Functional alleles & intermediate phenotypes in alcoholism and dyscontrol disorders

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The heritability of addictive disorders

\[ h^2 \pm \text{range} \]

Nature Genetics Reviews, 2005
Alcoholism and other addictions: The intermediate phenotypes

Frontal cortical function/behavioral inhibition
Drug metabolism and response/tolerance
Reward
Anxiety-dysphoria/stress response
Obsession/Craving

Electrophysiology
Imaging: brain structure and function
Neuropsychology
Metabolomics
Gene expression
Gene, stress, & substances in dyscontrol

**MAOA** rare & common alleles: GxE, fMRI

**COMT Val158Met**: Roles in cognition & resiliency

**HTTLPR**: GxE for depression and suicidality
Genes with alleles proven to modulate human behavior

Rare

*MAOA*
*HTT*
*HPRT*
*ERBA*

Deterministic

Brunner Syndrome
OCD
Lesch-Nyhan Syndrome
ADHD
Dyscontrol
Anxiety, OCD
Cognition, anxiety
Episodic memory
Alcoholism
Alcoholism

Common

*MAOA*
*HTT*
COMT
BDNF
ALDH2
ADH1B

Probabilistic

*Regulatory
For risk genes, odds ratios are larger for Intermediate Phenotypes than for Diseases (Wellcome Trust medians)
Brunner syndrome: X-linked dyscontrol due to the MAOA C936T stop-codon

Borderline mental retardation

Dyscontrol behaviors:
- Aggressive outbursts
- Arson
- Attempted rape
- Exhibitionism

No fibroblast MAOA activity

Abnormal monoamine metabolism:
- ↓ urinary HIAA, HVA, VMA
- ↑ urinary normetanephrine & tyramine

Brunner et al.,
Science, 1993
Expanding the stress connection to behavioral dyscontrol: Predisposition, early exposure, and substance abuse
Child sexual abuse and psychiatric disorders in females

- ASPD 2.9 [1.4-6.0]
- Alcoholism [Abuse +Dep] 2.1 [1.2-3.6]
- Substance abuse 4.2 [2.2-7.8]
- Affective disorder 2.3 [1.3-4.0]
- Anxiety disorder 1.8 [1.0-3.1]
- PTSD 5.3 [2.2-12.7]

Robin et al, Amer J. Psychiatry, 1997
Addictions: A cause and effect of stress/trauma and dyscontrol

- Key factor in accidents, violence and sexual trauma
- A consequence of trauma
- Consequences of underage drinking
- A cause of allostatic changes
- Genes mediate liability
Alcoholics Tend to Be Anxious

Ducci et al, 2007
Allostasis and Addiction

G. Koob, B. McEwen
A functional promoter polymorphism (*MAOA-LPR*) predicts MAOA expression.

MAOA promoter repeats:

- **3**
- **3.5**
- **4**
- **5**

**ACCGGCACCGGCACCGACCGGCACCGACCGAC**

Bar graphs showing luciferase activity in JAR, SY5Y, and SK-N-SH cells with different MAOA promoter repeat lengths (3, 3.5, 4, 5) and vector controls.
GxE interaction of *MAOA-LPR* and childhood maltreatment on antisocial behavior, in males

GxE interaction of \textit{MAOA-LPR} & childhood sexual abuse for ASPD & alcoholism

Ducci et al, Molecular Psychiatry, 2007
Non-additive interaction of **MAOA-LPR** and testosterone predicts antisocial behavior

**Low activity MAOA-LPR**

- $\beta_a \ (SE) = 3.49 \ (1.01); \ p=0.001$

**High activity MAOA-LPR**

- $\beta_a \ (SE) = -0.94 \ (1.04); \ p=0.37$

Sjoberg et al., *Neuropsychopharmacology* 2007
MAOA-LPR predicts differential fMRI activations to angry and fearful faces in limbic and paralimbic regions ($n = 142$)

Andreas Meyer-Lindenberg (2006) PNAS 103, 6269-6274
MAOA-LPR predicts fMRI limbic activations during retrieval of aversive memories (n = 90)

Andreas Meyer-Lindenberg (2006) PNAS 103, 6269-6274
Monoamine Oxidase A Gene Promoter Variation and Rearing Experience Influences Aggressive Behavior in Rhesus Monkeys

Timothy K. Newman, Yana V. Syagailo, Christina S. Barr, Jens R. Wendland, Maribeth Champoux, Markus Graessle, Stephen J. Suomi, J. Dee Higley, and Klaus-Peter Lesch

BIOL PSYCHIATRY 2005;57:167–172
© 2005 Society of Biological Psychiatry

\[ \text{rhMAOA-LPR} \]
COMT Val158Met: Apparent counterbalancing effects in cognition and stress/anxiety
COMT Val158Met and WCST

Perseverative Errors (t-scores)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Siblings</th>
<th>Patients</th>
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<tbody>
<tr>
<td>Val/Val</td>
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<tr>
<td>Val/Met</td>
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<tr>
<td>Met/Met</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

N = 449
F = 6.00
p = .003

Egan et al, PNAS, 2001
Fear of Uncertainty (HA2) and COMT Val158Met in females from two populations

Enoch et al, Psychiatric Genetics, 2003

Plains Indian
[N = 148, F=3.5, p=0.03, 2 df]

U.S. Caucasian
[N = 75, F=3.5, p=0.09, 2 df]
$^{11}\text{C}$-Carfentanil binding in brain

Source: Jon-Kar Zubieta
COMT Met158Val and μ-opioid system activation in response to sustained pain

HTTLPR: Still psychiatric genetics’ most popular locus

S allele

14 repeats

1200 nt

L allele

16 repeats

A>G

CCCCCTGCTGCACCCCCCGCAT

Hu et al, AJHG, 2006

HTT

AP2
GxE: Interaction of HTTLPR and stress in depression

Caspi et al, Science 2003
Gene x Environment (HTT x Childhood stress) predicts suicide attempts in abstinent, African American, Substance Dependent patients (N=306)

Roy et al, In press
Anxiety, Resiliency, Functional Allele to Complex Behavior

Environmental Factors

GCH1

BDNF Val66Met

HTT HTTLPR

MAOA

GABRA2

COMT Val158Met

Episodic Memory

Anxiety, Resiliency

Executive Cognition

OCD

Alcoholism

Pain

Schizophrenia

Marker phenotype

Trauma
Thanks!

Mary-Anne Enoch
Zhifeng Zhou
Ke Xu
Xianzhang Hu
Francesca Ducci
Robert Lipsky
Peihong Shen
Qiaoping Yuan
Colin Hodgkinson

Ahmad Hariri
Deborah Mash
Rajita Sinha
Jon-Kar Zubieta
Mary Heitzig
David Scott

Rob Robin
Bernard Albaugh
Alec Roy
Genetic Complexity

Polygenicity: Multiple genetic variants confer risk in combination.

Heterogeneity: Multiple genetic variants confer risk in different individuals.
Genetic complexity in affected populations

Polygenicity

Heterogeneity
Genetic complexity and twin concordance

Polygenicity

MZ

DZ

Heterogeneity

MZ

DZ

Affected

Unaffected
Lack of evidence for polygenic inheritance of addictions

Concordance Ratios

- Cocaine: MZ/DZ = 3.72
- Sedatives: MZ/DZ = 2.71
- Stimulants: MZ/DZ = 2.69
- Caffeine: MZ/DZ = 2.38
- Hallucinogens: MZ/DZ = 2.23
- Opiates: MZ/DZ = 2.19
- Gambling: MZ/DZ = 1.96
- Smoking: MZ/DZ = 1.84
- Alcohol: MZ/DZ = 1.73
- Cannabis: MZ/DZ = 1.52

Nature Genetics Reviews, 2005
Pain/Stress Challenge:
Hypertonic saline infusion to masseter muscle
COMT yin/yang haplotypes in five populations & Linkage to *Opioid addiction* & *Alcoholism*

<table>
<thead>
<tr>
<th>Case/Control</th>
<th>Population</th>
<th>p value</th>
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<td>477/361</td>
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<td>178/283</td>
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<td>175/175</td>
<td>Plains Indian</td>
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</table>
COMT Val158Met and Addiction

- Polysubstance abuse: Val158
  - Vandenburgh, Uhl and colleagues

- Late onset alcoholism: Met158
  - [Hallikainen et al, 2000] 62 early onset, 132 late onset, 267 controls. Odds ratio of 3 for late onset, p=0.017
  - [Tiihonen et al, 1999] 67 & 56 late onset, 3140 blood donors, 267 matched controls. Met/Met vs Val/Val Odds ratio 2.5, p =0.006, Attributable risk for Met/Met vs Val/Val 13.3%
COMT Val158Met

Val158

Behavioral Dyscontrol

Met158

High anxiety, Stress reactive

Alcoholism and other substance abuses
Replication of COMT in experimental pain in 202 females prospectively followed for TMJ

(Diatchenko et al, Hum Mol Genetics, 2005)

ANOVA: p=0.18

rs4818

p<0.01

rs6269

p<0.01
Chromosome 4

Long et al, 1998
GABRA2 LD and Alcoholism Linkages

Edenberg et al
Kranzler et al
Enoch et al

Same alleles,
Same haplotype

Haplotype Tagging

1121121
2212212
2212211
Addictions Array for 130 Candidate Genes

- 1536 SNPs
- Tagging of haplotypes > 0.6% in frequency
- Avg of >11 SNPs/gene, Range 4 - 35
- 186 “perfect” genomic control SNPs (AIMs)
  - Balanced set with cross-population $\Delta > 0.7$, and >10x
- $ < 0.05$/genotype
- 25,000 individuals genotyped (Yale, Rockefeller, Wash U, Columbia [2], Univ. Colorado, Emory, VCU, NICHD)
Assignment of ancestry with 186 Ancestry-informative SNPs (Structure2, Four-factor solution)

<table>
<thead>
<tr>
<th></th>
<th>Finns</th>
<th>Plains Indians</th>
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<th>African American</th>
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<tr>
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</tr>
</tbody>
</table>
Ethnic factor scores of 1051 individuals in 52 CEPH population with 186 AIMs
7-factor solution, Structure 2

Hodgkinson et al, unpublished
Repeats in the 5 Kb region upstream of 5-HTT in Macaca mulatta and Homo sapiens

20-22 bp Imperfect repeats

Sequence Identity
rh-HTTLPR has GxE effects on alcohol preference & stress response

Interaction Between Serotonin Transporter Gene Variation and Rearing Condition in Alcohol Preference and Consumption in Female Primates
Christina S. Barr, VMD, PhD; Timothy K. Newman, PhD; Stephen Lindell, BA; Courtney Shannon, BA; Maribeth Champoux, PhD; Klaus Peter Lesch, MD; Stephen J. Suomi, PhD; David Goldman, MD; J. Dee Higley, PhD

Rearing Condition and rh5-HTTLPR Interact to Influence Limbic-Hypothalamic-Pituitary-Adrenal Axis Response to Stress in Infant Macaques
Christina S. Barr, Timothy K. Newman, Courtney Shannon, Clarissa Parker, Rachel L. Dvoskin, Michelle L. Becker, Melanie Schwandt, Maribeth Champoux, Klaus Peter Lesch, David Goldman, Stephen J. Suomi, and J. Dee Higley

Biol Psych 55: 733, 2004
Arch Gen Psych 61: 1146, 2004
Serotonin Transporter Genetic Variation and the Response of the Human Amygdala

Science 2002 July 19; 297(5580):400-3

Ahmad R. Hariri,¹ Venkata S. Mattay,¹ Alessandro Tessitore,¹ Bhaskar Kolachana,¹ Francesco Fera,¹ David Goldman,² Michael F. Egan,¹ Daniel R. Weinberger¹

¹ Clinical Brain Disorders Branch, NIMH, NIH.
² Laboratory of Neurogenetics, NIAAA, NIH.
5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression

Lukas Pezawas, Andreas Meyer-Lindenberg, Emily M Drabant, Beth A Verchinski, Karen E Munoz, Bhaskar S Kolachana, Michael F Egan, Venkata S Mattay, Ahmad R Hariri & Daniel R Weinberger

Statistical functional connectivity maps between bilateral amygdala and perigenual anterior cingulate cortex
A common, functional NPY haplotype influencing anxiety and stress response
(Zhifeng Zhou et al, submitted)

• The common haplotype predicts reduced brain and lymphoblast mRNA levels and plasma NPY
• The reduction of function haplotype predicts:
  – Trait anxiety
  – Reduced amygdala emotional fMRI activation
  – Reduced amygdala pain/stress induced opioid release
• A functional promoter locus was identified via \textit{in vitro} reporter constructs
A functional human GCH1 haplotype predicts post-diskectomy clinical pain and experimental pain.

Tegeder et al, Nature Medicine, 2006

162 post-diskectomy patients

547 normal controls
GCH1 mRNA and protein in rat DRG are upregulated by nerve injury

Tegeder et al, Nature Medicine, 2006
Biopterin synthesis in rat DRG is upregulated by nerve injury and blocked by a GCH1 inhibitor
Rapid inhibition of pain and DRG neuronal activation by the GTP cyclohydrolase inhibitor, 2,4-diamino-6-hydroxypyrimidine (DAHP)

Tegeder et al, Nature Medicine, 2006
Genotype-predicted NPY expression predicts pain/stress induced opioid activation
Zhou et al, submitted
Genotype-predicted NPY expression predicts emotion-induced fMRI activation

Zhou et al, submitted
Genotype-predicted NPY expression predicts anxiety
Zhou et al, submitted
Functional Allele to Complex Behavior

- **GCH1**
- **BDNF Val66Met**
- **HTT**
- **HTTLPR**
- **NPY**
- **GABRA2**
- **COMT Val158Met**

- **Episodic Memory**
- **Anxiety, Resiliency**
- **Executive Cognition**
- **OCD**
- **Alcoholism**
- **Pain**
- **Schizophrenia**
- **Marker phenotype**

Environmental Factors

- **Trauma**
HTTLPR and anxiety

[Sen, Burmeister and Ghosh, 2004]

- 26 Studies, 5,629 subjects
- $p = 0.087$
- Substantial effect of inventory and inter-study heterogeneity
- $p < 0.000016$, NEO, corrected for heterogeneity
- 0.1 SD increment in TPQ Harm avoidance or NEO Neuroticism per ”s” allele
Triallelic Functionality at HTTLPR

- S and $L_G$ are equivalent in expression in lymphoblasts and raphe-derived neurons
- AP2 transcription factor binds to $L_G$ and acts as a repressor of transcription
  - Gel-shift and supershift assays
  - Allele-specific, AP2-specific decoy DNA eliminates the $L_A:L_G$ difference

Hu et al, AJHG, 2006
HTT

Ile425Val

Ozaki et al, Mol Psych, 2003
Replication of HTTLPR-OCD linkage in Parent/child trios
Collaboration with James Kennedy, Clarke Centre, Toronto

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>L(_G)</th>
<th>L(_A)</th>
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<tbody>
<tr>
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<td>11</td>
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<tr>
<td>Untransmitted</td>
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<td>41</td>
<td>20</td>
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Triallelic: \(p = 0.023\)
Low/High: \(p = 0.010\)

Hu et al, AJHG, 2006
## HTTLPR Genotype and Allele Frequencies in 169 OCD Patients and 253 Controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>OCD</th>
<th>Control</th>
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<tr>
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<tr>
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<tr>
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</table>

\[\chi^2 = 19.4\]  
\[p = 0.001\]

\[\chi^2 = 6.6\]  
\[p = 0.036\]
Non-additive interaction of *MAOA-LPR* and testosterone predicts antisocial behavior

\[
\beta_a (SE) = 3.49 (1.01); \ p = 0.001
\]

\[
\beta_a (SE) = -0.94 (1.04); \ p = 0.37
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Sjoberg et al., *Neuropsychopharmacology* 2007