Pathophysiology and genetics of cholelithiasis

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Outline of presentation

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- Background

- Biochemical aspects: “all you always wanted to ask about gallstone pathogenesis but did not dare to ask”

- Genetics (biliary lipid transport proteins)

- Nuclear receptors

Background

- In Western world:
  - 70% cholesterol gallstones and 30% black pigment gallstones
  - below age 50 yrs mainly cholesterol stones, increasing relative contribution of pigment stones at increasing age
  - main location in gallbladder.

- In Far East:
  - traditionally high frequency of brown pigment stones in bile ducts
  - in recent decades shift to cholesterol gallstones, related to western diet

Risk factors for black pigment gallbladder stones

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- Cystic fibrosis
- Hemolytic anemias
- Gilbert syndrome-associated UGT1A1 mutation

Pigment and cholesterol gallstones: ends of a spectrum of one single disease?

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- Cholesterol gallstones generally have small amounts of conjugated bilirubin at their center.
- Other factors (e.g. diet, apoE genotype) may determine whether cholesterol or pigment stones develop.
- Gilbert syndrome-associated UGT1A1 mutation is risk factor for **both** pigment **and** cholesterol gallstones (Buch et al. *Gastroenterology* 2010)

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Biliary cholesterol solubilization

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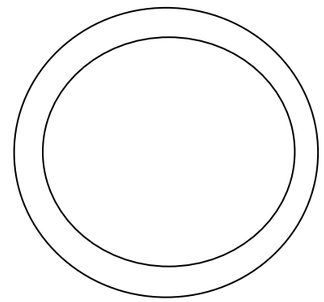
- Cholesterol solubilized in mixed micelles by bile salts and phospholipids

- Biliary cholesterol supersaturation (CSI > 1) if excess cholesterol can not be solubilized in mixed micelles (excess cholesterol secretion or diminished bile salt or phospholipid secretion)

- Excess cholesterol solubilized in cholesterol-phospholipid vesicles



- Nucleation of cholesterol crystals if excess cholesterol can not be kept in vesicles

Cholesterol crystallization: on gallstone surface versus in aqueous solution

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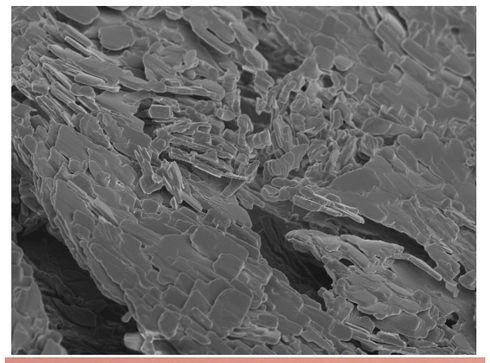
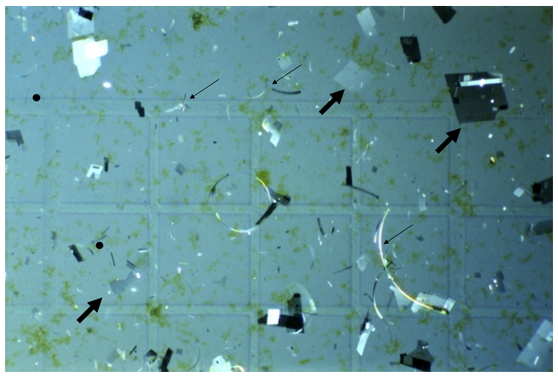
Cholesterol in supersaturated aqueous solution

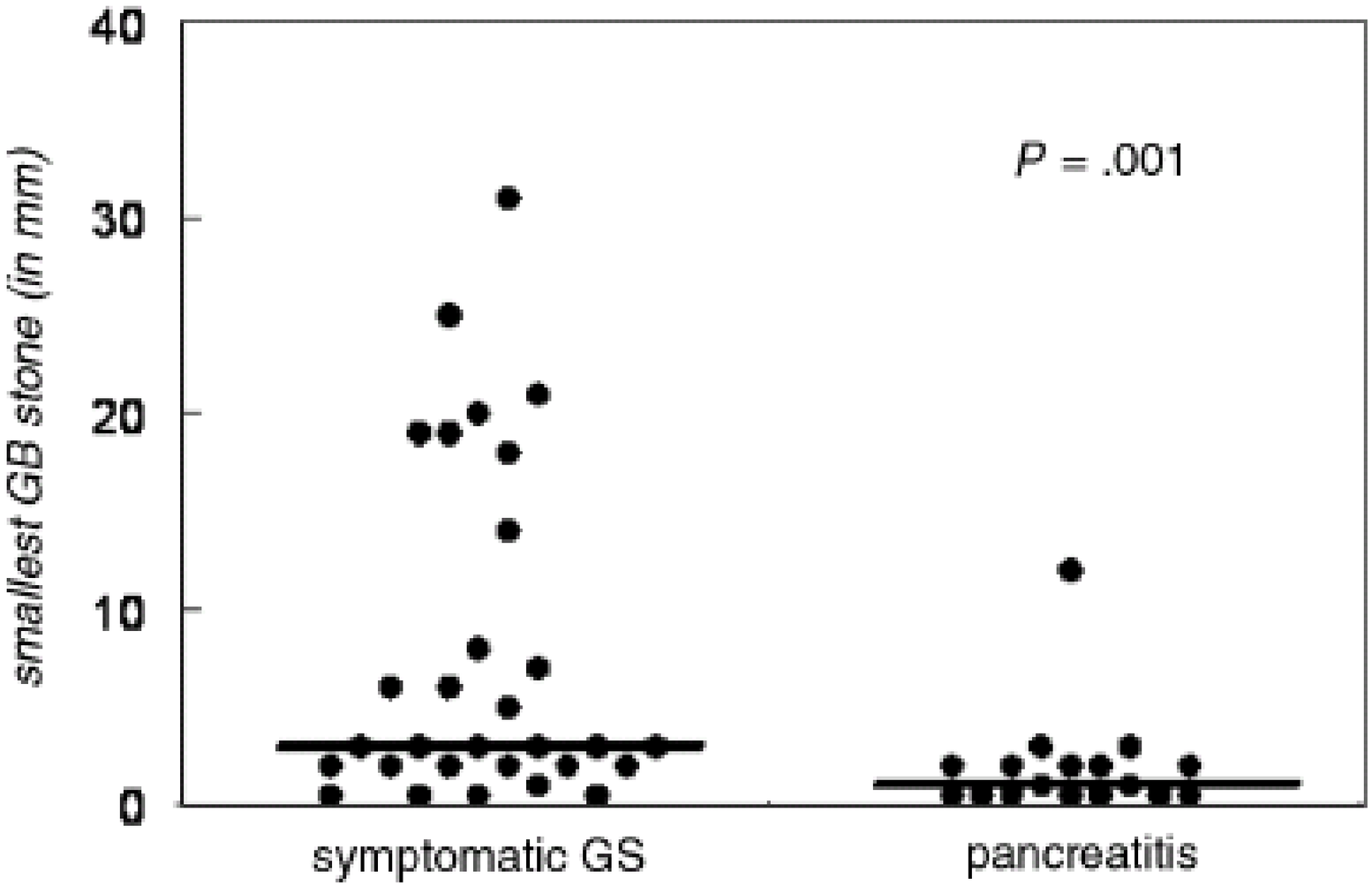
Nucleation on stone surface (gallstone growth)



Nucleation as free cholesterol crystals (microlithiasis, sludge, stones remain small)

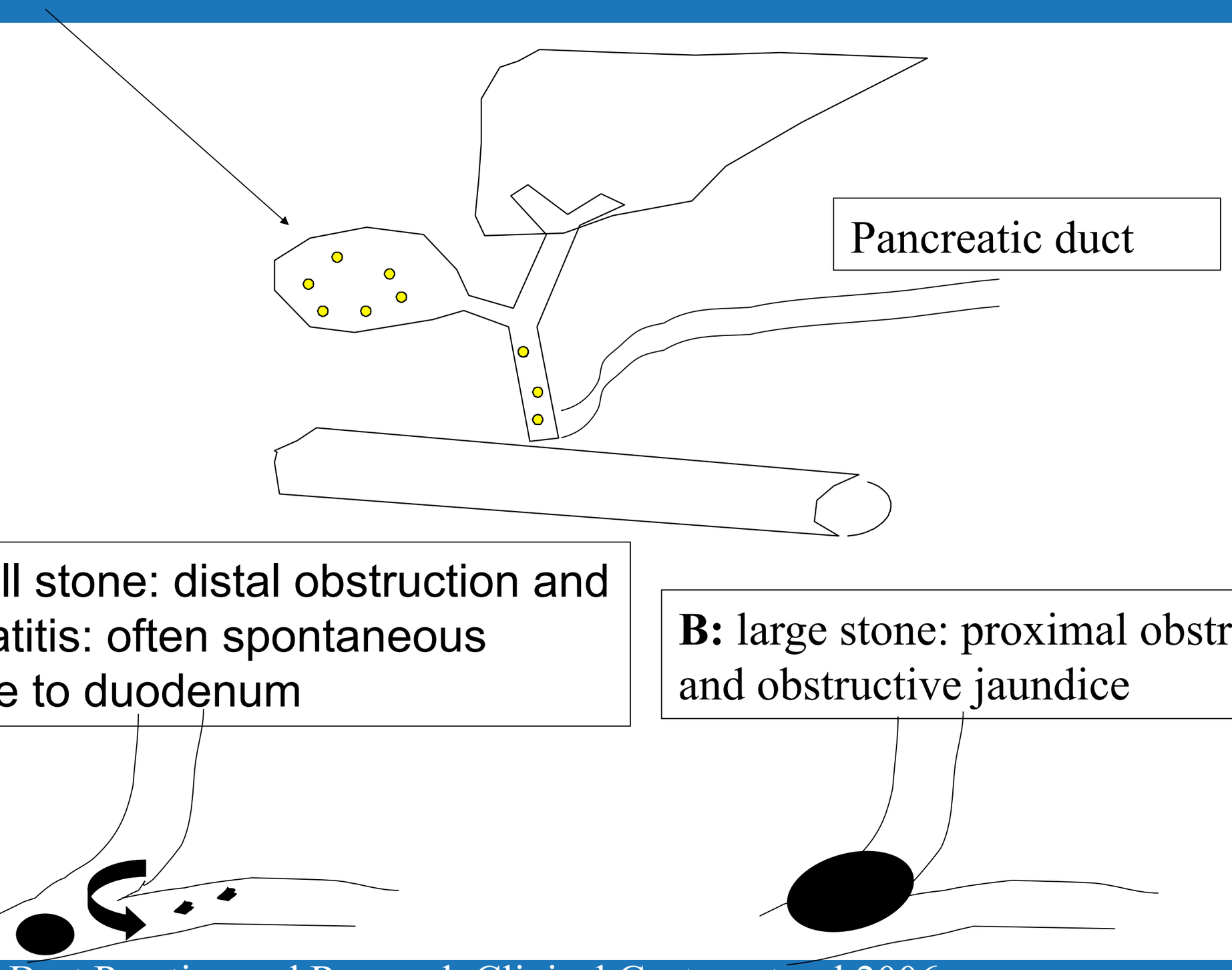


Small gallstones in biliary pancreatitis



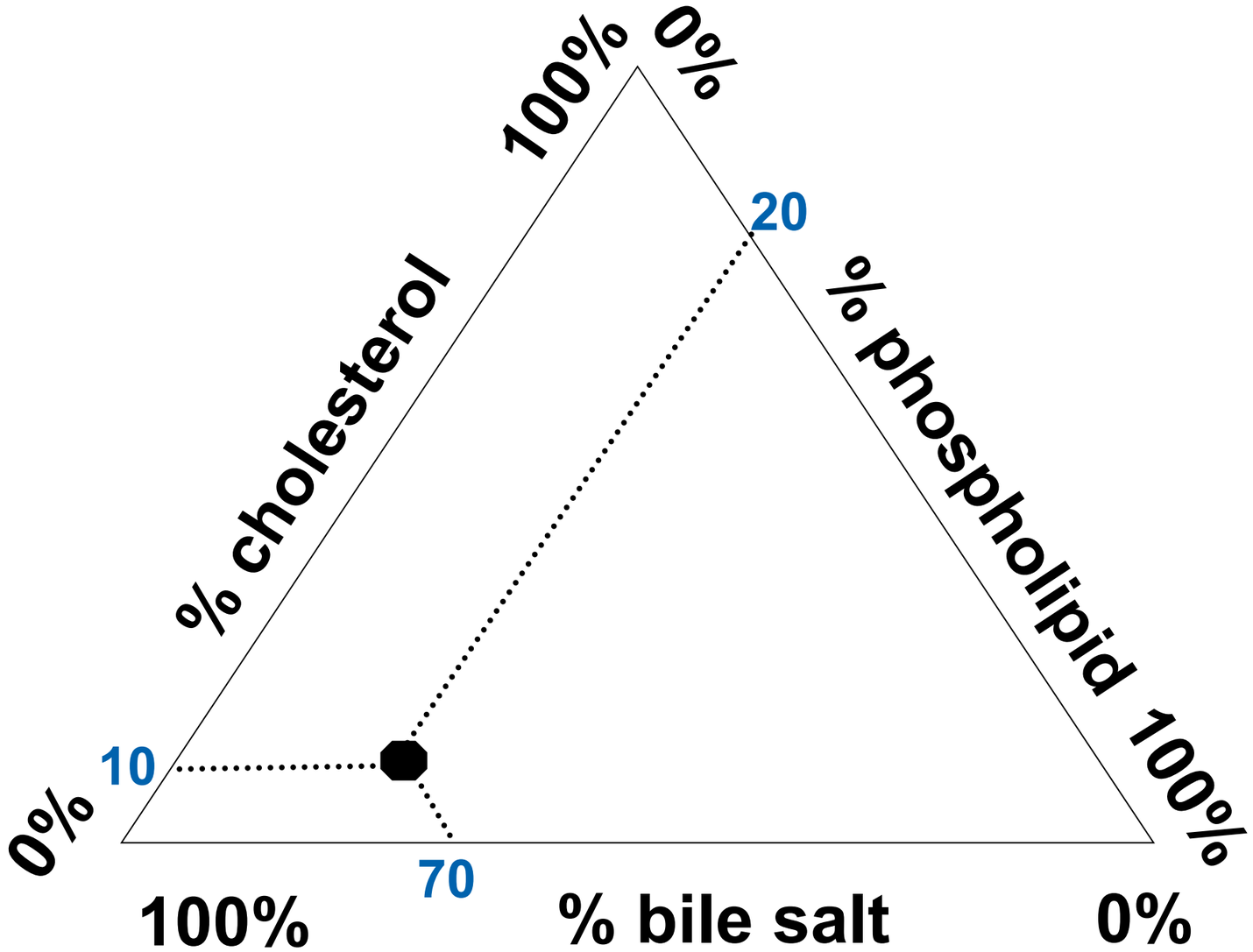
Venneman *et al*. Hepatology 2005;41:738-46

Small stone: more often migration to bile duct, especially in case of vigorous gallbladder emptying

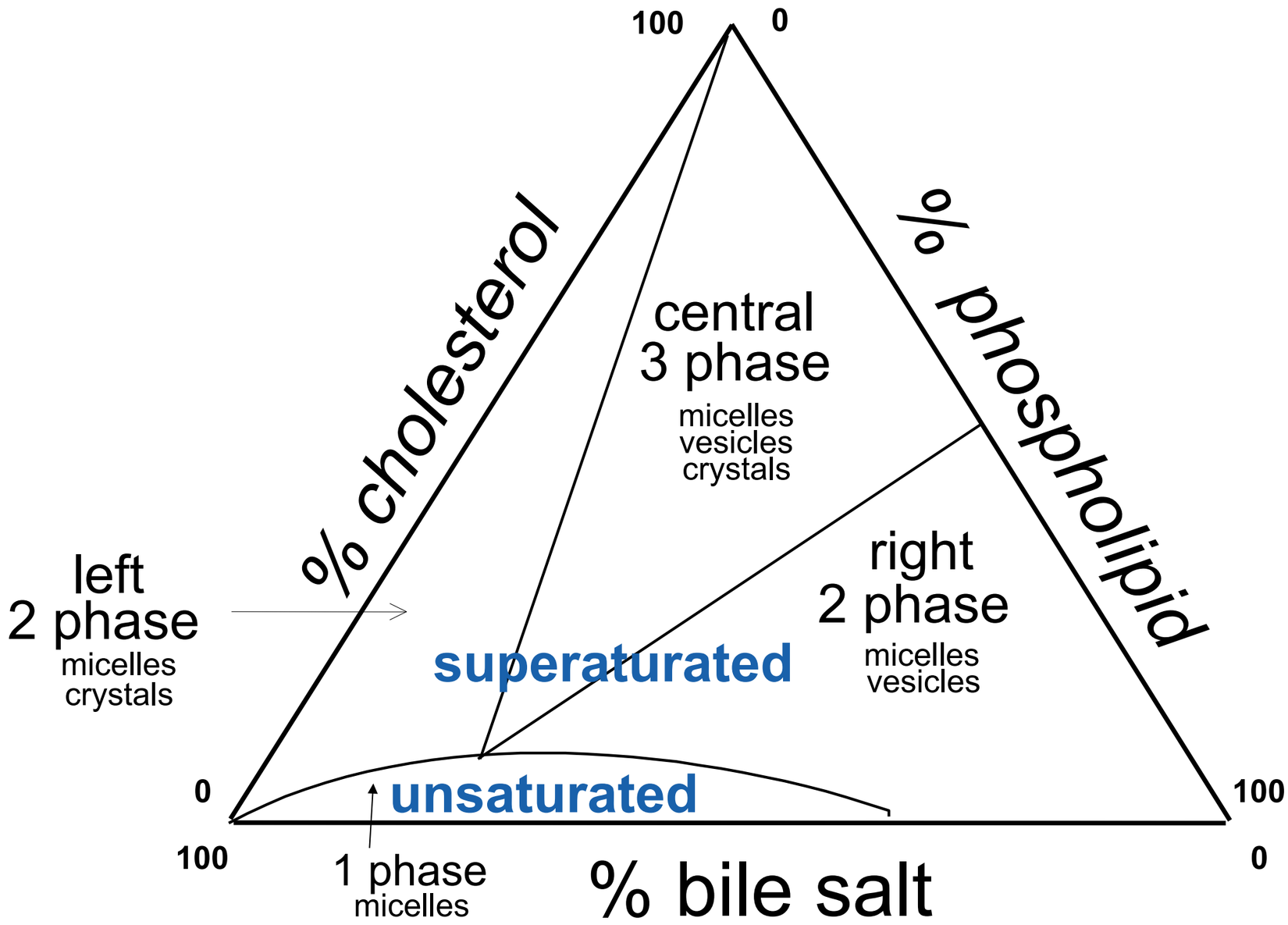


Van Erpecum. Best Practice and Research Clinical Gastroenterol 2006

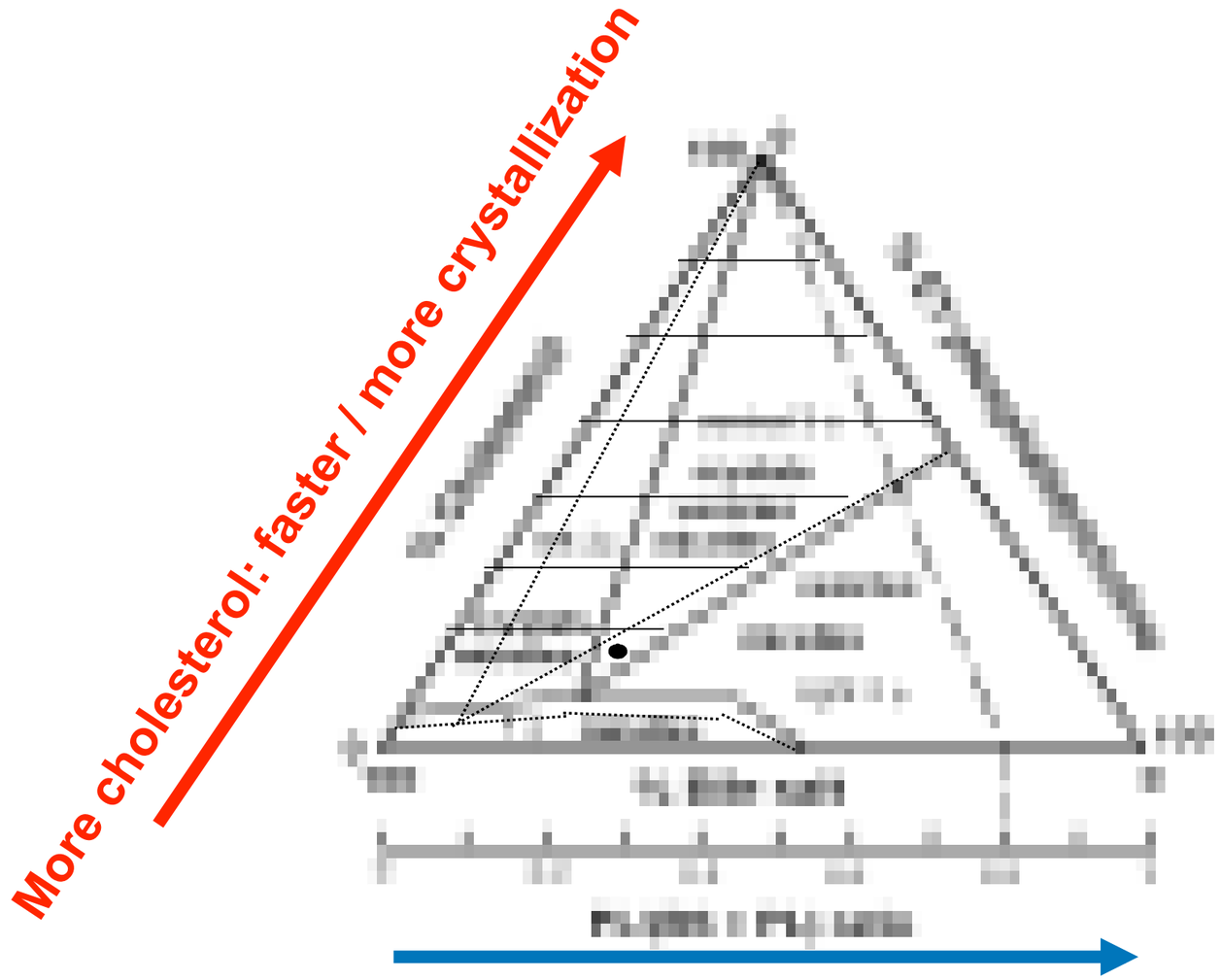
The bile salt –phospholipid-cholesterol phase diagram



Various zones in the bile salt –phospholipid-cholesterol phase diagram



Relative amounts of various lipids determine what happens



Relative amount of phospholipid versus bile salt determines speed of cholesterol crystallization

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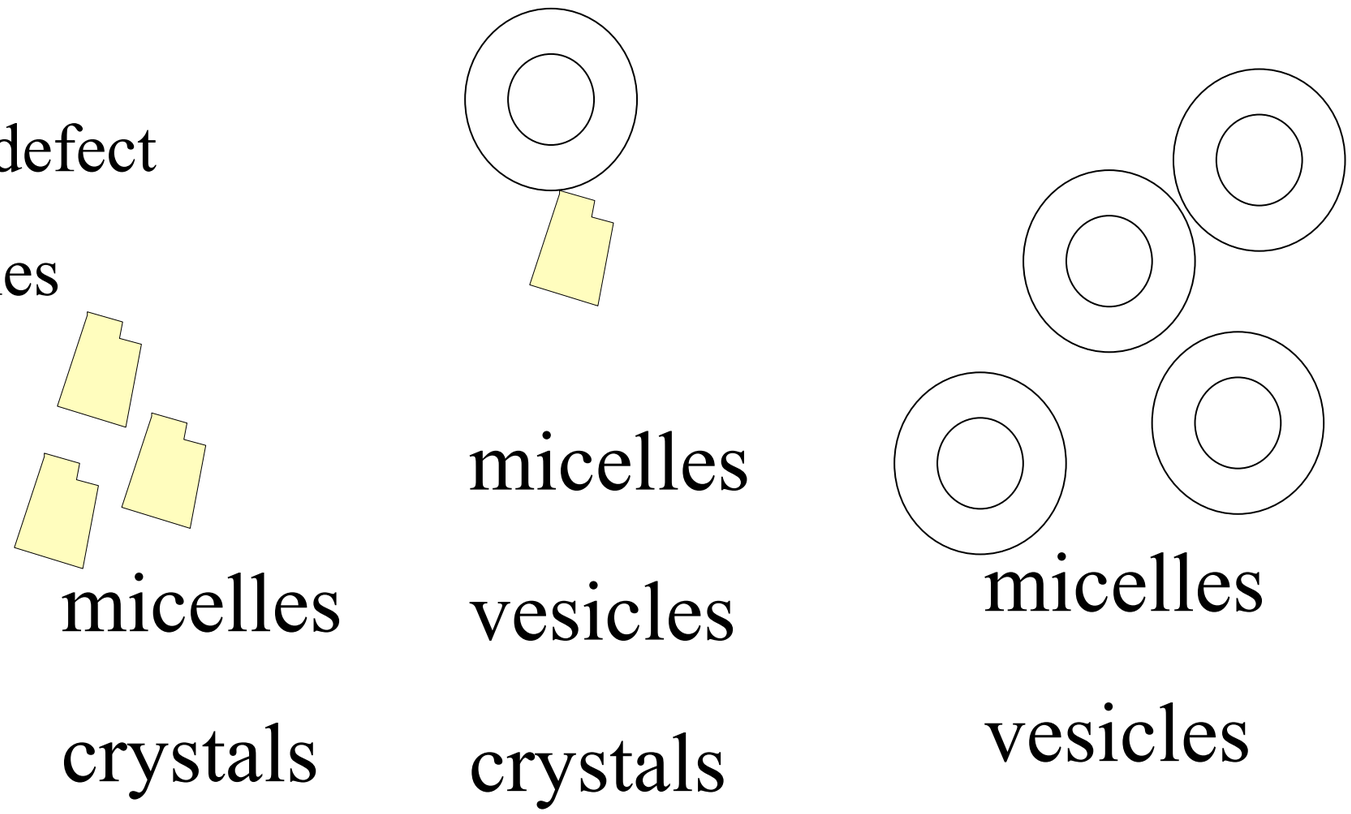
Mdr3 / ABCB4 defect

intrahepatic stones

pancreatitis?



Relative excess bile salt — **Relative excess phospholipid**

lower PL contents

faster nucleation

ABCB4 deficiency: Low phospholipid associated cholelithiasis (LPAC)

- Mutations of *ABCB4* gene encoding the hepatobiliary phosphatidylcholine floppase
- (relative) biliary phospholipid deficiency (?)

- **Clinical:**
- Age at onset of symptoms < 40 years

- Cholesterol gallbladder stones and intrahepatic sludge / microlithiasis (OR 6.1)
- Recurrence of biliary colic after cholecystectomy (OR 8.5)

- Positive family history

- Association with obstetric or mild chronic cholestasis (gGT$^{\uparrow}$)

- Treatment with ursodeoxycholic acid (?)

Rosmorduc et al. *Gastroenterology* (2003)

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Cholesterol gallstones: environmental factors (75%) versus genetic factors (25%).

- High-caloric, high-carbohydrate diet
  Leitzmann et al. *Gastroenterology* (2005)

- Low-fiber diet (*Bacteroides* enterotype ?)
  Tsai et al. *Am J Gastroenterol* (2004)

- Physical inactivity
  Leitzmann et al. *N Engl J Med* (1999)

- Drugs: estrogen progesteron, octreotide therapy
  Cirillo et al. *JAMA* (2005), Liu et al. *BMJ* (2008)

- Obesity / metabolic syndrome
  Völzke et al. *Digestion* (2005), Tsai et al. *Gut* (2006)

- Rapid weight loss / surgery for obesity
  Shiffman et al. *Ann Intern Med* (1995)

- "Weight cycling"
  Tsai et al. *Arch Intern Med* (2006)

Relationship between incidence of gallstones and rate of weight loss in various studies

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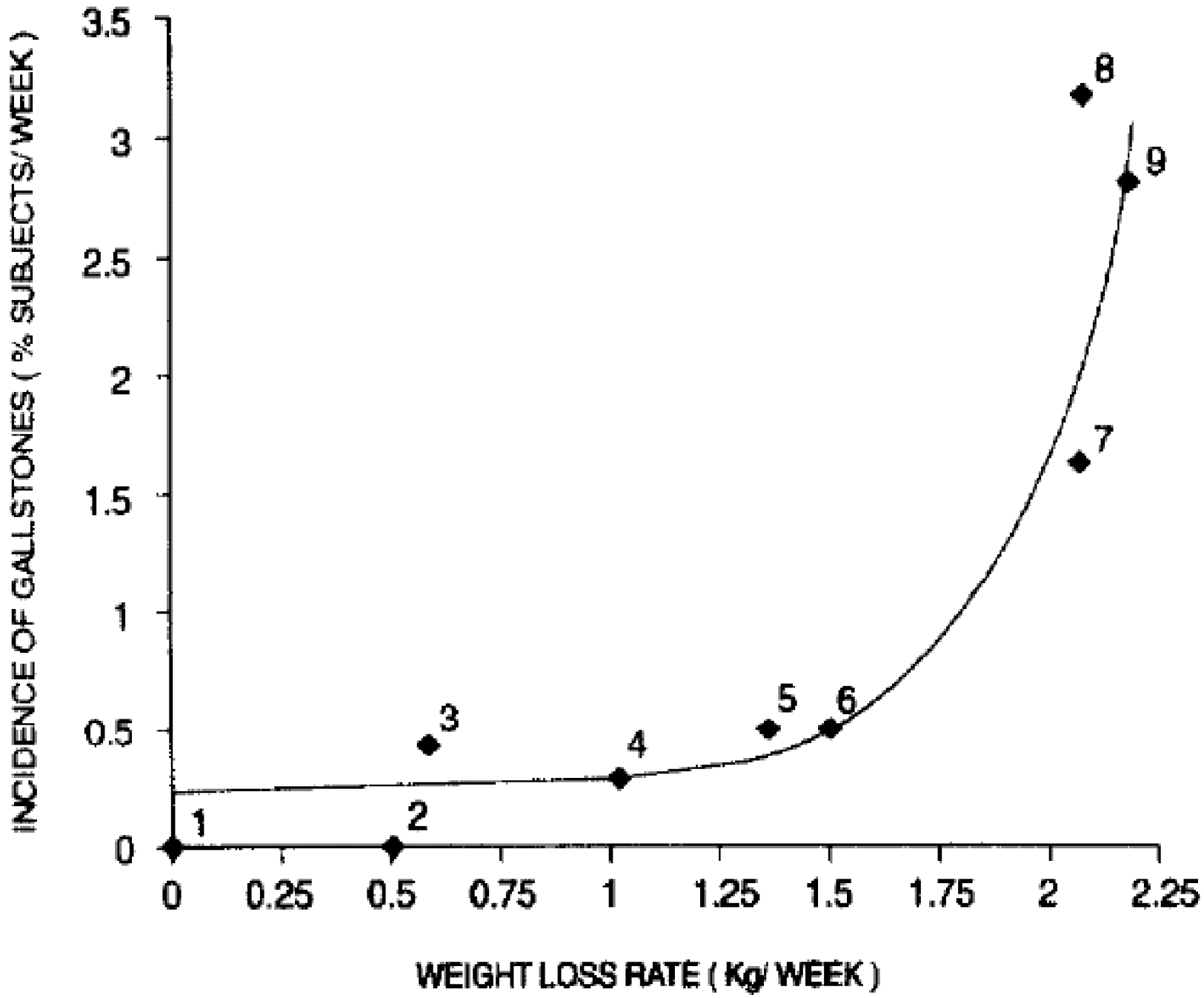

Weinsier. Am J Med 1995;98:115-7

Main genetic factor: *ABCG8* p.D19H

	Year	Population	N	OR	Risk allele frequency (%)
Buch et al.	2007	Germany	1,832	2.2	5.0
		Chile	167	1.9	7.0
Grünhage et al.	2007	Romania	178 (ASP)	3.0	8.5
Kuo et al.	2008	Chile	74	3.5	1.4
Katsika et al.	2010	Sweden	341	2.5	6.8
Siddapuram et al.	2010	India	226	2.3	8.2
Stender et al.	2011	Denmark	3,124	1.9	6.4

Krawczyk et al. *Semin Liver Dis* (2011)

10-year risk of gallstone disease

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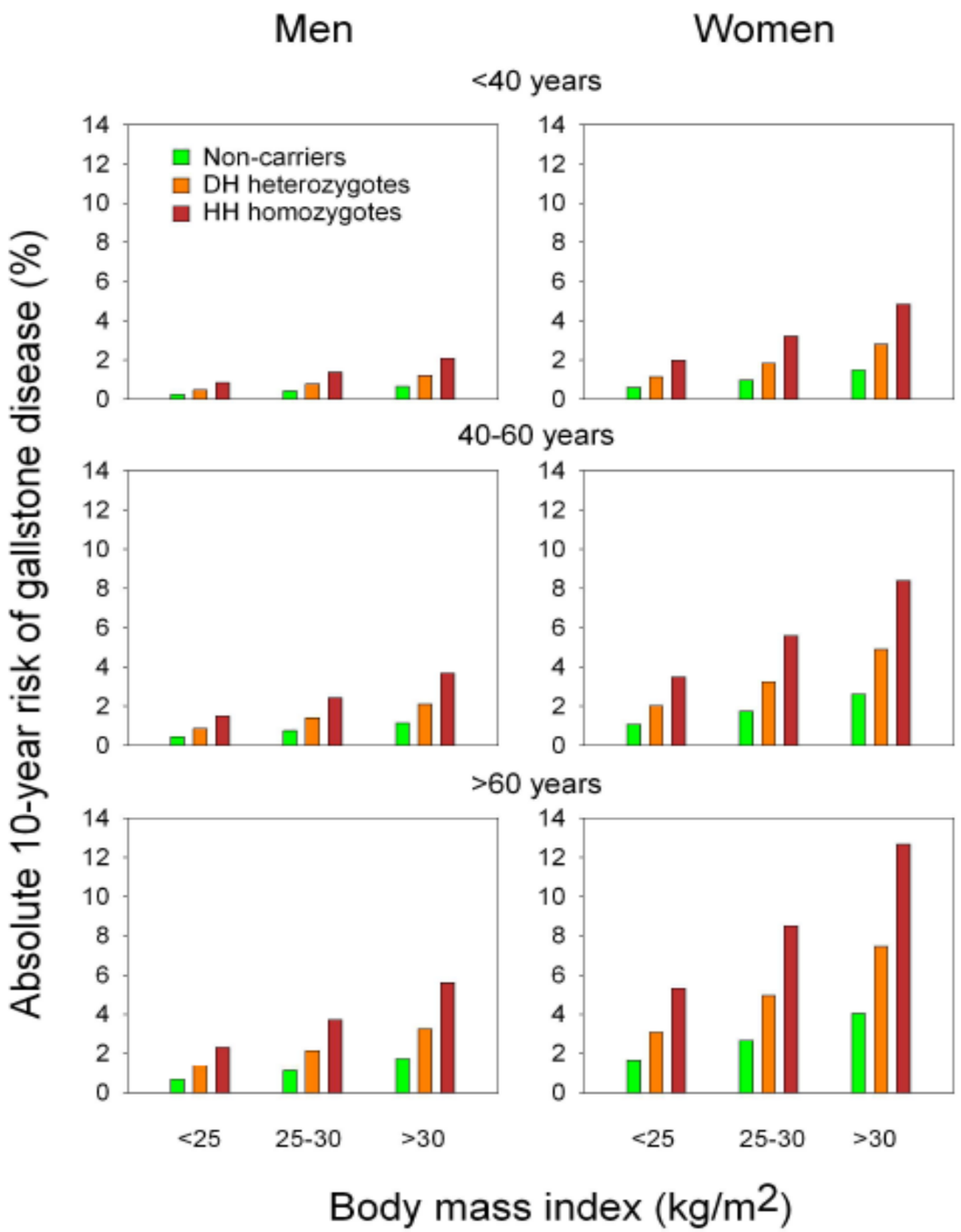

Stender et al. *Heptology* (2011)

Effects of *ABCG8* variant p.D19H

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ABCG8 p.D19H confers

- cholesterol gallstone susceptibility (more biliary secretion?)
- decreased intestinal cholesterol absorption (more transfer from enterocyte back to intestinal lumen?)

- *Secondary effects:*
- higher hepatic cholesterol neosynthesis
- (lower serum cholesterol levels)
- better response to statins

⇒ *ABCG8* p.D19H may represent a gain-of-function mutation that increases clearance of sterols from the body

Teupser et al. *Circulation* (2010) GWAS N = 4,412 Jakulj et al. *J Lipid Res* (2010) Meta-anaylsis N = 591

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Nuclear receptors

- FXR: regulates transport proteins ABCB11 (bile salt transporter) and ABCB4 (phospholipid transporter) at hepatocytic canalicular membrane.

- LXR: regulates cholesterol transporter (ABCG5/G8 at

Bile salt nuclear receptor FXR protects against various diseases

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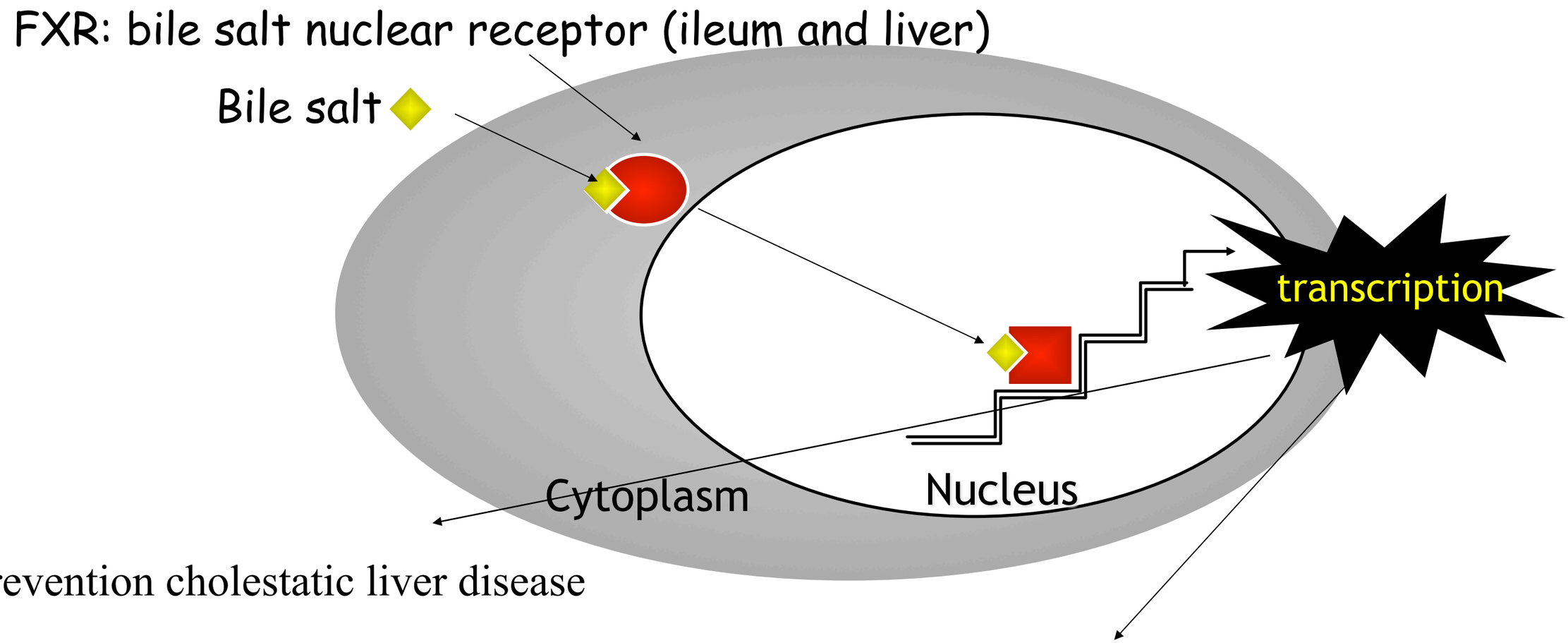


Prevention cholestatic liver disease

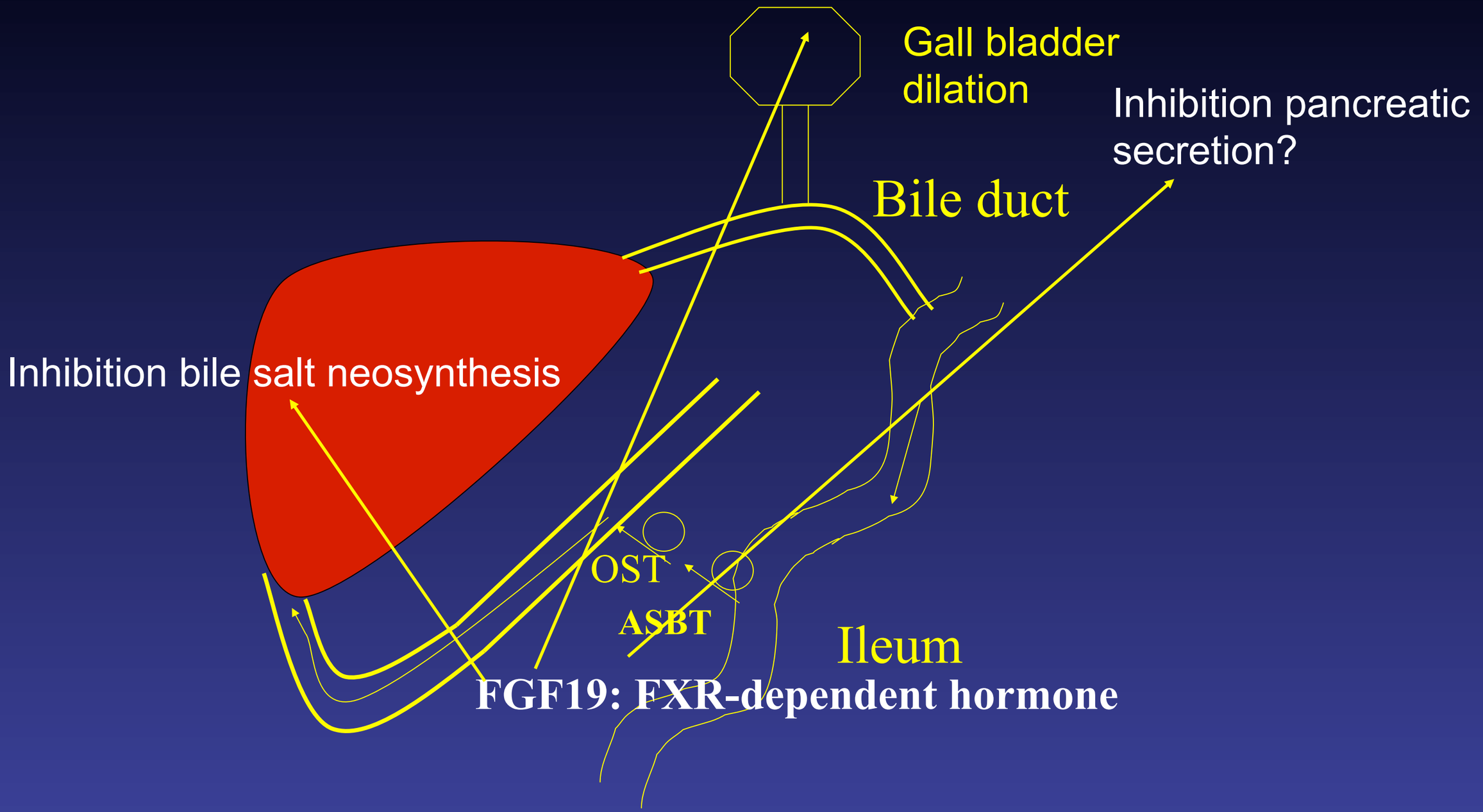
Prevention bacterial overgrowth, translocation and infaction

Role in Crohn/Colitis?

Prevention against colonic cancer?

Prevention gallstones?

Bile salt-FXR-FGF19 axis: master regulator of enterohepatic circulation



Moschetta et al, ***Gastroenterology****. 2012;142:355-365 and Nature Medicine* 2006;12,1253-1255

Effects of FXR loss of function variants and FXR agonists on gallstone formation

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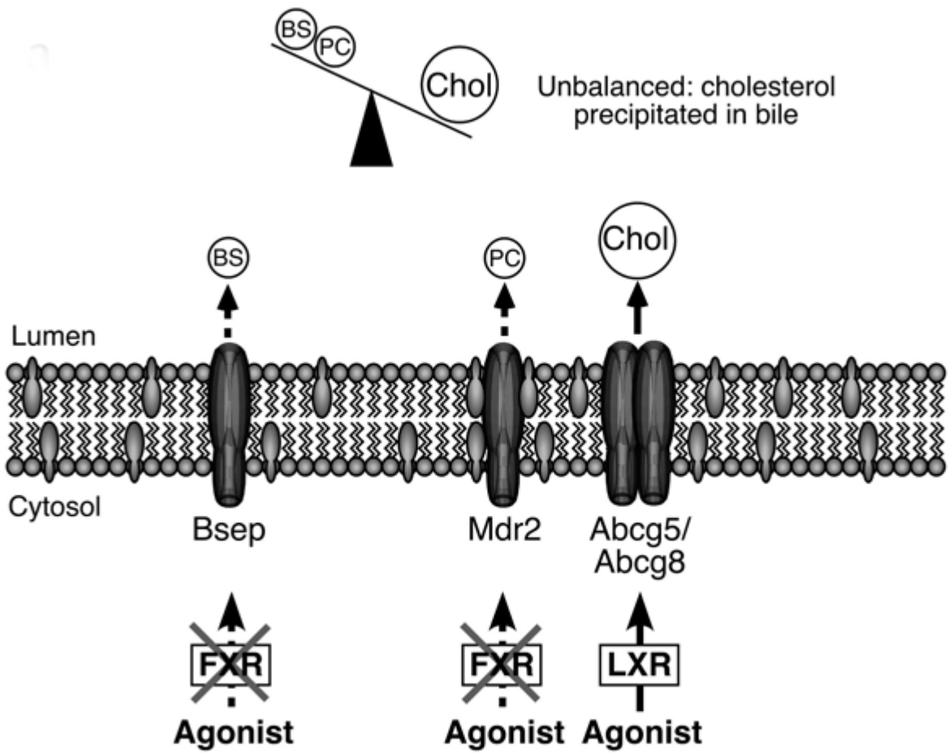
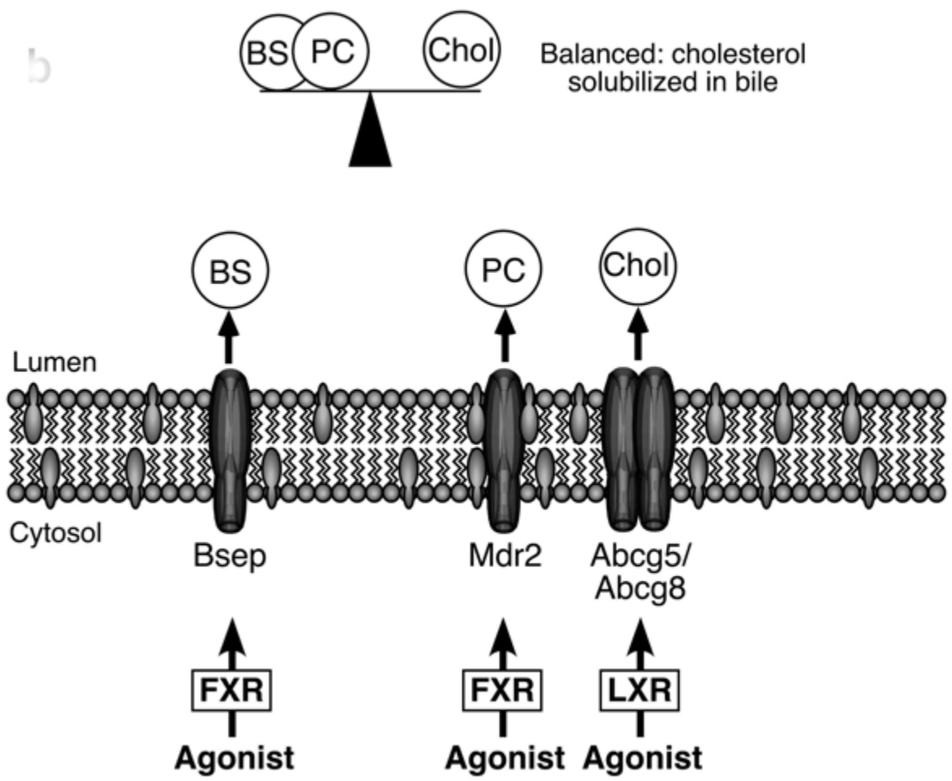
FXR loss of function



FXR agonist



Moschetta et al. *Nature Medicine* 2004;**10**, 1352 - 1358

FXR (-/-) mouse is more susceptible to gallstone formation than wild type mouse on lithogenic die

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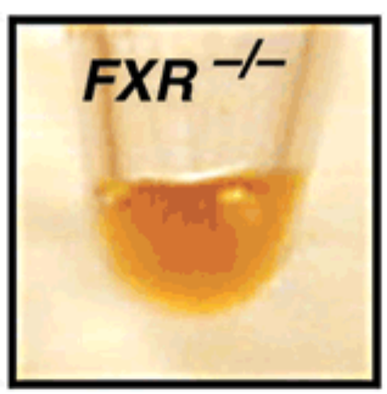

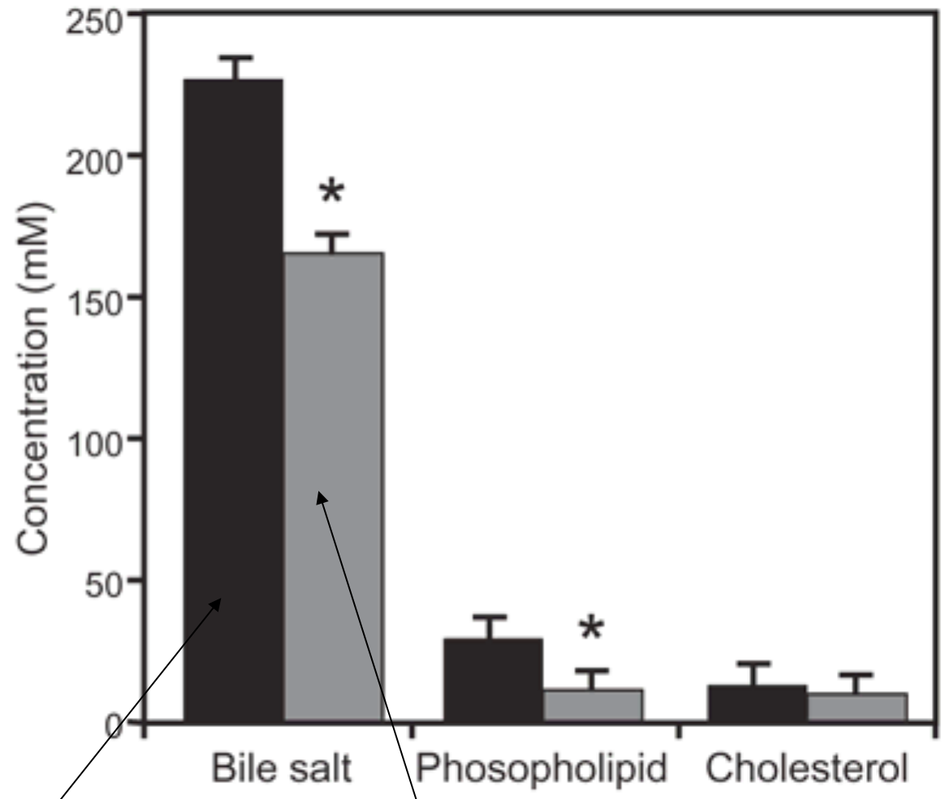

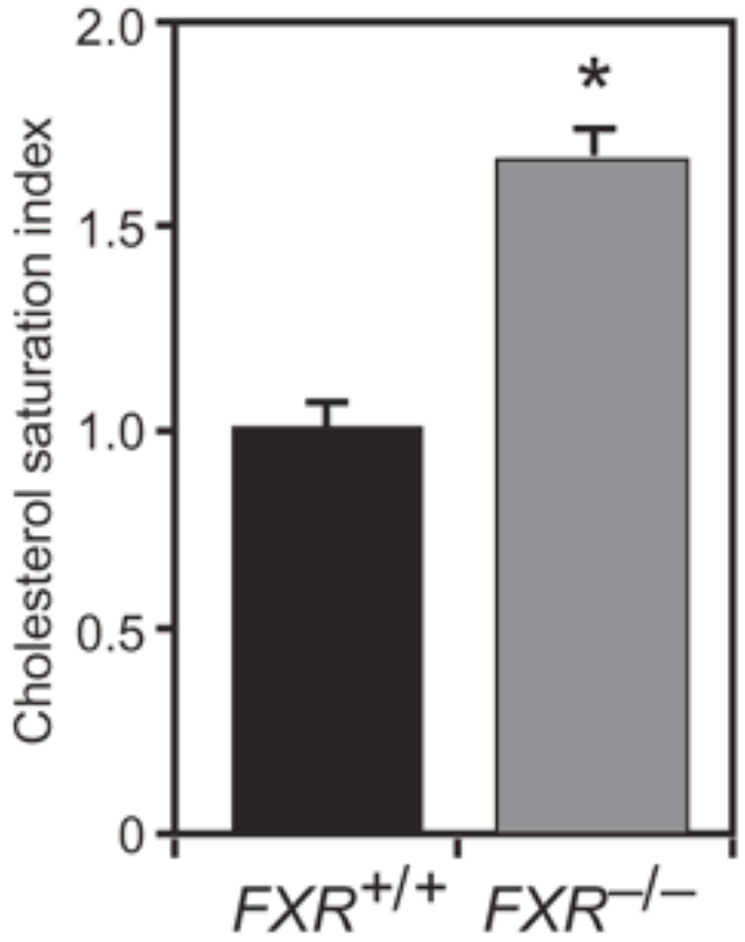

Moschetta et al. *Nature Medicine* 2004;**10**, 1352 - 1358

FXR agonist prevents gallstones in wildtype but not in FXR (-/-) mouse on lithogenic diet

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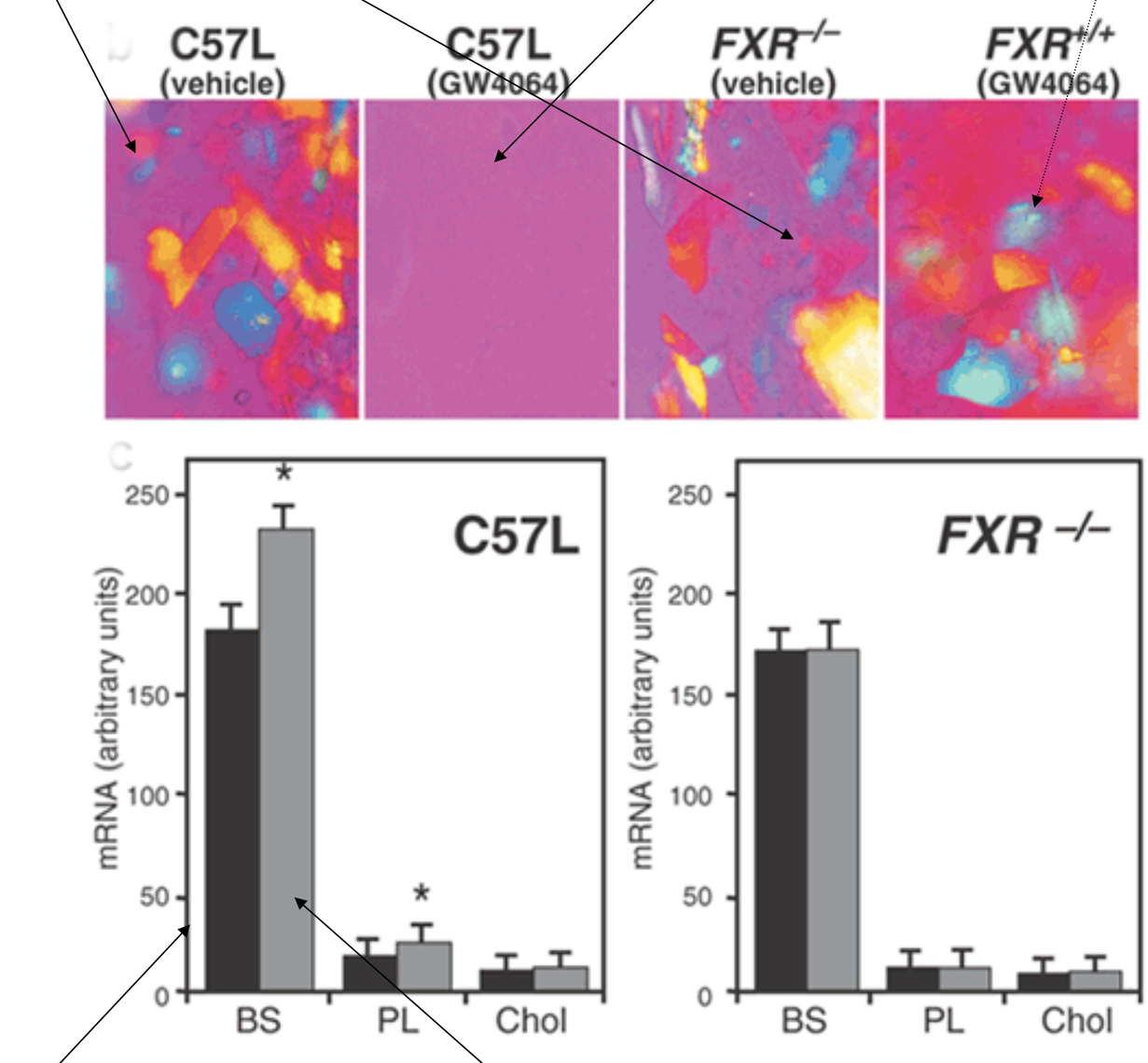
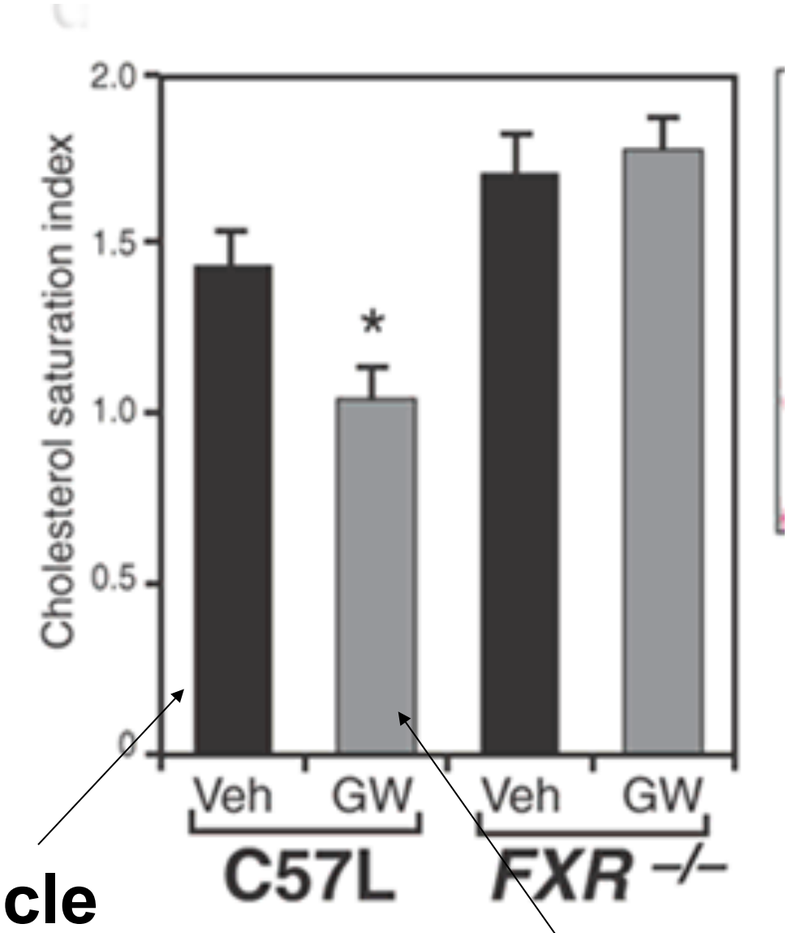
vehicle

FXR agonist





vehicle

FXR agonist

vehicle

FXR agonist

Moschetta et al. *Nature Medicine* 2004;**10**, 1352 - 1358

Conclusions

- Significant progess in understanding pathogenesis of gallstones
- Environmental factors determine 75% of gallstone formation (cave rapid weight loss)
- Genetic factors determine 25% of gallstone formation (ABCG8 cholesterol transporter 11%: UGT1A1 Gilbert variant 6%)
- Mutations in ABCB4 phospholipid transporter can lead to gallstone and liver disease.
- Role nuclear receptors in human gallstone formation to be investigated: possibly therapeutic consequences of synthetic FXR agonists

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