VNTR polymorphism of Dopamine Transporter

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

40 bp VNTR

5’ 3’ UTR

Variable number of tandem repeats 3-13 copies

AAA

Adapted from Miller & Madras, 2002; Fuke et al., (2001), Vandenberg et al., 1992
DRD4 VNTR Polymorphism in Exon 3

Translation start site

Exon 3 VNTR
Variable number of tandem repeats
2-11 copies

48 bp VNTR

Adapted from: D’Souza; van Tol et al., (1992)
Serotonin Transporter, SLC6A4
17q11.1-q12
minus strand

Overview of Chr17

[25,392,812 ▶

EFCAB5 → CCDC55 → SLC6A4 → BLMH → TMIGD1

[25,685,191 ▶

rs12945042
rs2066713
5HTTLPR
rs12945042
rs25531
MspI
L_A/L_G
rs6354
rs2020942
rs140700
rs2054847
rs1042173

5’

Coding Region

Untranslated Region

3’
VNTR and Ins/del Genotyping on ABI 3130xl

Genotyping four typical VNTR candidate genes
Dinucleotide Repeat Genotyping on ABI 3130xl
DRD4 Exon-3 48 bp VNTR

2R  379 bp
3R  427 bp
4R  475 bp
5R  523 bp
6R  571 bp
7R  619 bp
8R  667 bp
Sequence of the 5HTTLPR

TCTCCCGCCTGGCGTTGCCGCTCTGA ATGCCAGCACCTAACCCTCTTAATGT

Forward primer

Error in original sequence - this C was absent
Error in original sequence - this was T, not C.

R = SNP rs25531 (A/G)
Restriction site = 152 bp
CCAG or CCGG

Restriction site = 283 or 326 bp

Full length = 376 or 419 bp

Insertion/Deletion
5HTTLPR – “tri-allelic” determination of SNP rs25531

Step one – amplify full length PCR product of the 43 bp Insertion/deletion.

- Short Allele 376 bp
- Long Allele 419 bp

Step two – incubate the PCR products with MspI

- 152 bp fragment from $L_G$
- 283 bp fragment from $S$
- 326 bp fragment from $L_A$
Genotyping methods: STR/VNTRs, summary

- Short tandem repeats → length differences

- VNTR, insertion/deletion polymorphisms
  30 bp to 300 bp (DAT1, DRD4, 5HTTLPR)

What about sequence differences?
Types of genetic differences between people

Any differences in DNA segments between any two persons can be used as genetic markers. Fragments of DNA can be distinguished from one another because of differences in their nucleotide sequences.

Types:

1. **Single Nucleotide Polymorphisms** (SNPs). A single base pair change one strand of DNA. The most prevalent form of differences between any two individuals.

2. **Minisatellites**. 10-100 nucleotides repeated several times in tandem; bordered by unique DNA sequences. Variable Number Tandem Repeats (VNTR) is an example. There are about 50,000 VNTRs in the human genome.

3. **Microsatellites** or Short Tandem Repeats (STRs). Smaller sequence repeats than minisatellites. Di- and tetra-nucleotide sequence repeats are common.
The size of the repeat itself may not matter. The location and function are important.

Position of polymorphism could have effects when in:

1. **Regulatory regions**: influences the type of protein made.
2. **Coding regions**: influences the type of protein made.

- **Non-synonymous**
  - Nonsense mutations: causes premature stop signal
  - Missense mutations: changes in protein sequence

- **Synonymous “silent”**
  - Mutation *does not* alter protein, could affect mRNA stability & translation
SNP Genotyping

Three Methods

Restriction Endonuclease

Taqman Assays

Illumina Golden Gate
The table below summarizes the possible results of the example allelic discrimination assay shown above.

<table>
<thead>
<tr>
<th>A substantial increase in...</th>
<th>Indicates...</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIC fluorescence only</td>
<td>homozygosity for Allele 1.</td>
</tr>
<tr>
<td>FAM fluorescence only</td>
<td>homozygosity for Allele 2.</td>
</tr>
<tr>
<td>both fluorescent signals</td>
<td>heterozygosity.</td>
</tr>
</tbody>
</table>
Allelic Discrimination assay for COMT val$^{158}$met SNP

G/G, val/val
G/A, val/met
A/A, met/met

Not called
Controls
Four SNPs from the current Illumina Panel

**BDNF Val66Met**

**DRD2 TaqIA**

**COMT Val158Met**

**GABRA2**
ABI 7000 – Taqman Assay Instrument external view
ABI 7000 – Taqman Assay Instrument internal view

96 Well Sample Plate
Illumina Bead Xpress Reader
ABI Biotrove Open Array Reader
Beckman-Coulter Biomek 3000 Robot
Beckman-Coulter Biomek 3000 Robot
Beckman-Coulter Biomek FX Robot
Genotyping for Wave IV

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Additional SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT1</td>
<td>40 bp VNTR</td>
<td>+10 SNPs</td>
</tr>
<tr>
<td>DRD4</td>
<td>48 bp VNTR</td>
<td>+10 SNPs</td>
</tr>
<tr>
<td>SERT</td>
<td>43 bp ins/del</td>
<td>+10 SNPs</td>
</tr>
<tr>
<td>DRD2</td>
<td>TaqIA SNP</td>
<td>+10 SNPs</td>
</tr>
<tr>
<td>5HT2A</td>
<td>-1438 G/A</td>
<td>+10 SNPs</td>
</tr>
<tr>
<td>MAOA</td>
<td>30 bp VNTR promoter</td>
<td></td>
</tr>
<tr>
<td>MAOA</td>
<td>STR intron 2</td>
<td></td>
</tr>
<tr>
<td>DRD5</td>
<td>5’UTR STR</td>
<td></td>
</tr>
<tr>
<td>COMT</td>
<td>val^{158}met SNP</td>
<td></td>
</tr>
</tbody>
</table>

Genomic Control Panel
Polymorphisms genotyped in the Add Health Data Sibling pairs data set.

(by the Smolen Laboratory)
### Polymorphisms genotyped in the Add Health Data Sibling pairs data set

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine Transporter</td>
<td>DAT1</td>
<td>VNTR</td>
</tr>
<tr>
<td>Dopamine Receptor D4</td>
<td>DRD4</td>
<td>VNTR</td>
</tr>
<tr>
<td>Monoamine Oxidase A</td>
<td>MAOA</td>
<td>VNTR</td>
</tr>
<tr>
<td>Dopamine Receptor D2</td>
<td>DRD2</td>
<td>SNP</td>
</tr>
<tr>
<td>Cytochrome P450 - 2A6</td>
<td>CYP2A6</td>
<td>SNP</td>
</tr>
<tr>
<td>Serotonin Transporter</td>
<td>SLC6A4</td>
<td>VNTR</td>
</tr>
</tbody>
</table>

**Key point:** All of these polymorphisms have functional effects that effect gene levels and that may influence behavioral expression.
Polymorphisms genotyped in the Add Health Data Sibling pairs data set

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Previous behavioral Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT1</td>
<td>Drug use/misuse, hyperactivity</td>
</tr>
<tr>
<td>DRD4</td>
<td>Novelty seeking, risk taking behaviors</td>
</tr>
<tr>
<td>MAOA</td>
<td>Aggression, depression, suicide, tobacco use, stress</td>
</tr>
<tr>
<td>DRD2</td>
<td>Drug use/misuse, inattention</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Metabolizes nicotine</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>Depression, suicide, anxiety, violence, stress</td>
</tr>
</tbody>
</table>
Sampling Structure for the National Longitudinal Study of Adolescent Health

132 Schools Selected with Unequal Probability of Selection

1994-1995 In-School Questionnaire N=90,118

1995 Wave I In-Home Questionnaire N=20,745

1996 Wave II In-Home Questionnaire N=14,738

Alternate Schools

1994 School Administrator Questionnaire N=164

Chantala, K & Tabor J, 1999
Carolina Population Center
**National Longitudinal Study of Adolescent Health**

**Total Sample**

- Wave 1- 1995 (n=20,745)
- Wave 2- 1996 (n=14,738)
- Wave 3- 2001 (n=15,170)
- Wave 4- 2008 (n=17,000)

**Genetic supplemental sample** (N = 5470, 4984, 4356)*

- **Association analysis**
  - DNA obtained at Wave 3 (N=2612)
  - David Rowe
  - Genotyped for several VNTR and SNP polymorphisms

- **Biometrical analysis**
  - MZ pairs = 298, 270, 237
  - DZ pairs = 437, 393, 315
  - FS pairs = 1049, 920, 779
  - HS pairs = 332, 296, 233

*Courtesy of David Timberlake
This is a good summary of the design of the Add Health Study, and the data collected in Waves I, II and III
Dopaminergic System

- Dopamine found in neurons of nigrostriatal, mesocortical and mesolimbic systems.

- Role in motor function, reward, reinforcement, emotional expression, neuroendocrine release and behavioural homoeostasis.

from Dr. Ursula. M. D'Souza
Dopaminergic Receptor System

Transporters & Auto-transporters

Receptors

D\(_1\)-like (D1, D5)
D\(_2\)-like (D2, D3 and D4)

Missale et al., 1998: Physiology Reviews 78(1): 189-225
VNTR polymorphism of Dopamine Transporter

Variable number of tandem repeats 3-13 copies

40 bp VNTR

Adapted from Miller & Madras, 2002; Fuke et al., (2001), Vandenberg et al., 1992
DAT1 3’ VNTR Polymorphism

- 40 bp repeat, fragment sizes range from 400 - 520 bp
- 9R (440 bp) & 10R (480 bp) polymorphisms are most common in Caucasian, Hispanic, and African-American populations
- Functional effects:
  - Receptor densities
    - SPECT- individuals with 9/10 repeat *had decreased* DAT protein compared with 10-repeat (Heinz et al., 2000)
DAT1 expression by genotype in brain (cerebellum and temporal lobe)

Functional & behavioral effects of the 40 bp DAT1 VNTR

– Transcriptional efficiency
  • Michelhaugh et al., (2001) - 9 repeat enhances transcription in cells and also in dopamine neurons in neonatal rat midbrain slices.
  • Miller, GM & Madras, BK. (2002). 9 repeat shows significantly higher levels of luciferase production than 10 repeats in HEK293 cells. Cloned downstream
  • Fuke et al., (2001) – 10 repeat showed higher expression than the other repeats in COS-7 cells and human glioblastoma A172 cells.
  • Mill et al., (2002) - 10 repeat showed higher expression than other commonly express genes (housekeeping genes)

– Behavioral associations
  • Alcohol, tobacco, illicit drug use/misuse.
  • gambling
  • conduct problems, violence, delinquency
  • attention-deficit/hyperactivity
DRD4 VNTR Polymorphism of Exon 3

• DRD4 is a D$_2$-like receptor
• Fragment lengths vary between 379 bp - 811 bp
• 4R (475 bp) & 7R (619 bp) alleles are most common
  (64.3% and 20.6%, respectively)
• Results in variation in the 3$^{rd}$ cytoplasmic loop of the receptor affecting G-protein binding
• DRD4 is activated by dopamine
• Inhibits adenylate cyclase and reduces cAMP levels
• 7R allele exhibits blunted ability to reduce cAMP levels

Wang et al., (2004); DiMaio et al., (2003); Anchordoquy et al., (2003)
DRD4 VNTR Polymorphism in Exon 3

Translation start site

48 bp VNTR

Exon 3 VNTR
Variable number of tandem repeats
2-11 copies

Adapted from: D’Souza; van Tol et al., (1992)
Model of the DRD4 Receptor Protein

Cytoplasmic loops
DRD4 VNTR Polymorphism of Exon 3

- Fragment lengths vary between 379 bp - 811 bp
- $4R$ (475 bp) & $7R$ (619 bp) alleles are most common (64.3% and 20.6%, respectively)
- Results in variation in the 3$^{rd}$ cytoplasmic loop of the receptor ($D_2$-like) protein, affecting G-protein binding
- $7R$ allele exhibits blunted ability to reduce cAMP levels
- Highly expressed in frontal cortex, amygdala, and hippocampus
- Behavioral associations:
  - Novelty seeking
  - conduct problems, hyperactivity

Wang et al., (2004); DiMaio et al., (2003); Anchordoquy et al., (2003)
Question:

Does the dopamine transporter polymorphism affect whether people smoke?

Answer:

Yes. The 9 repeat form appears to be protective against becoming a smoker.
Within family transmission ($W_{ij}$) of the 9-repeat allele of the Dopamine Transporter by smoking status

There are fewer than expected 9 repeat alleles in chronic smokers

*The 9 repeat allele appears to protect against becoming a chronic smoker*
Question:

Do the DRD2 TaqI, DRD4, dopamine transporter or serotonin transporter polymorphisms affect alcohol consumption?

Answer:

Yes. For DRD2 and DAT1 9 repeat.

No. For DRD4 and SERT.
The DRD2 TaqI and DAT1 polymorphisms are associated with average number of drinks per drinking episode.
The Add Health sample was used to confirm an association between subjective effects to nicotine and the CHRN3B3 gene.
CHRNA5/A3/B4 Gene Cluster on chromosome 15q25.1

CHRNB3 and CHRNA6 genes on chromosome 8p11.2 – 8p11
Role of genotype in the cycle of violence in maltreated children.

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R.

“(They Studied) male children from birth to adulthood to determine why some children who are maltreated grow up to develop antisocial behavior… A functional polymorphism in… monoamine oxidase A (MAOA) was found to moderate the effect… Maltreated children with a genotype conferring high levels of MAOA expression were less likely to develop antisocial problems.

These findings may partly explain why not all victims of maltreatment grow up to victimize others, and they provide epidemiological evidence that genotypes can moderate children's sensitivity to environmental insults.”

Thus was begun anew the quest for GxE interactions.”
Maltreatment before the age of 7

Behavioral consequences:

- **Increased rates of conduct problems**
  

- **Substance misuse**
  

- **Depression**
  

- **Poor academic performance**
  

Neurological consequences:

- **Promotes adaptation in particular brain structures.**
  

- **Increased levels of neurotransmitters**
  
MAOA: Behavior and neurological effects

Prior evidence:

• **Aggression**

• **Increased neurotransmitter levels**

“…Maltreated children with a genotype conferring high levels of MAOA expression were less likely to develop antisocial problems…”
Question:

Is there a gene-by-environment interaction between early abuse and monoamine oxidase-A ("the Caspi hypothesis")?

Answer:

No. Early abuse leads to higher antisocial behavior in adulthood, but we found no interaction with MAOA.
MAOA and Antisocial Behavior: Effect of Early Maltreatment

Higher early maltreatment leads to higher antisocial behavior

We found no effect of MAOA activity
Childhood Adversity, Monoamine Oxidase A Genotype, and Risk for Conduct Disorder

Debra L. Foley, PhD; Lindon J. Eaves, PhD, DSc; Brandon Wormley, BS; Judy L. Silberg, PhD; Hermine H. Maes, PhD; Jonathan Kuhn, PhD; Brien Riley, PhD

Prevalence of conduct disorder as a function of monoamine oxidase A activity and level of exposure to childhood adversities.

Table 2. Prevalence of Conduct Disorder by MAO-A Genotype and Level of Exposure to Childhood Adversity*

<table>
<thead>
<tr>
<th>Level of Exposure to Childhood Adversity, No. (%)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low MAO-A</td>
<td>4/99  (4.04)</td>
<td>3/19  (15.79)</td>
<td>3/27  (11.11)</td>
<td>2/5   (40)</td>
<td>1/1   (100)</td>
</tr>
<tr>
<td>High MAO-A</td>
<td>22/249 (8.84)</td>
<td>13/53 (24.53)</td>
<td>10/47 (21.28)</td>
<td>1/10  (10)</td>
<td>0/4   (0)</td>
</tr>
<tr>
<td>P Value, 2-tailed Fisher exact test</td>
<td>P = .17</td>
<td>P = .53</td>
<td>P = .35</td>
<td>P = .24</td>
<td>P = .20</td>
</tr>
</tbody>
</table>
Dopamine D2 Receptor, DRD2
11q22-q23
minus strand

[112,690,461] TTC12 → DRD2 ← ANKK1 → [113,149,635]

rs1800497 T
rs1079597
rs6277
rs6275
rs1801028
rs1124492
rs4587762
rs17601612
rs427422
rs4350392
rs7122454
rs12363125
rs6277
rs427422
rs1800497 T

Coding Region
The DRD2 TaqIA A1 allele is associated with low verbal ability.
A Dopamine Gene (DRD2) Distinguishes Between Offenders Who Have and Have Not Been Violently Victimized

Jamie Vaske, John Paul Wright, and Kevin M. Beaver

“…offenders who are violently victimized are more likely to carry the DRD2 (A1) risk allele than offenders who have not been violently victimized.”
“DRD2 (TaqIA genotype) interacted with delinquent peers to predict victimization (in caucasian males).”
In addition to DRD2, includes MAOA, DRD4, DAT1 and 5HTTLPR to form a Genetic Risk Score

“...measures of genetic risk that are based on multiple polymorphisms can be employed to examine the gene×environmental basis to antisocial behavioral phenotypes.”
Males heterozygous for DRD2 (A1/A2) displayed more depressive symptoms than either homozygous males. Heterosis.

Interaction with DRD4 2R/2R.
“Contrary to hypothesis, presence of the A1 DRD2 allele was associated with having had fewer sex partners in the past year.”
“For DRD2, the trajectory of serious delinquency for the heterozygotes (A1/A2) is…higher than the A2/A2 (or) A1/A1 genotype(s)… Heterosis.”

DAT1 10R is associated with serious delinquency.

“…absence of…correlation between the two genetic variants.”
“(The) DRD2 A1 allele is associated with a decreased likelihood of school continuation...Mentors who are teachers compensate for this negative association (a G x E).”
Genetic association analyses using individuals with DNA and genotypes

Pitfalls and caveats
Polymorphisms available for association tests in the Add Health dataset

• Candidate genes: 10 polymorphisms
  DAT1, DRD2, DRD4, 5HTT (SLC6A4), CYP2A6, MAOA, CHRNA6 (2 SNPS), CHRNB3 (2 SNPS)

• Zygosity marker set: 12 polymorphisms
Tests of genetic association with 57 phenotypes (within family tests using QTDT variance components model)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>p&lt;.05</th>
<th>p&lt;.01</th>
<th>p&lt;.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidate genes</td>
<td>570</td>
<td>21</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>(10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zygosity panel</td>
<td>684</td>
<td>64</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>(12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1254</td>
<td>85</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>(22)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Caveats about heritability

1. **Heritability is not an absolute property** of a physical or behavioral characteristic. It is a function of the genetic and environmental variation for a given population in particular circumstances, and at a particular developmental stage.

2. **Estimates of heritability will typically be quite imprecise** --- with large standard errors of estimation, and will depend on how the phenotype is defined and assessed.

3. **The contribution of a risk factor** to population variation is a function of both the size of effect in individual cases, and the frequency of effect.
Caveats about association studies

- Low power in small samples;
- Multiple tests or ‘fishing’ should be avoided;
- Multiple papers avoid the Bonferroni correction;
- Many potential sources of error;
- Many false positives and negatives:
- = (very) poor track record of replication (especially for association studies of individual genes.)
Historical Performance of Genetic Association Studies
(Cardon, 2007)

- Pubmed: 27 Feb 2007. “Genetic association” gives 42,294 hits

- 1635 claims of ‘replicated’ genetic association (4%)

- 436 claims of ‘validated’ genetic association (1%)

- In reality, ~ 30-50 confirmed associations for complex traits prior to GWAS.
Spurious Genetic Associations
Patrick F. Sullivan (2007) Biological Psychiatry

• In genetically realistic simulations of 500 cases and 500 control subjects for 10 COMT SNPs, 968 of 1000 simulations (96.8%) produced at least one false positive at the $p = .05$ level of significance.
False positive findings from a study can often appear to be “compelling,” “noteworthy,” or “intriguing”;

False positive findings may propagate and confuse the field;

Replication is important, but the definition of replication should be precise: Is it really the same phenotype?
Genotype-by-Environment Interactions

- The study of genetic association allows the identification of individual genetic contributions to heritable variation.

- and genetic association allows for the study of gene by environment interactions during development.
Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene

Avshalom Caspi,¹,² Karen Sugden,¹ Terrie E. Moffitt,¹,²* Alan Taylor,¹ Ian W. Craig,¹ HonaLee Harrington,² Joseph McClay,¹ Jonathan Mill,¹ Judy Martin,³ Antony Braithwaite,⁴ Richie Poulton³

In a prospective-longitudinal study of a representative birth cohort, we tested why stressful experiences lead to depression in some people but not in others. A functional polymorphism in the promoter region of the serotonin transporter (5-HTT) gene was found to moderate the influence of stressful life events on depression. Individuals with one or two copies of the short allele of the 5-HTT promoter polymorphism exhibited more depressive symptoms, diagnosable depression, and suicidality in relation to stressful life events than individuals homozygous for the long allele. This epidemiological study thus provides evidence of a gene-by-environment interaction, in which an individual’s response to environmental insults is moderated by his or her genetic makeup.
Interaction Between the Serotonin Transporter Gene (5-HTTLPR), Stressful Life Events, and Risk of Depression
A Meta-analysis

Neil Risch, PhD
Richard Herrell, PhD
Thomas Lehner, PhD
Kung-Yee Liang, PhD
Lindon Eaves, PhD
Josephine Hoh, PhD
Andrea Griem, BS
Maria Kovaec, PhD
Jurg Ott, PhD
Kathleen Ries Merikangas, PhD

Context Substantial resources are being devoted to identify candidate genes for complex mental and behavioral disorders through inclusion of environmental exposures following the report of an interaction between the serotonin transporter linked polymorphic region (5-HTTLPR) and stressful life events on an increased risk of major depression.

Objective To conduct a meta-analysis of the interaction between the serotonin transporter gene and stressful life events on depression using both published data and individual-level original data.

Data Sources Search of PubMed, EMBASE, and PsycINFO databases through March 2009 yielded 26 studies of which 14 met criteria for the meta-analysis.

Study Selection Criteria for studies for the meta-analyses included published data on the association between 5-HTTLPR genotype (SS, SL, or LL), number of stressful life events (0, 1, 2, ≥3) or equivalent, and a categorical measure of depression defined by the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition)
Conclusion: This meta-analysis yielded no evidence that the serotonin transporter genotype alone or in interaction with stressful life events is associated with an elevated risk of depression in men alone, women alone, or in both sexes combined.

The results of this meta-analysis clearly demonstrate that stressful life events have a potent relationship with the risk of depression…

Addition of the serotonin transporter genotype did not improve the prediction of risk of depression…

The results…should not deter investigators from Including environmental risk factor information in their studies…

Risch et al, 2009
but: G x E interactions are real

- Behavior genetic studies suggest that both genetic and environmental influences are ubiquitous.
- Gene by environment interactions are a given -- heritability may be different in different environments, at different ages, at different stages of development.
- Genes often influence multiple aspects of behavior (pleiotropy).
G x E interactions involving measured genotypes and measured environments are hard to detect

- The effects of individual **genes** on behavior are usually very small.

- The effects of individual **polymorphisms** within those genes on behavior may be exceedingly small. Why pick on some poor, unsuspecting SNP?

- Genetic associations, including interactions, have a very poor track record of replication.

- We should be very cautious about the clinical importance of single gene associations for complex traits, and interactions with environmental influences.
Binge Eating as a Major Phenotype of Melanocortin 4 Receptor Gene Mutations

Ruth Branson, M.B., Ch.B., Natascha Potoczna, M.D., John G. Kral, M.D., Ph.D., Klaus-Ulrich Lentes, Ph.D., Margret R. Hoehe, M.D., Ph.D., and Fritz F. Horber, M.D.

Table 1. Mutations in the Melanocortin 4 Receptor Gene Identified among 469 Severely Obese Subjects and 25 Normal-Weight Controls.*

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Change in Amino Acid Sequence</th>
<th>No. Affected</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Obese Subjects</td>
<td>Controls</td>
</tr>
<tr>
<td>Known</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C728T</td>
<td>Thr112Met†††</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>C886T</td>
<td>Arg165Trp†††</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>A700G</td>
<td>Val103Ile†††</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>A1144C</td>
<td>Ile251Leu†††</td>
<td>5</td>
<td>0</td>
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<td>Novel</td>
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<tr>
<td>A424G</td>
<td>Thr11Ala†</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>T544C</td>
<td>Phe51Leu</td>
<td>1</td>
<td>0</td>
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<tr>
<td>A991G</td>
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<tr>
<td>C408T</td>
<td>Thr5Thr</td>
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</tr>
<tr>
<td>A1419G 3' UTR</td>
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<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
The way forward?

Not all genetic studies need measured genotypes:
   Biometrical studies of behavior (multivariate, developmental, environmental) can tell us a lot irrespective of which individual genes are involved.

Molecular genetic studies need:
   Adequate sample sizes.
   Multiple indices of behavior (across development).
   Good Environmental assessments.
   Improved genetic techniques (dense marker sets, GWAS).
   Appropriate statistical methods and tests.
   Rigorous \textit{a priori} replication.
   More scientific skepticism.

\textbf{Add Health Wave 4 provides a real opportunity to achieve all of these goals.}
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*Wave 4 co-funders
"Yes ... I believe there's a question there in the back."