Pathophysiology and genetics of cholelithiasis

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Outline of presentation

• **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
• 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

- Cystic fibrosis
- Hemolytic anemias
- Gilbert syndrome-associated UGT1A1 mutation
- 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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Genetics (biliary lipid transport proteins)

Nuclear receptors
Biliary cholesterol solubilization

• 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

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: distal obstruction and pancreatitis: often spontaneous passage to duodenum

A: Small stone: distal obstruction and pancreatitis: often spontaneous passage to duodenum

B: large stone: proximal obstruction and obstructive jaundice

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

- **1 phase**
  - 100% bile salt
  - 0% phospholipid and cholesterol

- **2 phase**
  - 50% bile salt
  - 50% phospholipid and cholesterol

- **3 phase**
  - 100% phospholipid
  - 0% bile salt and cholesterol

- **Central**
  - Micelles
  - Vesicles
  - Crystals

- **Left**
  - Super saturated
  - Micelles
  - Crystals

- **Right**
  - Super saturated
  - Micelles
  - Vesicles
Relative amounts of various lipids determine what happens.

More phospholipid compared to bile salt: slower / less crystallization

More cholesterol: faster / more crystallization
Relative amount of phospholipid versus bile salt determines speed of cholesterol crystallization.

Mdr3 / ABCB4 defect
intrahepatic stones
pancreatitis?

Relative excess bile salt
micelles
vesicles

crystals

Relative excess phospholipid
micelles
vesicles

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↑)
- Treatment with ursodeoxycholic acid (?)

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

- High-caloric, high-carbohydrate diet

- Low-fiber diet (*Bacteroides* enterotype ?)

- Physical inactivity

- Drugs: estrogen progesteron, octreotide therapy

- Obesity / metabolic syndrome

- Rapid weight loss / surgery for obesity

- "Weight cycling"
Relationship between incidence of gallstones and rate of weight loss in various studies

Main genetic factor: *ABCG8* p.D19H

<table>
<thead>
<tr>
<th>Year</th>
<th>Population</th>
<th>N</th>
<th>OR</th>
<th>Risk allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buch et al.</td>
<td>2007, Germany</td>
<td>1,832</td>
<td>2.2</td>
<td>5.0</td>
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<tr>
<td></td>
<td>Chile</td>
<td>167</td>
<td>1.9</td>
<td>7.0</td>
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<tr>
<td>Grünhage et al.</td>
<td>2007, Romania</td>
<td>178 (ASP)</td>
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<td>8.5</td>
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<tr>
<td>Kuo et al.</td>
<td>2008, Chile</td>
<td>74</td>
<td>3.5</td>
<td>1.4</td>
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<tr>
<td>Katsika et al.</td>
<td>2010, Sweden</td>
<td>341</td>
<td>2.5</td>
<td>6.8</td>
</tr>
<tr>
<td>Siddapuram et al.</td>
<td>2010, India</td>
<td>226</td>
<td>2.3</td>
<td>8.2</td>
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<tr>
<td>Stender et al.</td>
<td>2011, Denmark</td>
<td>3,124</td>
<td>1.9</td>
<td>6.4</td>
</tr>
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</table>
10-year risk of gallstone disease

Men

Women

Absolute 10-year risk of gallstone disease (%)

Body mass index (kg/m²)

Non-carriers
DH heterozygotes
HH homozygotes

<40 years

40-60 years

>60 years
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


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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 hepatocytic canalicular membrane).
Bile salt nuclear receptor FXR protects against various diseases

FXR: bile salt nuclear receptor (ileum and liver)

Prevention cholestatic liver disease

Prevention bacterial overgrowth, translocation and infaction

Role in Crohn/Colitis?

Prevention against colonic cancer?

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

FGF19: FXR-dependent hormone

Gall bladder dilation
Inhibition pancreatic secretion?

Inhibition bile salt neosynthesis

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

FXR loss of function

FXR agonist

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

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

Significant progress 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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