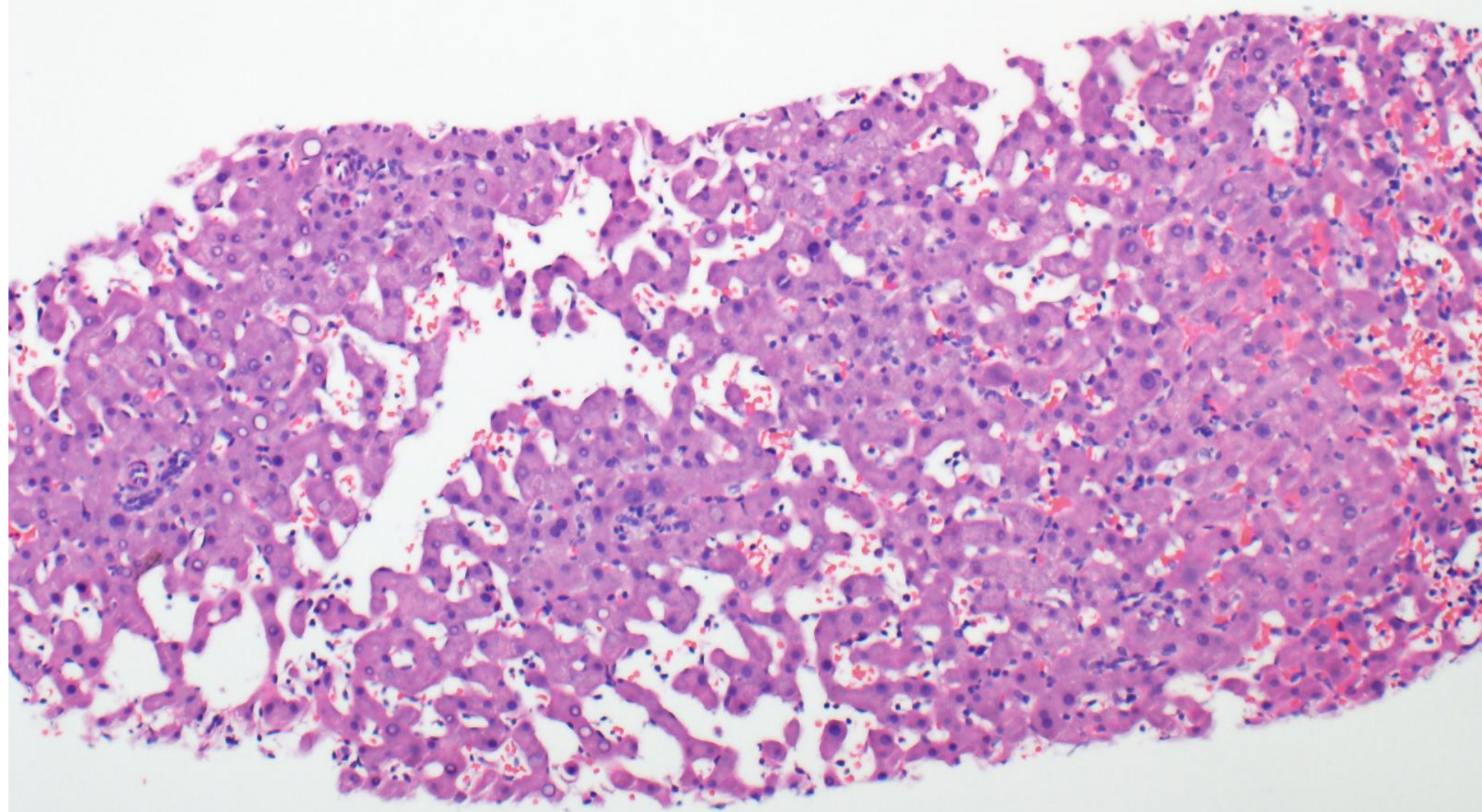
# Case #4: $\beta$ -catenin-activated hepatocellular adenoma

- Hepatocellular differentiation and loss of normal portal structures
- No cytologic atypia, architectural atypia, or reticulin loss
- Diffuse homogeneous glutamine synthetase staining
- Nuclear  $\beta$ -catenin staining
- Relatively high risk of malignant transformation

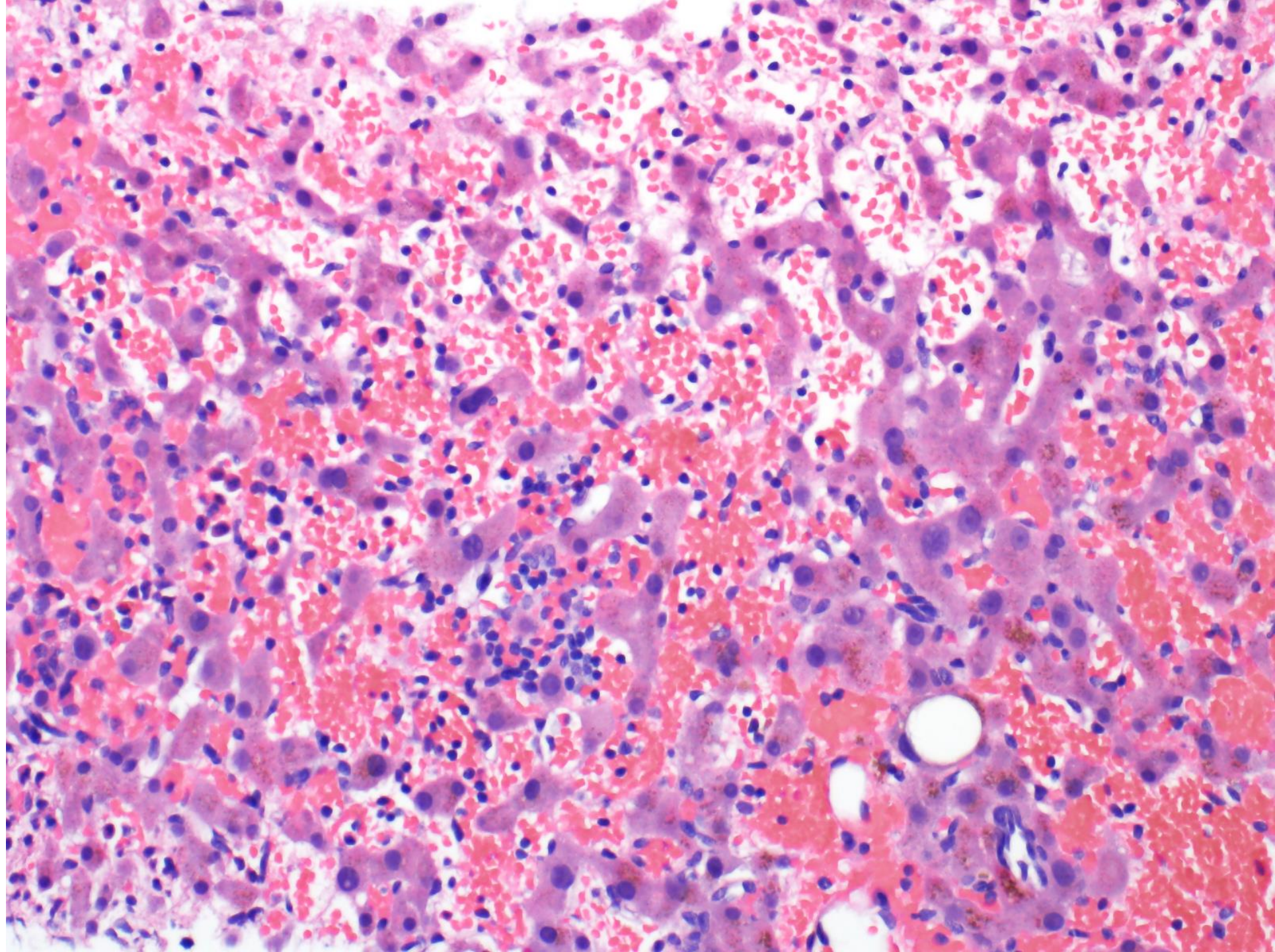
# Case #5

- 39-year-old woman with history of dysmenorrhea on hormone therapy
- Elevated alk phos and GGT
- Ultrasound showed 20+ liver masses up to 7.4 cm
- Background liver showed moderate steatosis with no fibrosis

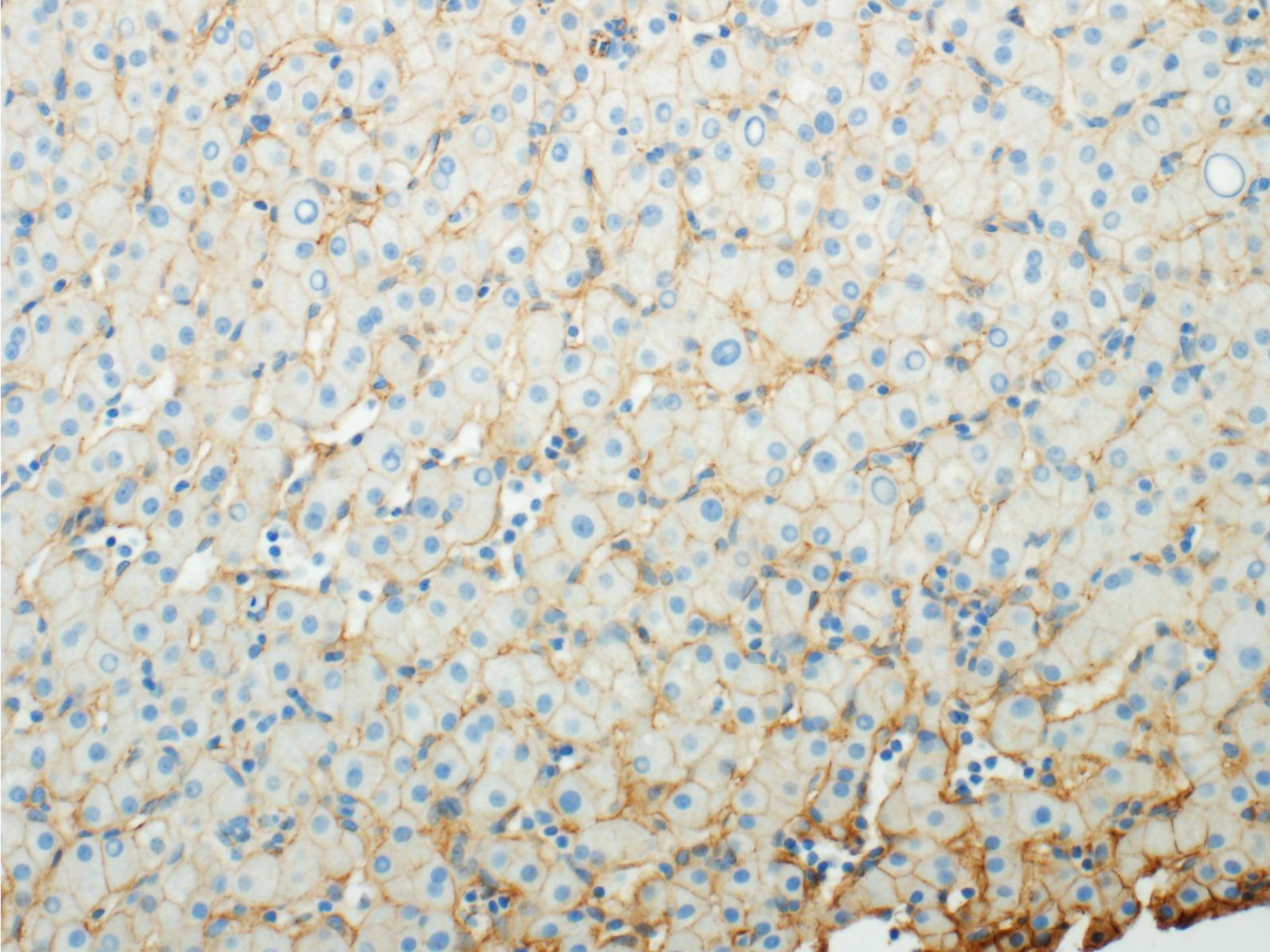






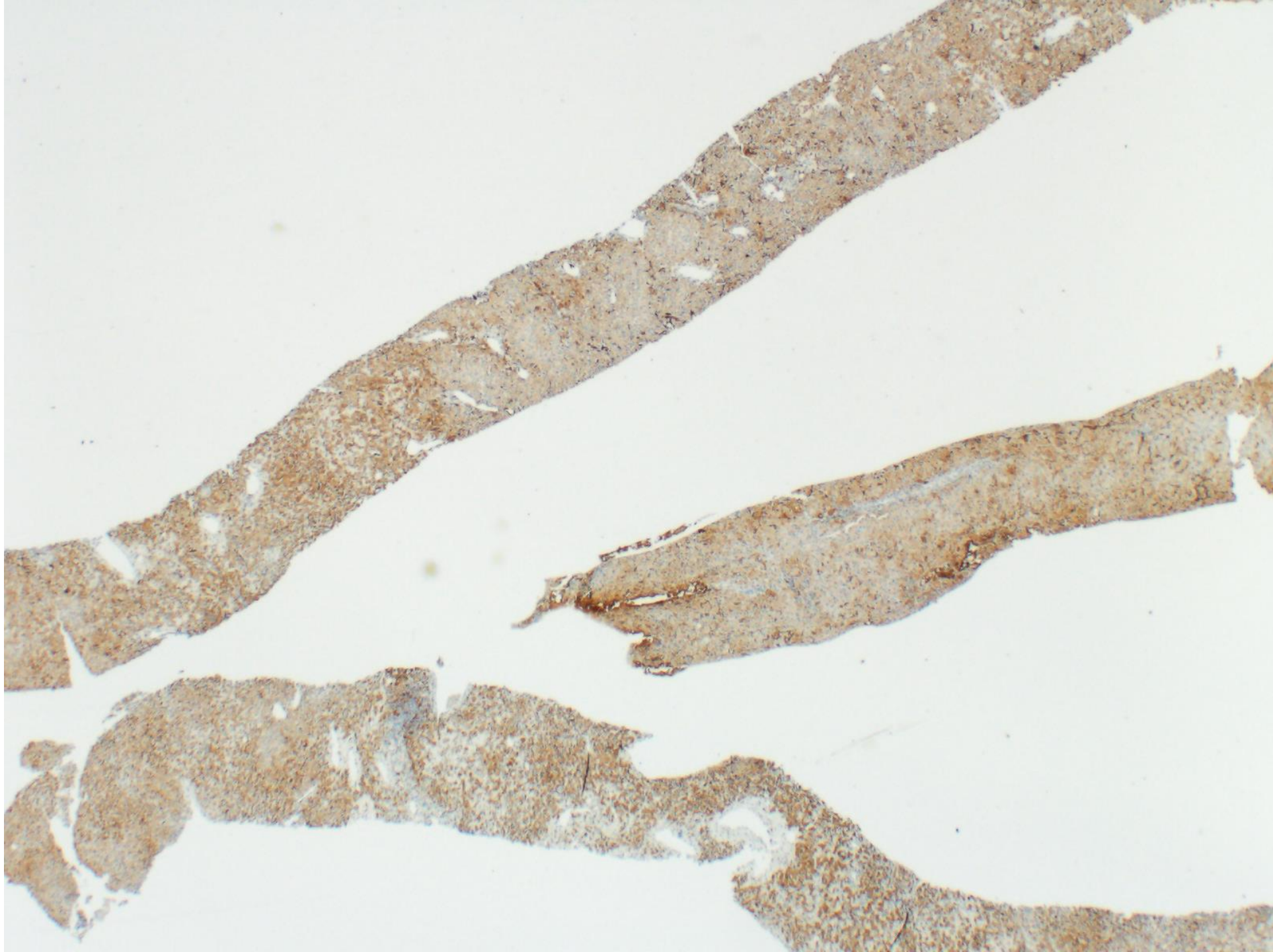






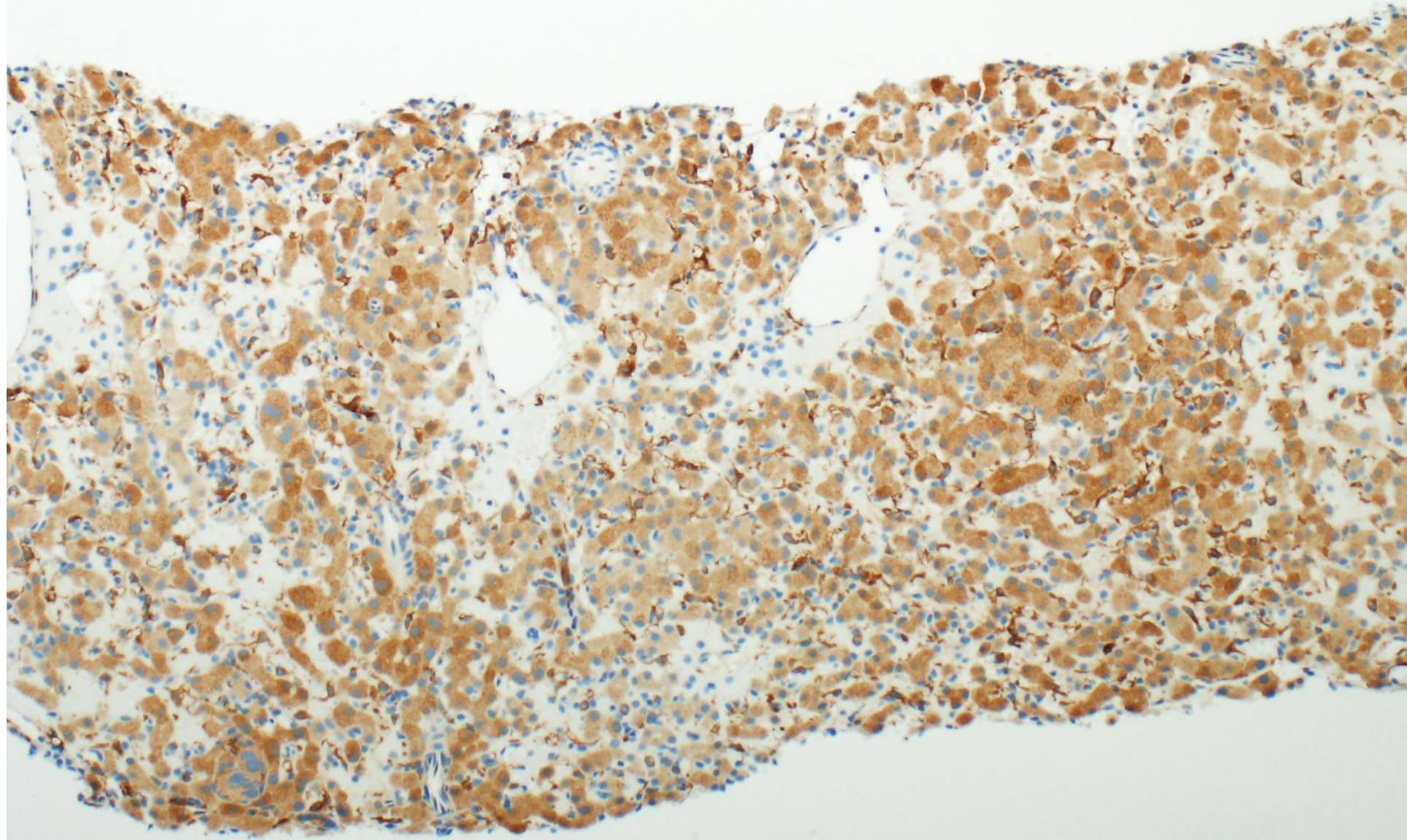
$\beta$ -catenin





Glutamine  
synthetase





Glutamine  
synthetase

# Case #5: Hepatocellular adenoma

- Hepatocellular differentiation and loss of normal portal structures
- No cytologic atypia, architectural atypia, or reticulin loss
- Diffuse heterogeneous glutamine synthetase staining
- Membranous  $\beta$ -catenin staining



# Patterns of glutamine synthetase staining

1. Map-like
2. Diffuse strong homogeneous
  - Moderate to strong staining in >90% of cells, but not map-like
3. Diffuse heterogeneous
  - Moderate to strong staining in 50-90% of cells
4. Patchy
  - Moderate to strong staining in <50% of cells, often perivascular
  - Weak staining (any extent)
  - Negative

# Patterns of glutamine synthetase staining

## 1. **Map-like = Focal nodular hyperplasia**

## 2. Diffuse strong homogeneous

- Moderate to strong staining in >90% of cells, but not map-like

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1. Map-like = Focal nodular hyperplasia

2. **Diffuse strong homogeneous**

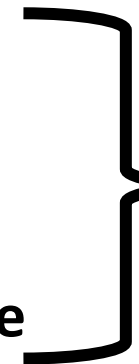
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- Weak staining (any extent)
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**β-catenin-activated**

# Patterns of glutamine synthetase staining

1. Map-like = Focal nodular hyperplasia

2. Diffuse strong homogeneous

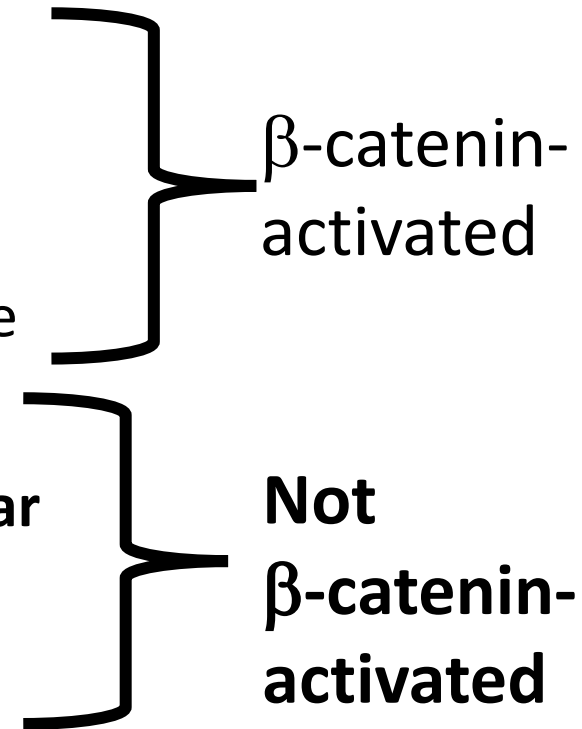
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- Moderate to strong staining in 50-90% of cells, but not map-like

4. **Patchy**

- **Moderate to strong staining in <50% of cells, often perivascular**
- **Weak staining (any extent)**
- **Negative**





Epithelioid lesion in liver (lack of portal tracts)

Is there obvious hepatocellular differentiation (bile)?

yes

no

Is there significant cytologic atypia and/or reticulin loss?

no

yes

Order  
glutamine  
synthetase and  
 $\beta$ -catenin

Do clinical  
history and  
radiology  
support HCC?

HCC

yes

Do clinical  
history and  
radiology  
support HCC?

Positive

Negative

Order  
other  
markers

Map-like

Other

Adenoma

Diffuse  
positive

no

HCC, hepatoblastoma,  
hepatoid carcinoma,  
angiomyolipoma

no

HCC, hepatoblastoma,  
hepatoid carcinoma

FNH

$\beta$ -catenin-activated  
hepatocellular adenoma

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