Genetics of Alcoholic Liver Disease

Felix Stickel

Hepatology Unit, Hirslanden Klinik Beau Site, Bern, Switzerland
&
Department of Clinical Research, University of Bern
90-100% of heavy drinkers reveal steatosis *(Teli et al 1995)*

10-35% develop alcoholic hepatitis *(Burt u. McSween 1999)*

8-20% progress to cirrhosis *(Bellentani et al 1997)*

Only a minority of heavy drinkers develop significant ALD
Individuals at risk for ALD
Risk factors of ALD progression

- Cirrhosis
- Alcohol
- Metabolic syndrome
- Chronic viral hepatitis
- Nutrition
- Host genetics
- Gender
- Ethnicity
- Genetic variants
- Comorbidity
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*)
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)
   ggc tgc atA/C aat gtc ttc ttt

2. Microsatellite
   tac aca cat gta CACACACACACACACACACACACA cca tga cct

3. Insertion
   AGG CC → AGG ATA CC

4. Deletion
   AGG TCC → AGGCC

Exonic – coding
Intronic – non-coding
Promoter – transcription
Intergenic – splicing
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*
Genetic case control studies

- 100s of studies
- Hypothesis-driven (role of gene in ALD development)
- Small sample size
- Inadequate characterization of cases and controls
- Chance association findings
- No confirmed genetic marker until recently
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}</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 rs738409 (G) allele frequency

**Clinical alcoholics**
- N = 1,043
- p = 1.6 x 10^-6

**At risk drinkers**
- N = 376
- p = 0.02

*Stickel et al. Hepatology 2011;53:86-95*
PNPLA3 variation and protein function

- Localised between membranes and lipid droplets
- PNPLA3 hydrolyses triglycerides in vitro (Lake et al, J Lipid Res 2005)
- PNPLA3 rs738409 (G/G) reduces triglyceride hydrolysis in vitro (He et al, J Biol Chem 2010;285:6706-15)
- PNPLA3 rs738409 (G/G) overexpressing mice reveal more steatosis than wild types (He et al, J Biol Chem 2010;285:6706-15)
PNPLA3 Variation – rs738409 C>G (I148M)
PNPLA3 variation – gain of function?

PNPLA3 variation – gain of function?

Cos-7 Zellen

PNPLA3 mRNA expression
Zucker rats (Alcohol 5.5% for 6 weeks)

X-fold expression

Lean Control
n=9

Lean EtOH
n=9

Fatty Control
n=8

Fatty EtOH
n=9

arb. units

n=9

n=9

n=8

ns

**

*
**PNPLA3 rs738409 GG and alcoholic cirrhosis**

\[ N = 3,746 \]

- **Tian et al.**
  - OR: 2
  - \( P = 1.7 \times 10^{-10} \)

- **Seth et al.**
  - OR: 7
  - \( P = 4.7 \times 10^{-5} \)
  - \( p = 0.0012 \)

- **Trepo et al.**
  - OR: 2
  - \( p = 0.02 \)

- **Stickel et al.**
  - OR: 4
  - \( p = 1.18 \times 10^{-5} \)
  - \( p = 0.04 \)
Imputation - Alcoholic cirrhosis

- Combined analysis of independent genome-wide data sets
- Reduces the problem of low sample sizes and insufficient power
- Identifies novel association signals via haplotype analysis
PNPLA3 genetic variation and HCC
ADH1C*1 homozygosity in alcohol-associated cancers

*Odds Ratio 3.6 (CI 1.4 - 7.5)

Homann, Stickel et al., Int J Cancer 118;2006:1998
A genetic variant of the *neurocan (NCAN)* gene is associated with HCC in alcoholic liver disease.
Carriage of both NCAN and PNPLA3 genetic risk variant increase the risk of HCC in alcoholic liver disease

Nischalke et al., J Hepatol 2014;June 16, in press
Hepatocyte

LSEC

Kupffer cell

ALDH

Lipid vesicle

PPAR-γ

Nucleus

ADH

CYP2E1

PNPLA3

ROS

DNA adducts

Lipid peroxides

Acetate

Acetaldehyde

Ethanol

FFA

TNFα

Endotoxin

ApoE

Lipogenesis ↑

Fatty acid oxidation ↑

Lipid storage ↑

Stickel & Hampe, GUT 2012;61:150-9

Intestinal lumen

Alcohol-induced barrier defect
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 secondary genetic risk factors