Who Gets Alcoholic Liver Disease?

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Alcoholic Fatty Liver (Steatosis)

Intracellular fat deposition

Fatty hepatocytes
Alcoholic SteatoHepatitis (ASH)

- Fat deposits
- Inflammation
- Fibrosis with necrosis
Cirrhosis

Nodules surrounded by fibrosis

Regenerative nodule

Fibrosis
Prevalence of disease stages in unselected heavy drinkers

- Normal (0-30%)
- Fatty Liver (60-100%)
- Cirrhosis (<10%)

WHY??
Non-genetic (inherited) factors

- Dose & pattern of alcohol intake
- Dietary factors
  - Specific nutrients?
  - General excess (obesity)
- Smoking
- Coffee drinking
- Gut bacteria (the “microbiome”)?

References:
- Becker 2002, Klatsky 2006
- Klatsky 2006
Cirrhosis death rate correlates with per capita alcohol intake

Leon et al Lancet 2006
ALD & Dose: individual level

% of category

Drinks per day

Bellentani et al 1997
ALD risk and pattern of intake

Risk of ALD increased by:
- Drinking outside meal-times
  Risk↑: 3.4[1.7-6.6]  
  Bellentani 1997
- Drinking beer/spirits rather than wine
  Risk↑: 2.5[1.7-3.3]  
  Becker 2002
- Daily versus weekend drinking
  Risk↑: 2.5 [1.8-4.6]  
  Stokkeland 2008
Is wine vs beer effect due to confounding “lifestyle” factors?
Diet and ALD: the role of obesity

- 1604 heavy drinkers
- Overweight = minimum BMI>25 women, >27 men in previous 10yr
- In multivariate analysis obesity/glucose best predictors of cirrhosis (Risk $\uparrow > x 2$ for obesity)

Naveau et al 1997
Raynard et al 2002
Rates of liver cirrhosis in 1,230,662 UK women (mean age 56yrs) followed up for mean 6.2 years

Liu  Br Med J 2010
Relative risks of contributions of BMI and alcohol to liver disease mortality (adjusted for all risk factors).

9,559 Scottish men followed up for mean 29 years
Obesity & ALD: ?explanation for association

- Mechanisms of ALD & obesity related liver disease (NAFLD) very similar:
  - Metabolic overload
  - Oxidative, ER and cytokine mediated stress
  - The microbiome/endotoxin

- Direct fibrogenic (scar producing) effects of obesity
  - High Insulin/glucose
  - “Adipokine” profile
Risk of ALD and coffee drinking

<table>
<thead>
<tr>
<th>Coffee intake</th>
<th>Relative Risk of ALD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/seldom</td>
<td>1.0</td>
</tr>
<tr>
<td>&lt;1</td>
<td>0.7 [0.4-1.1]</td>
</tr>
<tr>
<td>1-3</td>
<td>0.6[0.4-0.8], p&lt;0.001</td>
</tr>
<tr>
<td>≥4</td>
<td>0.2[0.1-0.4], p&lt;0.001</td>
</tr>
<tr>
<td>Per cup/day</td>
<td>0.8[0.7-0.9], p&lt;0.001</td>
</tr>
</tbody>
</table>

Adjusted for age, weight, smoking, gender, ethnicity

Klatsky 2006
Gut bacteria - the “microbiome” - and ALD susceptibility?

- Abundant evidence that the gut microbiome may be involved in ALD pathogenesis. 
  *Purohit 2008*

- Non-alcoholic steatohepatitis (NASH) as a transmissible disease? 
  *Henao-Mejia Nature 2012*

- What about ALD?
  - Faeces from ALD patients induce ALD in alcohol fed mice. 
    *Llopis 2013*
Gender and ALD

- Females develop ALD at “lower” intake
- Explanation: ↑ blood alcohol levels for same dose per body weight
  - ↓ Vol distribution

- Estrogen sensitises the liver (Kupffer cells) to bacterial (endo) toxins
  - ↑ gut permeability to endotoxin
  - ↑ Endotoxin receptors (CD14) & release of inflammatory mediators from liver inflammatory cells (eg. TNFα)

*Marshall 1983
*Thurman et al 1999
Are genes important?

Concordance rates (%) among 15,924 male twin pairs age 67-77. Alcoholism and cirrhosis ↑ 25%.

Ethnic variation in susceptibility


Alcoholism genetic

ALD genetic

Risk “over-and-above” the risk of alcoholism

Hrubec 1981

Alcoholism genetic

ALD risk now shared with alcoholism

No longer a significant independent genetic risk for ALD

Reed 1996
Finding the genes

- **Hypothesis-driven**
  - Candidate gene case-control studies
    - All studies in ALD thus far
    - Almost all underpowered (too small)

- **Hypothesis-free (generating)**
  - “Whole” genome scanning
    - None in ALD as yet (underway) – BUT:
      - Clues from studies in NAFLD
Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease

Stefano Romeo¹,², Julia Kozlitina²,³,⁸, Chao Xing¹,², Alexander Pertsemidis¹, David Cox⁴, Len A Pennacchio⁵, Eric Boerwinkle⁶, Jonathan C Cohen¹ & Helen H Hobbs¹,⁷

- USA Study population: (Black n=1032, White n=636, Hispanic n=383).
- 9229 non-synonymous exonic SNPs.
- Steatosis defined non-invasively by ¹H-MRS.

PNPLA3 (I148M) – Carriage of G allele associated with increased steatosis (p=5.9 x 10⁻¹⁰)
Presence of NASH

OR 1.5 (1.12-2.04)

Fibrosis >F1

OR 1.5 (1.09-2.12)
PNPLA3 - Adiponutrin

But why the link with NASH & scarring?

He et al, 2010
Huang et al, 2010
Huang et al, 2011
Pirazzi et al, 2012

Anstee et al, 2013
PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells

Carlo Pirazzi¹,²,³, Luca Valenti¹,²,³, Benedetta Maria Motta¹,³,⁴, Piero Pingitore²,⁴, Kristina Hedfalk², Rosellina Margherita Mancina¹,³, Maria Antonella Burza¹, Cesare Indiveri⁴, Yvelise Ferro⁵, Tiziana Montalcini⁵, Cristina Maglio¹, Paola Dongiovanni³, Silvia Fargion³, Raffaela Rametta³, Arturo Pujia⁵, Linda Andersson¹, Saswati Ghosal¹, Malin Levin¹, Olov Wiklund¹, Michelina Iacovino⁵, Jan Borén¹ and Stefano Romeo¹,⁵,*

Human Molecular Genetics, 2014
doi:10.1093/hmg/ddu121
Carriage of the PNPLA3 rs738409 C>G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma


Journal of Hepatology 2014 vol. 61 | 75–81
Case control studies of PNPLA3 in ALD

<table>
<thead>
<tr>
<th>Study</th>
<th>Numbers</th>
<th>Ethnicity</th>
<th>Associated phenotype</th>
</tr>
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<tbody>
<tr>
<td>Tian, 2010</td>
<td>1,221</td>
<td>Mexican-European</td>
<td>↑ Risk of ALD and cirrhosis</td>
</tr>
<tr>
<td>Seth, 2010</td>
<td>548</td>
<td>UK</td>
<td>↑ Risk of ALD and cirrhosis</td>
</tr>
<tr>
<td>Stickel, 2010</td>
<td>1,043/376</td>
<td>German</td>
<td>↑ Risk of ALD and cirrhosis</td>
</tr>
<tr>
<td>Trepo, 2011</td>
<td>658</td>
<td>Belgian</td>
<td>↑ Risk of ALD and cirrhosis</td>
</tr>
<tr>
<td>Trepo 2012</td>
<td>571</td>
<td>Belgian and French</td>
<td>↑ Risk of HCC</td>
</tr>
<tr>
<td>Nischalke 2011</td>
<td>160</td>
<td>Germany</td>
<td>↑ Risk of HCC</td>
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Exome-wide association study identifies a \textit{TM6SF2} variant that confers susceptibility to nonalcoholic fatty liver disease

Julia Kozlitina$^{1,8}$, Eriks Smagris$^{1,8}$, Stefan Stender$^2$, Børge G Nordestgaard$^{3-5}$, Heather H Zhou$^6$, Anne Tybjærg-Hansen$^{2,3,5}$, Thomas F Vogt$^6$, Helen H Hobbs$^{1,7}$ & Jonathan C Cohen$^1$

\textit{TM6SF2} (E167K) – Carriage of minor allele associated with increased steatosis ($p = 5.7 \times 10^{-8}$)

– Carriage of major allele increased cholesterol and cardiovascular disease

See also: Holmen et al, Nature Genetics, 2014
Multivariate analysis (additive model) incorporating Age, Gender, BMI, T2DM and PNPLA3 rs738409 genotype.

i.e. OR per copy of the minor allele carried
Does TM6SF2 genotype determine the CAUSE of death in NAFLD/ALD?
TM6SF2 gene variant determines risk of liver and heart disease
Conclusions

- ALD is a typical “complex” disease with susceptibility likely due to a combination of genetic/environmental factors.
- Several modifiable environmental risk factors such as diet/coffee drinking identified.
- Role of the microbiome as a modifiable and transmissible risk factor worthy of further study.
- PNPLA3 is the only robust genetic association reported thus far and is also associated with HCC.
- Genetic factors may explain CAUSE of death in heavy drinkers.
- Genome wide scans are awaited with interest.