Addiction Treatments of the Future:
The Role of Genetics

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### Prevalence of Specific Drug Abuse and Vulnerability to Develop Addictions

<table>
<thead>
<tr>
<th>Drug Use</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol Use – ever</td>
<td>~ 203 million</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>~ 18.8 million</td>
</tr>
<tr>
<td>Cocaine Use – ever</td>
<td>~ 35 million</td>
</tr>
<tr>
<td>Cocaine Addiction</td>
<td>~ 2 to 3 million</td>
</tr>
<tr>
<td>Heroin Use – ever</td>
<td>~ 3.7 million</td>
</tr>
<tr>
<td>Heroin Addiction</td>
<td>~ 1 million</td>
</tr>
<tr>
<td>Illicit Use of Opiate Pain Medication – ever</td>
<td>~ 33.4 million</td>
</tr>
<tr>
<td>Addiction to Illicit Use of Opiate Pain Medications</td>
<td>~ 1.6 million</td>
</tr>
</tbody>
</table>

#### Development of Addiction After Self Exposure

<table>
<thead>
<tr>
<th>Addiction</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholism</td>
<td>~ 1 in 8 to 1 in 15</td>
</tr>
<tr>
<td>Cocaine Addiction</td>
<td>~ 1 in 8 to 1 in 15</td>
</tr>
<tr>
<td>Heroin Addiction</td>
<td>~ 1 in 3 to 1 in 5</td>
</tr>
</tbody>
</table>

*SAMHSA National Survey on Drug Use and Health, 2005 and 2006; NIDA, Others, 1998-2006*
Development of Methadone Maintenance Treatment – 1964 Onward

Hypothesis (1964)

Heroin (opiate) addiction is a disease – a “metabolic disease” – of the brain with resultant behaviors of “drug hunger” and drug self-administration, despite negative consequences to self and others. Heroin addiction is not simply a criminal behavior or due alone to antisocial personality or some other personality disorder.

1964: Initial clinical research on development of treatment using methadone maintenance pharmacotherapy and on elucidating mechanisms of efficacy.

Dole, Nyswander and Kreek, 1966, 2008
Number of patients currently in treatment:

- USA: ~250,000
- Europe: ~500,000
- Rest of world: ~200,000

Efficacy in “good” methadone treatment programs using adequate doses (80 to 150mg/d):

- Voluntary retention in treatment (1 year or more): 50 – 80%
- Continuing use of illicit heroin: 5 – 20%

Actions of methadone treatment:

- Prevents withdrawal symptoms and “drug hunger”
- Blocks euphoric effects of short-acting narcotics
- Allows normalization of disrupted physiology

Mechanism of action: Long-acting narcotic provides steady levels of opioid at specific receptor sites.

- Methadone found to be a full mu opioid receptor agonist which internalizes like endorphins
- Methadone also has modest NMDA receptor complex antagonism

Kreek, 1972; 1973; 2008
Prevalence of HIV-1 Infection in Intravenous Drug Users
New York City: 1983 - 1984

Protective Effect of Methadone Maintenance Treatment

Percent of IV Drug Users Infected with HIV-1

50% – 60% Untreated, street heroin addicts: positive for HIV-1 antibody

9% Methadone maintained since <1978 (beginning of AIDS epidemic): less than 10% positive for HIV-1 antibody

Kreek with Des Jarlais and others, 1984
Natural History of Drug Abuse and Addictions

Initial Use of Drug of Abuse

Sporadic Intermittent Use

Regular Use

Addiction

Early Withdrawal (abstinence)

Protracted Abstinence

> 80% without pharmacotherapy relapse to addiction

< 20% sustain abstinence with no specific medications

Kreek et al., Nature Reviews Drug Discovery, 1:710, 2002
Few Targeted Pharmacotherapies Available for Specific Addictive Diseases

I. Opiate Addiction (Heroin and Illicit Use of Opiate Medications)
   a. METHADONE (50-80%)**
   b. BUPRENORPHINE (+ NALOXONE) (40-50%)*
   [c. NALTREXONE ( <15%)]

II. Nicotine Addiction (Primarily Tobacco Smoking)
   a. NICOTINE – DIVERSE DELIVERY SYSTEMS (?)
   b. BUPROPRION (?)
   c. VARENICLINE (?)

III. Alcoholism
   a. NALTREXONE (30-40%)*
   b. ACAMPROSATE (?)

IV. Cocaine, Amphetamines and Other Stimulants
   NONE

(%) is % of unselected persons with specific addictions who can be retained voluntarily in treatment for 3 months (*) or 12 months (**) with moderate to high success in eliminating specific drug use.

Kreek, 2008
Factors Contributing to Vulnerability To Develop a Specific Addiction

Use of the drug of abuse essential (100%)

Genetic (25-60%)
- DNA
- SNPs
- other polymorphisms

Drug-Induced Effects (very high)
- mRNA levels
- peptides
- proteomics

Environmental (very high)
- prenatal
- postnatal
- contemporary
- cues
- comorbidity
- stress-responsivity

Kreek et al., 2000; Kreek et al., *Nature Reviews Drug Discovery*, 2005
Development of an Addiction

• Drugs alter normal brain networks and chemicals

• “Rewarding” or “pleasurable” effects of drugs (the so-called “reinforcing effects”) involve:
  – Dopamine
  – Endorphins (acting at Mu Opioid Receptors)

• “Countermodulatory” response to reward involves:
  – Dynorphins (acting at Kappa Opioid Receptors)
REWARD – Basal and Cocaine-Induced Increases in Extracellular Dopamine Levels Become Attenuated After Chronic “Binge” Pattern Cocaine Administration: Microdialysis (Nucleus Accumbens) Study in C57BL/6 Mice

Zhang et al., Synapse, 50:191, 2003
Endogenous Opioids ("Endorphins" – 3 classes) and their Opioid Receptors (3 types)

LaForge, Yuferov and Kreek, 2000
REWARD — Mu Opioid Receptor-Endorphin System: Chronic Cocaine in Rat Increases Mu Opioid Receptor Density, But With No Increase in Mu Endorphins

Unterwald et al., Brain Res., 584:314 1992

Relative “endorphin deficiency” develops and persists for an extended time.
COUNTERMODULATION – Chronic Cocaine Increases Kappa Opioid Receptor Density in Rat, But Kappa Opioid Receptor Directed “Dynorphins” Also Increase

Dynorphin Acting at the Kappa Opioid Receptor Lowers Dopamine Levels and Prevents Surge After Cocaine

Atypical responsivity to stress and stressors may, in part, contribute to the persistence of, and relapse to, self-administration of drugs of abuse and addictions.

Such atypical stress responsivity in some individuals may exist prior to use of addictive drugs on a genetic or acquired basis, and lead to the acquisition of drug addiction.
Hypothalamic Pituitary Adrenal (HPA) Axis

- Endogenous Opioids (mu)
- CRF
- β-Endorphin
- ACTH
- Adrenal
- Cortisol
- Kidney
Heroin, Cocaine, and Alcohol Profoundly Alter Stress Responsive Hypothalamic-Pituitary-Adrenal (HPA) Axis: Normalization during methadone treatment

- Acute effects of opiates
- Chronic effects of short-acting opiates (e.g., heroin addiction)
- Opiate withdrawal effects *
- Opioid antagonist effects
- Cocaine effects *
- Alcohol effects
- Chronic effects of long-acting opiate (e.g. methadone in maintenance treatment)

* Our challenge studies have shown that a relative and functional “endorphin deficiency” develops.

Kreek, 1972; 1973; 1987; 1992 ... 2008
“On / Off” versus “Steady-State”

Disruption *versus* Normalization

- levels of gene expression and gene products (peptides)
- receptor mediated events
- physiology
- behaviors

Kreek, 1987; 2001
Normalization of Heroin Disruption Physiology During Methadone maintenance treatment: PET studies of mu opioid receptors in human brain regions

Kling et al., 2000

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Specific Binding (ml plasma/ml tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThI</td>
<td>Normal volunteers n=14</td>
</tr>
<tr>
<td>Amy</td>
<td>MMTP volunteers n=14</td>
</tr>
<tr>
<td>Caud</td>
<td>(~20-30 percent reduction in mu-opioid receptor binding)</td>
</tr>
<tr>
<td>Ins</td>
<td></td>
</tr>
<tr>
<td>ACg</td>
<td></td>
</tr>
<tr>
<td>Put</td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td></td>
</tr>
<tr>
<td>MFr</td>
<td></td>
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<tr>
<td>Par</td>
<td></td>
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<tr>
<td>Crb</td>
<td></td>
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<tr>
<td>IT</td>
<td></td>
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<tr>
<td>Hip</td>
<td></td>
</tr>
<tr>
<td>WMt</td>
<td></td>
</tr>
</tbody>
</table>

Area related to pain response
Area with dopamine terminals involved in “reward”, mood, and decision-making
Area with dopamine terminals involved in memory, learning, and movement

Kling et al., 2000
Methadone Maintenance Treatment Allows Normalization of Endogenous Opioid-Related Physiological Functions Disrupted During Chronic Heroin Use

Neuroendocrine Function
- **Hypothalamic-Pituitary-Adrenal Axis** – Stress Responsivity: levels and circadian rhythm of release of POMC peptides ($\beta$ Endorphin; ACTH and cortisol)
- **Hypothalamic-Pituitary-Gonadal Axis** – Reproductive Biology: levels and pulsatile release of LH and testosterone levels

Immune Function
- **Natural Killer Cell Activity**
- **Absolute Numbers of Cells:** T cells; T cell subset levels; B cells; NK cells
- **Immunoglobulin Levels (M and G)**

Genetic vulnerability to develop an addiction once self-exposed probably due to:

- Multiple variants (different types) and of
- Multiple genes (as with any complex disorder, e.g., hypertension, diabetes)
- Probably shared and unique variants for each specific addiction
- Genetic contributions of comorbid conditions and personality types may play a role

Kreek, 2008
Basic Principles: DNA

- Genetic information is encoded in long, threadlike molecules called deoxyribonucleic acid (DNA).

- During fertilization, DNA comes from egg and sperm; developing offspring inherits genetic material from each parent. Thus, DNA transmits genetic information from one generation to the next.

- The entire sequence of DNA molecules in an organism is called its genome.

Hassin and Kreek, 2004
The Human Genome (as currently understood)

- In the human genome, there are ~3 billion bases (nucleotides)
- In humans, there are estimated to be ~25,000-35,000 genes
- Each gene is a sequence of bases or nucleotides

Hassin and Kreek, 2004
Single Nucleotide Polymorphisms (SNPs) in Genes: Definitions

• SNP — a single nucleotide polymorphism, that is, a gene variant involving one nucleotide or base of any base pair

• Allelic Frequency:
  <1% low or rare
  1–5% intermediate
  >5% high, frequent

Hassin and Kreek, 2004
SNPs and Other Polymorphisms (i.e., allelic variants of genes):

• usually neither “good” nor “bad”

• may (or may not) have any functional significance (e.g., yield different peptides and proteins; alter levels of gene expression)

• may (or may not) contribute to altered response to therapeutic agents, i.e., medications, “pharmacogenetics” and “pharmacogenomics”

• may (or may not) contribute to altered response to endogenous peptides (e.g., hormones, enzymes)—“physiogenetics” and “physiogenomics”
Role of Mu Opioid Receptor and Related Endorphin Systems in Normal Physiological Functions*

- Neuroendocrine Functions
  - Stress responsive systems including hypothalamic-pituitary-adrenal axis
  - Reproductive function including hypothalamic-pituitary-gonadal axis
- Response to Pain
- Immunological Function
- Gastrointestinal Function
- Cardiovascular Function
- Pulmonary Function
- Mood, Affect; Cognition

* All disrupted by chronic abuse of the short acting opiate, heroin

Kreek, 2000
Mu Opioid Receptor Knock-Out Mice

- No morphine or other mu agonist analgesia
- No heroin or morphine self-administration
- No heroin or morphine induced conditioned place preference
- Attenuated self-administration of cocaine
- Attenuated self-administration of alcohol

Reviewed in Kreek et al., Nature Reviews Drug Discovery, 1:710-726, 2002

[Different knock-out constructs and multiple research groups, including Kieffer, Uhl, Yu. Pintar, Loh, with, e.g., Maldonado, Pasternak, Hoellt, Roberts]
Genetic Variants of the Mu Opioid Receptor: Single Nucleotide Polymorphisms in the Coding Region Including the Functional A118G (N40D) Variant

HYPOTHESIS

Gene variants:

• Alter physiology
  “PHYSIOGENETICS”

• Alter response to medications
  “PHARMACOGENETICS”

• Are associated with specific addictions

Bond, LaForge... Kreek, Yu, PNAS, 95:9608, 1998
Binding and Coupling to G Protein-Activated, Inwardly Rectifying $K^+(GIRK)$ Channels by Endogenous Opioid Peptides to the Prototype and A118G Variant Mu Opioid Receptor

\[ \text{Percent Bound} \]

\[ \text{Log } [\text{Endomorphin -1(M)}] \]

\[ \text{Log } [\beta \text{ Endorphin (M)}] \]

\[ \text{Fraction Maximum Current Response} \]

\[ \text{Log } [\text{Endomorphin -1(M)}] \]

\[ \text{Log } [\beta \text{ Endorphin (M)}] \]

_Bond, Laforge et al., PNAS, 1998_
Association Between a Functional Polymorphism in the mu Opioid Receptor Gene and Opiate Addiction in Central Sweden

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All Subjects</th>
<th>Swedish with Both Parents Swedish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n=170)</td>
<td>Opiate Dependent (n=139)</td>
</tr>
<tr>
<td>A/A</td>
<td>147 (0.865)</td>
<td>98 (0.705)</td>
</tr>
<tr>
<td>A/G</td>
<td>21 (0.123)</td>
<td>39 (0.281)</td>
</tr>
<tr>
<td>G/G</td>
<td>2 (0.012)</td>
<td>2 (0.014)</td>
</tr>
</tbody>
</table>

RR = 2.86  \chi^2 (1) = 13.403  P = 0.00025*  RR = 2.97  \chi^2 (1) = 8.740  P = 0.0031*

Thus, in the entire study group in this central Swedish population, Attributable Risk due to genotypes with a G allele in this population: 18%

Attributable Risk due to genotypes with a G allele in Swedes w/ Swedish parents: 21% (with confidence interval ranges from 8.0 to 28.0%)

ACTH and Cortisol Levels 6 Hours After Administration of Naltrexone or Placebo: Effects of Alcohol Consumption with “Priming Drink” and Up to 4 Drinks in Each of Two 2 Hour Consecutive Sessions

- Naltrexone, n = 7 (1.9 +/- 0.72 drinks/2h choice)
- Placebo, n = 9 (4.6 +/- 0.85 drinks/2h choice)

O’Malley et al, Psychopharmacology, 2002
Alcohol Urge Questionnaire (AUQ) 6 Hours After Oral Naltrexone or Placebo During Two 2 Hour Consecutive Drinking Choice Sessions

O’Malley et al, Psychopharmacology, 2002
“Physiogenetics” and “Pharmacogenetics” Related to A118G Variant of Human Mu Opioid Receptor Gene – Altered Stress Responsivity

Basal plasma levels of cortisol significantly higher in persons with the A118G variant.  
*Bart et al., 2006*

Serum Cortisol (ug/dl)

- **P** = Placebo
- **N** = Naloxone
- **A/A** (n=29)
- **A/G** (n=7)

Cumulative Survival (Time to Relapse)

- Naltrexone/ A/G, G/G (n=23)
- Naltrexone/ A/A (n=48)
- Placebo/ A/G, G/G (n=18)
- Placebo/ A/A (n=41)

*N = Naloxone  
P = Placebo*

*Wand et al., 2002*

*Osling et al., 2003*
Association Between a Functional Polymorphism in the mu Opioid Receptor Gene and Alcoholism in Central Sweden

<table>
<thead>
<tr>
<th></th>
<th>Swedish with two Swedish parents</th>
<th>Non-Swedish without Swedish Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol Dependent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=193)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A118</td>
<td>158</td>
<td>104</td>
</tr>
<tr>
<td>A118G, G118G</td>
<td>35</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=120)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A118</td>
<td>90</td>
<td>141</td>
</tr>
<tr>
<td>A118G, G118G</td>
<td>16</td>
<td>7</td>
</tr>
</tbody>
</table>

\[ OR=1.92 \quad \chi^2_{(1)} = 7.18, p = 0.0074 \]

Thus, in the entire study group in this central Swedish population:

**Attributable Risk due to genotypes with a G allele: 11.1%**
(with confidence interval ranges from 3.6 to 18.0%)

*Bart G, Kreek MJ, LaForge KS... Ott J, Heilig M, et al., Neuropsychopharmacology, 2005*
Repeat Polymorphism of Dynorphin Gene – Grouped by Frequency of Cocaine Use and Cocaine/Alcohol Dependent versus Controls

**TGACTTA**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cocaine / Alcohol dependent</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long</td>
<td>Long/Short</td>
</tr>
<tr>
<td>Caucasian n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>American</td>
<td>46%</td>
<td>46%</td>
</tr>
<tr>
<td>African ** n</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>American</td>
<td>41%</td>
<td>43%</td>
</tr>
<tr>
<td>Hispanic n</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>American</td>
<td>40%</td>
<td>40%</td>
</tr>
</tbody>
</table>

**Significant difference between control and cocaine and alcohol dependent. Fisher’s Exact test, p = 0.01.**

**Significant difference between control and cocaine and alcohol dependent.**

_Fisher's Exact test, p = 0.01._

**Significant difference between control and cocaine and alcohol dependent.**

_Fisher's Exact test, p = 0.01._

*Williams et al, 12:496, 2007*
Impulsivity* (genetics?)

Risk Taking* (genetics?)

Comorbidity (genetics)

Stress Responsivity-atypical (genetics)

Environmental Factors (~100% contribution to addiction)

Drug Induced Effects (w/ some genetic factors) (~100% contribution to addiction)

Genetic Factors for addiction (30-60% contribution to addiction)

** Relative scale of contributors to stage of drug use/addiction:

0 ↓ ↓ ↓ ↓

Kreek, Nielsen, Butelman & LaForge, Nat Neurosci., 8:1450, 2005
P-glycoprotein (MDR1, ABCB1)

P-gp is expressed in tissues with barrier function like the endothelial cells lining of the Blood-Brain Barrier


Adapted from Ho et al. *Clinical Pharmacology & Therapeutics* (2005)
Selected *ABCB1* SNPs and Study Design

**Coding sequence**

1. 1236C/T
2. 2677G/T (A893S)
3. 3435C/T

**Intronic**

1. rs3789243
2. rs2520464
3. rs1922242
4. rs2235067
5. rs2032583
6. rs6949448

**Methadone dose**

- “Low”
- “High”

- 150 mg/day

**Tests for association:**

- Single SNP
  - Allele frequency
  - Genotype frequency

- Multi locus genotype pattern:
  - 3 - locus (coding region)
  - 9 - locus

*Levran et al., 2008*
Natural History of Drug Abuse and Addictions

Primary Prevention

Initial Use of Drug of Abuse

Possible Utility of Vaccines and Selected Medications

Sporadic Intermittent Use

Regular Use

Medications Useful and Needed

Addiction

Early Withdrawal (abstinence)

Protracted Abstinence

Progression

> 80% without pharmacotherapy relapse to addiction

< 20% sustain abstinence with no specific medications

Kreek et al., Nature Reviews Drug Discovery, 1:710, 2002