Pathogenesis of Gallstone Formation

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Gallstones - a Common Disease

• Gallstone prevalence in Europe and United States: 10 - 20%

• > 190,000 cholecystectomies per year in Germany
  Lammert et al. S3-Guidelines (2007)

• Occlusion or transection of the common bile duct 0.15%
  BQS Germany (2005)

• Costs (United States): $10 billion / year
  Maurer et al. Gastroenterology (2007)
Gallstones: Classification

- Brown pigment stones
- Caution bilirubinate
- Infected bile ducts
- > 90%
- Black pigment stones
- Bilirubin polymers
- Gallbladder (and bile ducts)
- 2%
- Gallbladder (and bile ducts)
- 10%

Schafmayer et al. BMC Gastroenterol (2006)

N = 1025
Cholesterol Gallstones: Molecular Mechanisms

- Biliary cholesterol hypersecretion
- Gallbladder hypomotility
- Slow intestinal transit
- Increased deoxycholate levels

CCK + FGF19 Control Gallbladder Motility

*Fgf15*^/-^ Mice

T-Cells are Critical for Gallstone Formation

**Rag2⁻⁻ Mice**

**Experimental design:**

1. Lithogenic diet (1% cholesterol + 0.5% cholic acid) for 8 wks
2. Adoptive transfer of immune cells 1 wk before initiation of lithogenic diet

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Gallstone Prevalence in Human Populations

Gallstones - a Complex Disease

**Swedish Twin Registry**

- Monozygotic twins: N = 11,073
- Dizygotic twins: N = 18,183

**Concordance rate (%)**

- < 65 yrs
- ≥ 65 yrs

**Structural Equation Modelling**

- Genetic factors: = Lith (lithogenic) genes (25%)
- Environmental factors: (62%)
- Common factors: (13%)


Environmental Factors: Recent Studies

- **High caloric / high carbohydrate diet**

- **Low fiber diet**

- **Physical inactivity**

- **Metabolic syndrome / abdominal obesity**

- **Rapid weight loss**

- **"Weight cycling"**

- **Estrogen substitution**
  Cirillo et al. *JAMA* (2005)
Genetic Factors: Inbred Mouse Model

Lithogenic diet
(1% cholesterol, 0.5% cholic acid, 15% fat)

⇒ Genetic factors (*Lith* genes) determine the formation of cholesterol gallstones in mice

Inbred mouse strains
= homozygous for all alleles

Susceptible
- C57L
- C57BL/6
- SWR

Resistant
- AKR
- 129
- A/J

Quantitative Trait Locus (QTL)-Analysis

Inbred Mouse Strains

Gallstone-susceptible × Gallstone-resistant

F1 × F1

F2 (N > 300)

Gallstones

- + + + + -

Genetic linkage analysis

Lammert et al. Gastroenterology (2001)
**Lith Genes: ABC Transporters**

ABC = *ATP Binding Cassette*

Cholesterol → ABCG5/G8

Bile salts → ABCB11

Phosphatidylcholine → ABCB4

ABCG8 - a Lith gene in Mice ...

Cholesterol $\xrightarrow{\text{ABCG5/G8}}$

Lith9 Locus

ABCG5 Gene  ABCG8 Gene

Exons

**ABCG8 - a Lith gene in Mice and Humans**

### Affected Sib Pair (ASP) Analysis (N = 178):

**Strategy:** If the disease is linked to a locus, affected sib pairs are more likely to carry the same allele than is to be expected by chance.

### Table

<table>
<thead>
<tr>
<th></th>
<th>Triglycerides (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
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<tbody>
<tr>
<td><strong>ABCG8</strong></td>
<td></td>
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<tr>
<td>A632V</td>
<td>CT/TT=28</td>
<td>155.5±57.3</td>
<td>205.7±32.6</td>
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<td>CC=44</td>
<td>154.1±67.3</td>
<td>204.6±39.2</td>
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<td>T400K</td>
<td>CA/AA=30</td>
<td>168.5±76.9</td>
<td>209.9±37.5</td>
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<td>CC=42</td>
<td>144.7±50.1</td>
<td>201.5±35.8</td>
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<tr>
<td>Y54C</td>
<td>AG/GG=44</td>
<td>145.5±58.4</td>
<td>202.7±39.6</td>
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<td></td>
<td>AA=28</td>
<td>170.6±68.3</td>
<td>210.3±32.8</td>
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<tr>
<td>D19H</td>
<td>GC/CC=12</td>
<td>130.9±52.6</td>
<td>192.5±27.3</td>
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<tr>
<td></td>
<td>GG=60</td>
<td>159.4±64.5</td>
<td>208.3±38.4</td>
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<tr>
<td><strong>ABCG5</strong></td>
<td></td>
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<tr>
<td>604E</td>
<td>CG/GG=24</td>
<td>127.4±51.9</td>
<td>191.7±35.1</td>
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<tr>
<td></td>
<td>CC=48</td>
<td>168.2±64.5</td>
<td>212.7±36.3</td>
</tr>
</tbody>
</table>


### Non-parametric linkage (NPL)

Score for ABCG8 D19H = 7.1 (⇔ P = 4.6 ×10^-13)

**ABCG8 - a Lith gene in Mice and Humans**

A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease


- **Strategy:** If the disease is associated with a locus, cases are more likely to carry the same allele than controls.

- **Genome wide association (GWA) scan**
- **Screening panel:** 280 cases and 360 controls
- **464,585 ("500 K") single nucleotide polymorphisms**
- **Association with ABCG8 D19H** ($P = 7.7 \times 10^{-9}$)
- **Replication panels:** 1,832 German and 167 Chilean patients

**Buch et al. Nat Genet (2007)**
ABCG8 - a *Lith* gene in Mice and Humans

- Cholesterol

- ABCG5/G8

- Chromosome 2p21
  - *ABCG5 Gene*
  - *ABCG8 Gene*

- Risk variant D19H

- Exons

- • Genome wide quantitative trait locus (QTL) analysis in mice
  - • Genome wide association (GWA) scan in humans
  - • Affected sib pair (ASP) analysis in humans
  - • Odds ratio for D19H 2.2 - 3.0
  - • Population attributable fraction 8 - 11%

**Abcb4 (Mdr2) Knockout Mouse**

- Phosphatidylcholine: ABCB4
- Cholesterol: ABCG5/G8
- Bile salts: ABCB11

→ No mixed micelles
→ Chronic cholangitis
→ Transient cholesterol intermediates

Cholelithiasis

Gallstones: *From Bench to Bedside*
Gallstones: Case #1

37 y o female

- Bile duct stones ("string of pearls") → ERC and sphincterotomy

- Intrahepatic cholestasis of pregnancy (ICP) at 27 and 30 weeks of gestation, low γ-GT → premature deliveries

- Liver biopsy: intrahepatic cholestasis, mild portal hepatitis and fibrosis

37 y o female

- Bile duct stones ("string of pearls") → ERC and sphincterotomy

- Intrahepatic cholestasis of pregnancy (ICP) at 27 and 30 weeks of gestation, low γ-GT → premature deliveries

- Liver biopsy: intrahepatic cholestasis, mild portal hepatitis and fibrosis

**Gallstones: Case #1**

- **Phosphatidylcholine**
  - ABCB4
  - c.957C>T (stop codon)

- **Cholesterol**
  - ABCG5/G8

- **Bile salts**
  - ABCB11
  - c.1331T>C (V444A)

**ABCB4 Deficiency**

- Mutations of the phosphatidylcholine transporter *ABCB4*
- Age at onset of symptoms < 40 yrs
- Cholesterol gallbladder stones and intrahepatic sludge or microlithiasis (OR 6.1)
- Recurrence of biliary symptoms after cholecystectomy (OR 8.5)
- Mild chronic cholestasis
- Association with intrahepatic cholestasis of pregnancy

**References**

Lithogenic Gene Signature: $ABCB4 + ABCB11$

- Mutations of the phosphatidylcholine transporter $ABCB4$
- Age at onset of symptoms < 40 yrs
- Cholesterol gallbladder stones and intrahepatic sludge or microlithiasis (OR 6.1)
- Recurrence of biliary symptoms after cholecystectomy (OR 8.5)
- Mild chronic cholestasis
- Association with intrahepatic cholestasis of pregnancy


Gallstones: Case #2

23 y o female

- Cystic fibrosis (CF)
- Focal biliary fibrosis
- Gallstones
- *CFTR (ABCC7)* mutations class IV

- Gallstone prevalence in CF: 4 - 27%
- Black pigment stones
Black Pigment Stones: Pathophysiology

Cystic fibrosis (CF)
Crohn's disease
Liver cirrhosis
↓
**Bile salt loss**

Absorption of unconjugated bilirubin in the colon
↓
Enterohepatic circulation of bilirubin
↓
'Hyperbilirubinbilia'
↓
Bilirubin precipitation

Unconjugated bilirubin
Bile salts
Gallbladder
Ileum
Liver
Colon

Vitek & Carey
**Lithogenic Gene Signature: CF + Gilbert**

UDP glucuronosyltransferase  
*UGT1A1*

**Exons**

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<tr>
<th>Promoter</th>
<th>1A1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
</table>

**TATA box**

A(TA)₆TAA  wild-type  
A(TA)₇TAA  Gilbert polymorphism

**Genotype** | **Bilirubin (mg/dl)** |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>(TA)₆(TA)₆</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>(TA)₆(TA)₇</td>
<td>0.54 ± 0.05*</td>
</tr>
<tr>
<td>(TA)₇(TA)₇</td>
<td>0.72 ± 0.08*</td>
</tr>
</tbody>
</table>

**CF**

- stones  
+ stones

N = 52  
Pathogenesis of Gallstone Formation

Genetic factors

Monogenic cholelithiasis
- ABCB4
- ABCB11
- CFTR (ABCC7)
- ...

Polygenic (common) cholelithiasis
- ABCG8
- ...

Environmental factors

- Diet
- Physical inactivity
- Estrogens
- ...

modified from H. Witt
Individual "lithogenic signatures"
- Genetic factors: *ABCG8, ABCB4, ABCB11, UGT1A1* ...
- Environmental factors: estrogens, enterohepatic bacteria ...

**Speculation: Future Developments**

**Normal risk**
- Primary prevention without drugs
  - Weight reduction, sports, diet modification

**Defined high risk groups**
- Prevention with drugs (or prophylactic cholecystectomy ?)
  - UDCA, nuclear receptor ligands, statins ?, ezetimibe ?

**Primary prevention without drugs**

**Prevention with drugs**

Friedrich August Walter
Anatomisches Museum (1796)

Friedrich Theodor Frerichs
Klinik der Leberkrankheiten (1861)