The Genetics of Alcoholism

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Genetics, Genomics and Alcoholism

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Burden of Disease (DALYs)
U.S., Canada, and Western Europe 15-44 years old

- Mental Illness*
- Injuries, including self-inflicted
- Alcohol and drug use
- Malignant neoplasms (cancer)
- Cardiovascular disease
- Respiratory disease
- Musculoskeletal disease
- Sense organ disease
- Digestive disease

Source: WHO World Health Report 2002
Burden of Disease by Specific Illness – DALYs
United States, Canada, and Western Europe
15-44 years old

- Unipolar depression
- Alcohol use
- Road traffic accidents
- Drug use
- Self-inflicted injuries
- Bipolar disorder
- Migraine
- Schizophrenia
- Hearing loss
- COPD

Source: WHO World Health Report 2002
Impact of Research on Heart Disease

- 63% decrease in mortality
- ~ 1 million early deaths averted per year
- $2.6 trillion in economic return
- New, effective treatments and prevention strategies

Courtesy of Tom Insel, M.D.
Environment

Drug

Biology
Genes

Addiction
Brain Changes in Detoxified Alcoholic

Control (male 46 yr)

Orbitofrontal Cortex

Alcoholic (male 50 yr)

Cerebellum
Dopamine D2 Receptors are Lower in Addiction

Cocaine

Alcohol

Heroin

control

addicted

Non-Drug Abuser

Drug Abuser
Effects of Increasing Brain DA D2 Receptors in Alcohol Drinking Behavior

**D2 Receptors**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>% Change in D2R</th>
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<tbody>
<tr>
<td>4</td>
<td>p &lt; 0.0005</td>
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<tr>
<td>6</td>
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<tr>
<td>8</td>
<td>p &lt; 0.005</td>
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<tr>
<td>10</td>
<td>p &lt; 0.10</td>
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**Alcohol Intake**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>% Change in Alcohol Intake</th>
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<tbody>
<tr>
<td>4</td>
<td>p &lt; 0.001</td>
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<tr>
<td>6</td>
<td>p &lt; 0.001</td>
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<tr>
<td>8</td>
<td>p &lt; 0.01</td>
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<tr>
<td>20</td>
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<tr>
<td>24</td>
<td>p &lt; 0.001</td>
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Mol Psychiatry.
Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112,117).

Genes Implicated: ADH1B/ADH1C/ADH5, KLB, GCKR, CADM2,FAM69C, DRD2 & PDE4B.
The Problem – Part 1

Farris et al. 2015
The Problem – Part 2
The Problem – Part 3
Excessive Ethanol Consumption
(Integration – Phenotype and Model)

- Risk
- Individual Variation
- Chronic Exposure

GWAS QTL Selection

Epigenetic & Residual Genetic Variation

Microarrays RNA-Seq Brain Regions

Coding vs Non-Coding RNAs

Biomarkers vs Causal Elements

Gene Networks & Key Hub Nodes
Effect size

High
Intermediate
Modest
Low

Rare alleles causing Mendelian disease
Few examples of high-effect common variants influencing common disease
Low-frequency variants with intermediate effect
Common variants implicated in common disease by GWA

Rare variants of small effect very hard to identify by genetic means

Very rare
Rare
Low frequency
Common

Allele frequency
ETIOLOGY OF ALCOHOL RELATED DISORDERS

- Biological theories – genetic factors
- Psychological theories
- Sociocultural theories
- Psychodynamic theories
Twin Studies: Concordance rates for DSM-III alcohol abuse/alcohol dependence among identical and fraternal twins.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Male Subjects</th>
<th>Female Subjects</th>
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<tr>
<td></td>
<td>Identical</td>
<td>Fraternal</td>
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<tr>
<td>Alcohol abuse and/or alcohol dependence</td>
<td>0.76</td>
<td>0.61</td>
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<tr>
<td>Alcohol dependence</td>
<td>0.59</td>
<td>0.36</td>
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</table>

Behavioral Sensitivity (Schuckit, 1984)

Fig 2.—Mean self-ratings on 0 to 36 scale for drug effect after placebo and after 0.75 mL/kg of ethanol for 23 matched pairs with positive (closed circles) and negative (open circles) family histories. Bars indicate SEs; and B, baseline.
Linkage vs. Association... (ish)

- Linkage analysis
- Association studies

Effect vs. Frequency

Common disease, common variant hypothesis
Table 2. Sib Pair Linkage Analysis of *HTR1B G861C* and *D6S284* to Antisocial Alcoholism in the Finnish Families and in the Southwestern American Indian Tribe*

<table>
<thead>
<tr>
<th></th>
<th><em>HTR1B G861C</em></th>
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<th><em>D6S284</em></th>
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<tr>
<td></td>
<td>No.</td>
<td>Sharing IBD</td>
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<tr>
<td><strong>Finnish Families</strong></td>
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<tr>
<td>Unaffected</td>
<td>220</td>
<td>0.501</td>
<td>198</td>
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<tr>
<td>Discordant</td>
<td>79</td>
<td>0.459</td>
<td>85</td>
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<tr>
<td>Affected</td>
<td>51</td>
<td>0.504</td>
<td>41</td>
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\[ df = 159, ~slope = -0.213, ~P = .04 \]

\[ df = 146, ~slope = -0.137, ~P = .06 \]

<table>
<thead>
<tr>
<th></th>
<th><em>HTR1B G861C</em></th>
<th></th>
<th><em>D6S284</em></th>
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<tr>
<td></td>
<td>No.</td>
<td>Sharing IBD</td>
<td>No.</td>
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<tr>
<td><strong>Southwestern American Indian Tribe</strong></td>
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<tr>
<td>Unaffected</td>
<td>223</td>
<td>0.497</td>
<td>221</td>
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<tr>
<td>Discordant</td>
<td>71</td>
<td>0.421</td>
<td>63</td>
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<tr>
<td>Affected</td>
<td>11</td>
<td>0.603</td>
<td>10</td>
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</table>

\[ df = 180, ~slope = -0.312, ~P = .01 \]

\[ df = 161, ~slope = -0.238, ~P = .01 \]

*Unaffected, discordant, and affected refer to sib pairs in which neither sibling has the trait, only 1 sibling has the trait, and both siblings have the trait, respectively. No. is the number of sib pairs; df is the effective degrees of freedom, corrected for multiplex sibships, for the regression analysis; slope is the slope of the regression line; sharing IBD is the proportion of alleles identical by descent shared by sib pairs; P is the sib pair linkage P value. For each locus or population pair, IBD sharing is substantially less than 50% in the discordant sib pairs, thus suggesting that these pairs were most informative for the regression analysis.
Alcoholism Is A Complex Disease

GWAS suggests many variants of small effect.

Many genes* linked to AD
- **ALDH2** (aldehyde dehydrogenase), **ADH1B, ADH1C, ADH4**,
- **CHRM2, nAChRs A3A5B4**
- **OPRK1, OPRM1** (opioid), **PDYN**
- **5-HTTLPR**
- **NMDAR1,NMDAR2B**
- **GABA-A: α2, β1, β3, γ3**
- **GABA-B**
- **MAO-A, MAO-C,DβH, COMT**
- **DAT (SLC6A3), DRD2, DRD4**
- **GRIK1** (glutamate)

* - listed genes have both positive and negative association findings, and should be carefully interpreted.
**Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112 117).**


Genes Implicated: ADH1B/ADH1C/ADH5, KLB, GCKR, CADM2,FAM69C, **DRD2** & PDE4B.
Transcriptome and Excessive Ethanol Consumption
(Cross Species Integration and Validation)

Selection
- P/NP
- HDID
- HAP/LAP
- HS-CC
- F₂
- HS4

Male vs Female
Inbred Strains
Controlled Exposure
CIE
Target Validation

Rodent (mouse & rat)

Rhesus & Cynomolgus Macaques

Human

Biopsy & Necropsy Samples
NHP Model
MRI (Structural & DTI)
CIE

Human Specific Patterns of Gene Expression
Correlation with fMRI. DMN, Structural and DTI

Human Specific Patterns of Gene Expression
Correlation with fMRI. DMN, Structural and DTI
WGCNA Assumes that the Gene Co-Expression Network is Scale Free

(a) Random network  (b) Scale-free network
Evolutionary Conservation of the Brain Across Species

Despite some known differences between species (e.g. anatomical) brain circuity and functionality is generally well-conserved

adapted from Rakic Nature Reviews Neuroscience 2009; Janak & Tye Nature 2015
Integrated genetic/genomic interaction analysis

Multiple Bioinformatics tools (target prediction databases, WGCNA co-expression analysis, enrichment, e.g.)

Cross-Species Overlapping Networks

Animal Model
Human Alcoholic
Common Networks
The Presidential Election of 1992

George H.W. Bush vs. Bill Clinton vs. Ross Perot
Review

Use of recombinant inbred strains to identify quantitative trait loci in psychopharmacology

Grazyna Gora-Maslak¹, Gerald E. McClearn¹, John C. Crabbe², Tamara J. Phillips², John K. Belknap², and Robert Plomin¹

¹ Center for Developmental and Health Genetics, Pennsylvania State University, University Park, PA 16802, USA
² VA Medical Center and Departments of Medical Psychology and Pharmacology, Oregon Health Sciences University, Portland, OR 97201, USA
Heterogeneous Stock
HS-CC

(C57BL/6J, A/J, 129S1/SvImJ, NOD/LtJ, NZO/HILtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ).

Colville et al. 2016
Comparing the High and Low preference lines identified 224 genes that showed a significant difference in variance (FDR < 0.05).

<table>
<thead>
<tr>
<th>GO Term</th>
<th>Description</th>
<th>P-value</th>
<th>FDR q-value</th>
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<tbody>
<tr>
<td>GO:0007267 cell-cell signaling</td>
<td>9.68E-06</td>
<td>5.22E-02</td>
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<tr>
<td>GO:0009719 response to endogenous stimulus</td>
<td>1.07E-05</td>
<td>3.85E-02</td>
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<tr>
<td>GO:0007165 signal transduction</td>
<td>1.51E-05</td>
<td>4.06E-02</td>
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<tr>
<td>GO:0038023 signaling receptor activity</td>
<td>2.40E-05</td>
<td>5.19E-02</td>
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</table>

Adra1a, Chrna7, Gabrb2, Grin2a, Grin2b, Htr2a & Oprd1
Differential Wiring (DW) is a measure of the change in connectivity between genes. It is defined here as a change in the Pearson correlation of > 0.5 when comparing the High and Low lines. Of the 7546 genes entered into the network analyses, 1971 showed a significant change in edges, ranging from 141 to 2071.

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<th>Description</th>
<th>P-value</th>
<th>FDR q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0098742</td>
<td>cell-cell adhesion via plasma-membrane adhesion molecules</td>
<td>2.09E-07</td>
<td>2.26E-03</td>
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<tr>
<td>GO:0007156</td>
<td>homophilic cell adhesion via plasma membrane adhesion molecules</td>
<td>2.51E-06</td>
<td>1.36E-02</td>
</tr>
<tr>
<td>GO:0044708</td>
<td>single-organism behavior</td>
<td>2.58E-05</td>
<td>9.31E-02</td>
</tr>
<tr>
<td>GO:0007610</td>
<td>behavior</td>
<td>2.83E-05</td>
<td>7.66E-02</td>
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<tr>
<td>GO:0097458</td>
<td>neuron part</td>
<td>3.10E-06</td>
<td>4.42E-03</td>
</tr>
</tbody>
</table>

Includes *Cadm3, Cdh 7,8 &12, Nlgn1* and 19 *Pcdh* genes; total genes in this category (37).

Colville et al. 2017
Regional Effects of Selection for Ethanol Preference from HS-CC Mice. Colville et al. 2018 (under review)

- RNA-Seq data were collected from the CeA, NAcS and PL.
- In all three regions, selection had marked effects on the wiring of genes with the GO of synapse.
- In each region, the annotation was highly significant (CeA – [N=74] – FDR < 2e-9; PL [N=66] – FDR < 6e-14; NAcS [N=77] – FDR < 1e-9).
- Only seven synaptic genes were common to all three regions: Chrna7, Dnm3, Egrf, Psd3, Ppp1r9a & Slc1a2.
- The synaptic genes in all regions were enriched in modules with significant annotations for the cadherins (FDR < e-7 or better).

(van der Vaart et. Al. 2017)
Some of the positively correlated genes (Grm2 and Nf1) have been previously identified as being associated with ethanol consumption (Zhou et al. 2013; Repunte-Cononio et al. 2015).

- For the negatively correlated genes, three related categories (structural constituent of ribosome, ribosome and translation) were significantly enriched at $p < 2 \times 10^{-14}$ (FDR $3 \times 10^{-11}$) or better.

- Genes in these categories were comprised primarily of ribosomal proteins (both mitochondrial and non-mitochondrial) and translation initiation factors.

- For the positively correlated genes there was significant enrichment in several membrane related categories including plasma membrane $p < 2 \times 10^{-14}$ (FDR $3 \times 10^{-11}$). Embedded in this group were a subset of genes associated with neuron projection ($p < 3 \times 10^{-5}$; FDR $4 \times 10^{-2}$).

- The list includes cell adhesion and post-synaptic density genes (Amigo1, Ctma2, Ncam1, Nrcam, and Shank1), glutamate receptors (Grin3a, Grm1 and Grm2), the GABA transporter (Stc6a1) and neurofibromin (Nf1).

Iancu et al. 2017 – Addiction Biology
Specific Aim 2. To analyze in collaboration with INIA-Stress samples from rhesus macaques chronically exposed to ethanol. (All animals will have a biopsy/necropsy sample from area 12/46).
Sustained increased intakes (3 month averages) after abstinence mostly due to heavy drinkers (red symbols).

Average intakes in first 4 weeks following abstinence increased in all monkeys.

Example of daily ethanol intakes prior to and between abstinence (red) and water intake during abstinence (blue).
Alcohol/Withdrawal: Biopsy vs Necropsy

- CAMK2A - calcium/calmodulin-dependent protein kinase II alpha
- DLG2 - discs large homolog 2 (Drosophila)
- GRIK2 - glutamate receptor, ionotropic, kainate 2
- KCNJ10 - potassium inwardly-rectifying channel, subfamily J, member 10
- NTRK2 - neurotrophic tyrosine kinase, receptor, type 2
- SHANK1 - sh3 and multiple ankyrin repeat domains 1
Rhesus: Cohort 10

Ethanol intake - 84 sessions

Sustained increased intakes (3 month averages) after abstinence mostly due to heavy drinkers (red symbols)

Average intakes in first 4 weeks following abstinence increased in all monkeys

Example of daily ethanol intakes prior to and between abstinence (red) and water intake during abstinence (blue)
Alcohol/Withdrawal: Biopsy vs Necropsy

- CAMK2A - calcium/calmodulin-dependent protein kinase II alpha
- DLG2 - discs, large homolog 2 (Drosophila)
- GRK2 - G-protein coupled receptor kinase 2
- KCNJ10 - potassium inwardly-rectifying channel, subfamily J, member 10
- NTRK2 - neurotrophic tyrosine kinase, receptor, type 2
- SHANK1 - shank and multiple ankyrin repeat domains 1
Effect of Chronic Ethanol Exposure in Cynomolgus Macaques

• The significantly affected genes were divided into those that were down- and up-regulated. Among the down-regulated genes (N=129 - lower in the necropsy sample) there was an enrichment in the GO annotation for inflammatory response (FDR $– 2 \times 10^{-8}$). Genes in this category included CCL2, CCL4, CCL8, IL1B, S100A8, STAB1 and TNF. Among the up-regulated genes (N=165) there was an enrichment in GO annotation for intrinsic to plasma membrane (FDR $– 2 \times 10^{-3}$). Genes in this category included DRD5, GABRG2, GLRB, GRIN2A, GRIN2B, HTR2A, SNAP25 and SYT1.

CCL2 – Chemokine (C-C motif) ligand 2; Monocyte chemoattractant protein 1.
Effect of Chronic Ethanol Exposure in Cynomolgus Macaques on Regional Gene Expression: CeA, NAcC & NAcS.

- Cohort 9: DE for CeA (N=45), CeA (N=86) & NAcS (N= 1601) [FDR < 0.05]; no overlap among regions.
- Data in shell grouped as to those down-(N=705) and up-regulated (N=896) by ethanol exposure.
- The down-regulated genes showed an enrichment in immune system process (FDR < 1e-6); structural constituent of ribosome (FDR < 2e-4) and extracellular exosome (FDR < 2e-13).
- The up-regulated genes showed an enrichment in genes associated with synaptic signaling (FDR < 1e-11); ion-gated channel activity (FDR < 8 e-12) and synapse part (FDR < 1e-17).
- Signaling genes include \textit{Cdh8, Pcdhb12, Pcdhb2 & Pcdhb9}. Opiate genes are also well represented: \textit{Oprk1, Oprm1, Pdyn} & \textit{Penk}.
Effect of Chronic Ethanol Exposure in Cynomolgus Macaques on Regional Gene Expression: Cont’d.

### Synaptic Signaling

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<tr>
<th>Protein1</th>
<th>Protein2</th>
<th>Protein3</th>
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<tbody>
<tr>
<td>AKAP5</td>
<td>GPR88</td>
<td>PDE7B</td>
</tr>
<tr>
<td>AKAP9</td>
<td>GRIA1</td>
<td>PDYN</td>
</tr>
<tr>
<td>CACNA1E</td>
<td>GRIA2</td>
<td>PENK</td>
</tr>
<tr>
<td>CACNB2</td>
<td>GRN2A</td>
<td>RASD2</td>
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### Ion-Gated Channel Activity

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### Synapse Part

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<th>Protein4</th>
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### PPI Hub Proteins

- CALM3, GRIN1, GRIN2B
- PRKACA, DLG4
- GRIN1, DLG4, GRIN2B, YWHAB

PPI Hub Proteins – FDR < 10^{-8} or better
Alcohol dependence is a heterogeneous psychiatric disorder characterized by high genetic heritability and neuroadaptations occurring from repeated drug exposure. Through an integrated systems approach we observed consistent differences in transcriptome organization within postmortem human brain tissue associated with the lifetime consumption of alcohol. Molecular networks, determined using high-throughput RNA sequencing, for drinking behavior were dominated by neurophysiological targets and signaling mechanisms of alcohol. The systematic structure of gene sets demonstrates a novel alliance of multiple ion channels, and related processes, underlying lifetime alcohol consumption. Coordinate expression of these transcripts was enriched for genome-wide association signals in alcohol dependence and a meta-analysis of alcohol self-administration in mice. Further dissection of genes within alcohol consumption networks revealed the potential interaction of alternatively spliced transcripts. For example, expression of a human-specific isoform of the voltage-gated sodium channel subunit SCN4B was significantly correlated to lifetime alcohol consumption. Overall, our work demonstrates novel convergent evidence for biological networks related to excessive alcohol consumption, which may prove fundamentally important in the development of pharmacotherapies for alcohol dependence.
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Acute pharmacology

Chronic exposure (neuroadaptation)

Forward and reverse genetics

Functional/biophysical analysis

Pharmacological, biochemical, molecular biological analyses

Transgenics, knockouts, QTL analysis, etc.

Direct alcohol targets

Alcohol-associated proteins

Preclinical drug screening

Pharmacotherapeutic target candidates