Genetics of alcoholic liver disease

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Alcoholic Liver Disease – Phenotypes may vary…
Alcoholic Liver Disease (ALD)

- **Healthy Liver**
- **Alcohol**
- **Alcoholic Fatty Liver / Fibrosis / Hepatitis**
- **Alcohol**
- **Liver Cirrhosis**

90-100% of alcoholics reveal steatosis
10-35% show signs of alcoholic fibrosis / hepatitis
10% develop cirrhosis

Only a minority of heavy drinkers develop severe liver disease!
Factors Influencing the Progression of ALD

Environmental Factors

- Drinking pattern (amount, continuity, type of beverage ?, relation to meals)
- Metabolic syndrome
- Coinfection with hepatitis C (and B ?) virus
- Age (cirrhosis)
- High fat diets
- Smoking ?
- Cannabis ?
- Coffee

Genetic factors ?

Rotily et al. 1990
Klatsky et al. 1992
Becker et al. 1996
Bellentani et al 1997
Becker et al. 2002
Raynard et al. 2002
Hezode et al. 2005
Johansen et al. 2006
Klatsky et al. 2006
Genetic risk of alcoholic liver disease
Evidence from epidemiology

- Females are more susceptible towards equal amounts of alcohol (Pares et al. 1986, Sato et al. 2001)

- Hispanics are more prone to developing ALD than Blacks and Whites (Wickramasinghe et al. 1995, Stinson et al. 2001)

- Monozygotic twins have a 3-fold higher prevalence of alcoholic cirrhosis than dizygotic twins (Hrubec et al. 1981, Reed et al. 1996)
Genetics of ALD: Why Bother?

Genetic Background of ALD

- Identify causal gene(s)/genetic variant(s)
- Diagnostic/Prognostic Testing
  - Prevention
- Better Insight to Pathophysiology
  - Drug therapy
  - Gene therapy
Mendelian vs. Complex Diseases

**Mendelian Inheritance**
- Mutation
- Genotype
- Phenotype
- Dominant, recessive, X-linked inheritance

**Complex Inheritance**
- Genetic variant
  - Gene A
  - Gene B
- Environment / Behavior
- Phenotype
Types of Genomic Variation

1. Single Nucleotide Polymorphism (SNP)

\[\text{ggc tgc atA/C aat gtc ttc ttt}\]

2. Microsatellite

\[\text{tac aca cat gta CACACACACACACACACACACACA cca tga cct}\]

3. Insertion

\[\text{AGG CC } \rightarrow \text{AGG ATA CC}\]

4. Deletion

\[\text{AGG TCC } \rightarrow \text{AGCC}\]
Types of Genetic Studies

- Twin studies
- Family linkage studies
- Genetic association studies (case control studies)
  - Candidate gene association studies
    *Hypothesis-driven*
  - Genome-wide association studies
    *Hypothesis-free/-generating*
Genetics of Alcohol-Related Liver Damage
Twin Studies

- 15.924 Twin pairs (National Academy of Sciences-National Research Council, USA)
- Prevalence of alcoholic cirrhosis 17.7/1,000
- Rate of concordance: monozygotic 16.9, dizygotic 5.3 (p < 0.001)
- Genetics contribute to 50% of variability to develop alcoholic liver cirrhosis

Candidate Gene Association Studies in ALD

Pubmed: "alcoholic liver disease" and "polymorphism" 1,358 hits

Candidate genes already tested
ADH1B, ADH1C, ALDH2, CYP2E1, CYP1A1, NAT2, GSTA1, GSTM1, GSTM3, GSTT1, GSTP1, ApoE, SLC6A4, MnSOD, IL-10, IL-1R antagonist, TGFbeta1, IL-1beta, CD14-ET-R, CTLA-4, MPO, HFE, UCP.. No confirmed genetic risk factor for ALD!

Candidate genes that could be tested
MMPs, TIMPs, collagens, CTGF, leptin, leptin-R, adiponectin, CPT1A, PPARs, SREBP-1, MTP, acyl-CoA oxidase, 11beta-HSD, SCD-1, PEMT, angiotensinogen, TLRs, MCP, CYP4A, RAR, MAT1, insulin-R, etc..
Genetic Association Studies:
Problem of replication validity

Ioannidis et al., Nature Genetics 2001;29:306-9
Meta-analysis

- SNPs of ADH1B, ADH1C, CYP2E1, ALDH2 and the risk of alcoholism and alcoholic liver disease
- 50 association studies (1990-2004)
- Exploration of heterogeneity, bias, power, compliance with Hardy-Weinberg equilibrium, subgroup analyses (ethnicity, gender)
- Associations for ADH1B*1, ADH1C*2 and ALDH2*1 and the risk of alcoholism (ORs 1.89, 1.32, and 4.35, respectively)
- Subgroup analysis: associations of ALDH2*2 and ADH1C*2 with alcoholism restricted to Asian men
- **No associations** for any of the tested genetic variants with regard to alcoholic cirrhosis
Genes associated with alcohol dependence
Genes associated with alcohol dependence

Chromosome 4

No association with ALD!

Fehr et al. Psychiatr Genetics 2006;16:9-17
### Genome-wide association studies in liver diseases

<table>
<thead>
<tr>
<th>Author</th>
<th>Disease</th>
<th>Patients (n)</th>
<th>Risk variant(s)</th>
<th>Genome-wide significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buch et al. 2007</td>
<td>Gall stones</td>
<td>2,280 cases 2132 controls</td>
<td>ABCG8</td>
<td>1.4 x 10^{-14}</td>
</tr>
<tr>
<td>Romeo et al. 2008</td>
<td>Fatty liver</td>
<td>9,229</td>
<td>PNPLA3 (I148M)</td>
<td>5.9 x 10^{-10}</td>
</tr>
<tr>
<td>Huang et al. 2007</td>
<td>Hepatitis C - progression</td>
<td>574 patients</td>
<td>7-gene signature</td>
<td>na</td>
</tr>
<tr>
<td>Ge et al. 2010</td>
<td>Hepatitis C – response to therapy</td>
<td>2,612 patients</td>
<td>IL28B</td>
<td>1.37 x 10^{-28}</td>
</tr>
<tr>
<td>Fellay et al. 2010</td>
<td>Hepatitis C - side-effects (RBV)</td>
<td>1,286 patients</td>
<td>ITPA</td>
<td>1.1 x 10^{-45}</td>
</tr>
<tr>
<td>Melum et al. 2011</td>
<td>PSC</td>
<td>1,740 cases 5,136 controls</td>
<td>MST1, BCL2L11</td>
<td>1.1 x 10^{-16} 4.1 x 10^{-8}</td>
</tr>
<tr>
<td>Mells et al. 2011</td>
<td>PBC</td>
<td>1,840 cases 5,163 controls</td>
<td>STAT4, DENND1B, CD80, IL7R, CXCR5, TNFRSF1A, CLEC16A and NFKB1</td>
<td>5 x 10^{-8}</td>
</tr>
</tbody>
</table>
Steatosis in NAFLD
PNPLA3 (Adiponutrin) rs738409 (G/C → I148M) and liver injury

- Genome-wide association study in patients with NAFLD (n=9,229)
- 2-fold higher hepatic fat content in homozygous carriers of PNPLA3 rs738409 (G) allele

PNPLA3 Variation – rs738409 C>G (I148M)
PNPLA3 variation and protein function

- Localised between membranes and lipid droplets

- PNPLA3 hydrolyses triglycerides \textit{in vitro} \cite{Lake2005}

- PNPLA3 rs738409 (G/G) reduces triglyceride hydrolysis \textit{in vitro} \cite{He2010}

- PNPLA3 rs738409 (G/G) overexpressing mice reveal more steatosis than wild types \cite{He2010}
PNPLA3 variation – Steatogenesis?

PNPLA3 variation and ALD

Multi-center cohort (alcoholics)
- Recruitment between 2000-2009
- Alcoholics (n=1043; German/Swiss ancestry) from
  - GI/Hepatology (Bern, Kiel, Erlangen, Heidelberg, Regensburg, Frankfurt, Homburg)
  - Addiction Medicine units (Bern, Regensburg, Mannheim)
- Inclusion criteria: heavy alcohol consumption (>60g/day ♀; >80g/day ♂ for >10 years)
- Standard laboratory (ALT, AST, GGT, AP, bilirubin, INR, albumin, platelets), US
- Exclusion of CHB+C, hemochromatosis, autoimmune hepatitis
- Cirrhosis as per (1) biopsy (Ishak 4-6), (2) complications of cirrhosis, (3) unequivocal US or CT imaging and/or esophageal varices

Population-based cohort (at-risk drinkers)
- SHIP cohort (n=376 / 4319; recruitment 1996)
- At-risk drinkers (median alcohol consumption 300g/day)
- Standard laboratory; US

Genotyping / Statistics
- TaqMan PCR (Applied Biosystems; Foster City, CA) (Hampe et al; Bioinformatics 2001;17:654-55)
- Case-control design; Chi² test; Fisher exact test
- Population-attributable risk

\[ PAR\% = \frac{f_{GT}(RR - 1)}{f_{GT}(RR - 1) + 1} \cdot 100 \]
PNPLA3 rs738409 (G) allele frequency

Stickel et al. Hepatology 2011;53:86-95
**PNPLA3 rs738409 GG and alcoholic cirrhosis**

N=3,746

- **Tian et al.**
  - $P = 1.7 \times 10^{-10}$
  - $P = 4.7 \times 10^{-5}$

- **Seth et al.**
  - $p = 0.0012$

- **Trepo et al.**
  - $p = 0.02$

- **Stickel et al.**
  - $p = 1.18 \times 10^{-5}$
  - $p = 0.04$
Hypothesis
Summary

- Environmental and host factors modulate evolution and progression of ALD

- Genetic background important modulator of susceptibility

- Quest for genetic risk factors if ALD has only identified/confirmed few candidate genetic variants

- Carriage of PNPLA3 rs738409 (G) allele and GG genotype first confirmed genetic risk factor

- Genome-wide association studies are under way to search for yet unknown genetic variants