Using genetic data in the Add Health Sample

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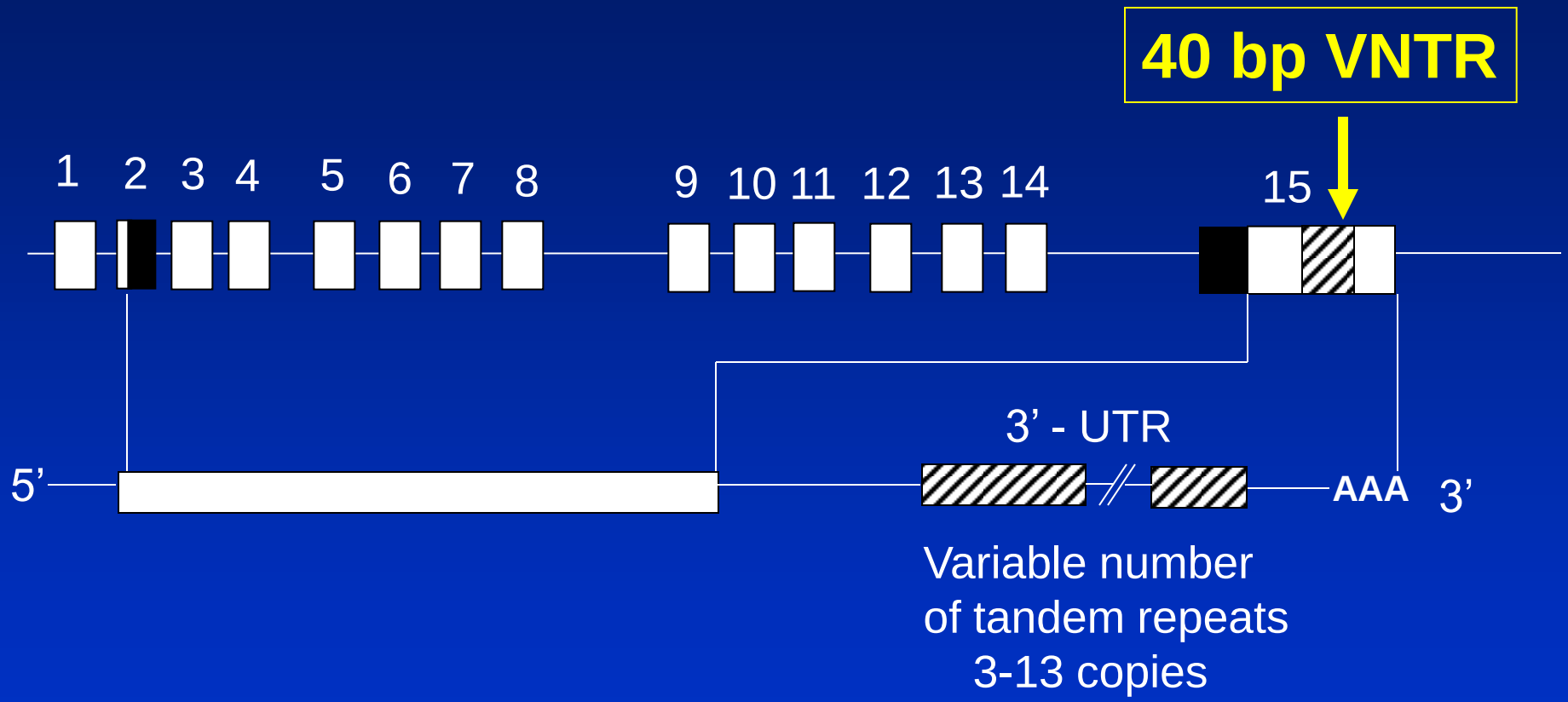
Institute for Behavioral Genetics, University of Colorado at Boulder

Add Health Users Workshop, July 2010

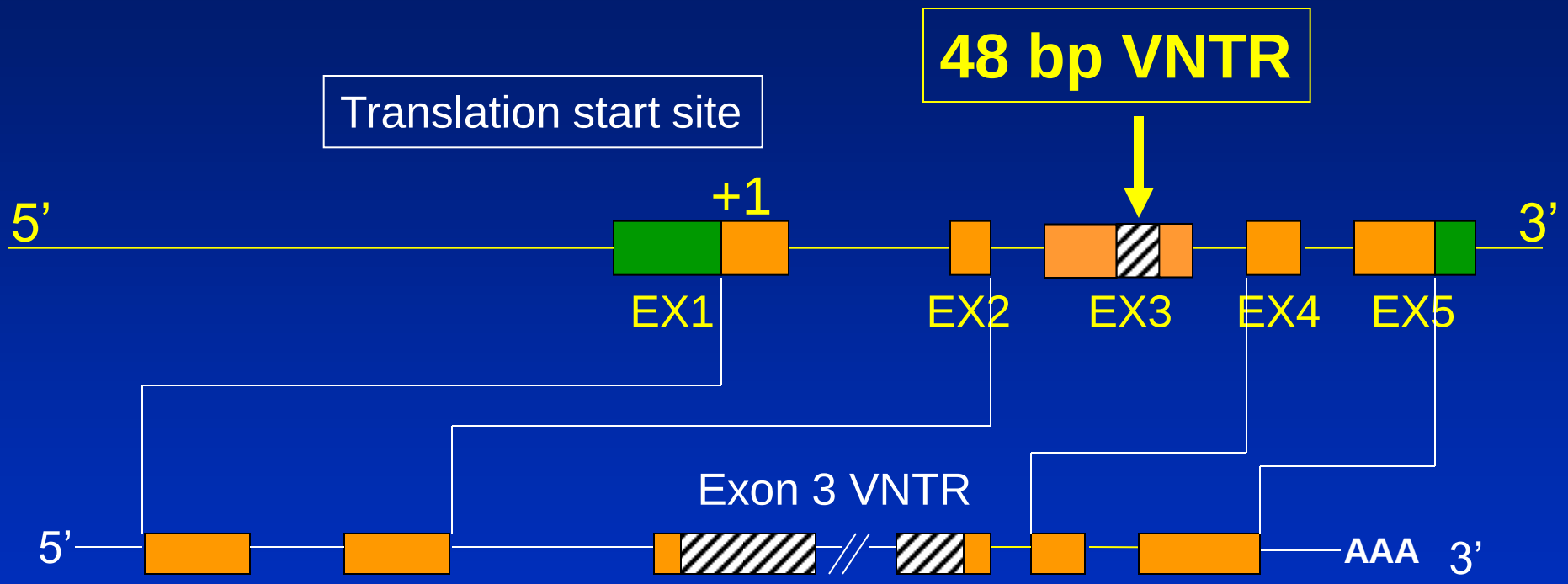
Bethesda, Maryland

VNTR polymorphism of Dopamine Transporter



Adapted from Miller & Madras, 2002; Fuke et al., (2001), Vandenberg et al., 1992

DRD4 VNTR Polymorphism in Exon 3

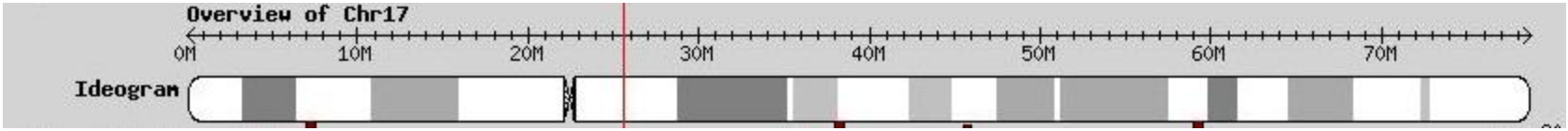


Adapted from: D’Souza; van Tol et al., (1992)

Serotonin Transporter, SLC6A4

17q11.1-q12

minus strand



[25,392,812

[25,685,191

EFCAB5

SLC6A4

TMIGD1

CCDC55

BLMH

rs2066713

25,586,831]

25,549,032]

5’

3’

5HTTLPR

1a

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

rs12945042

rs25531
MspI
L_A/L_G

rs6354

rs2020942

rs140700

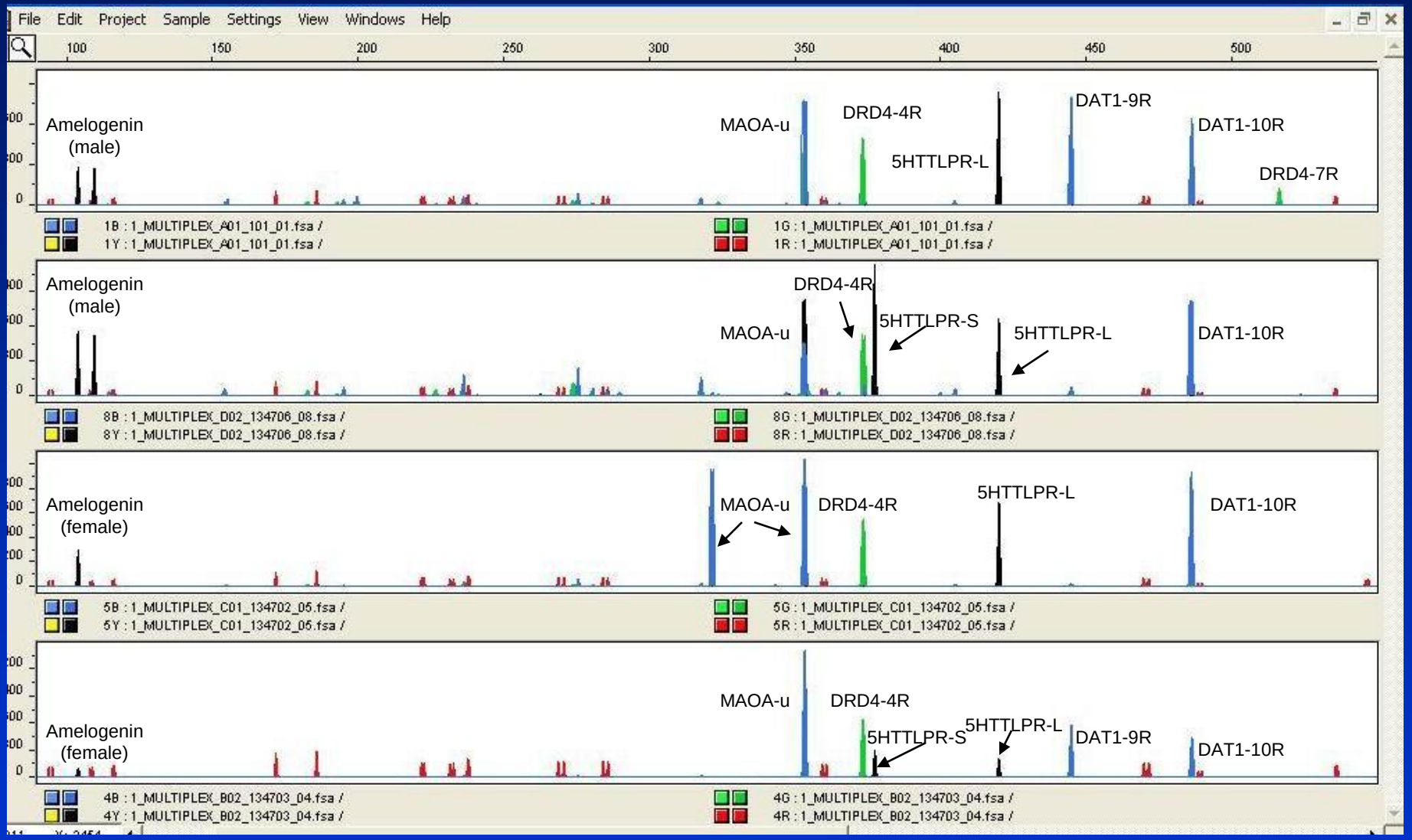
rs2054847

rs1042173

Coding Region

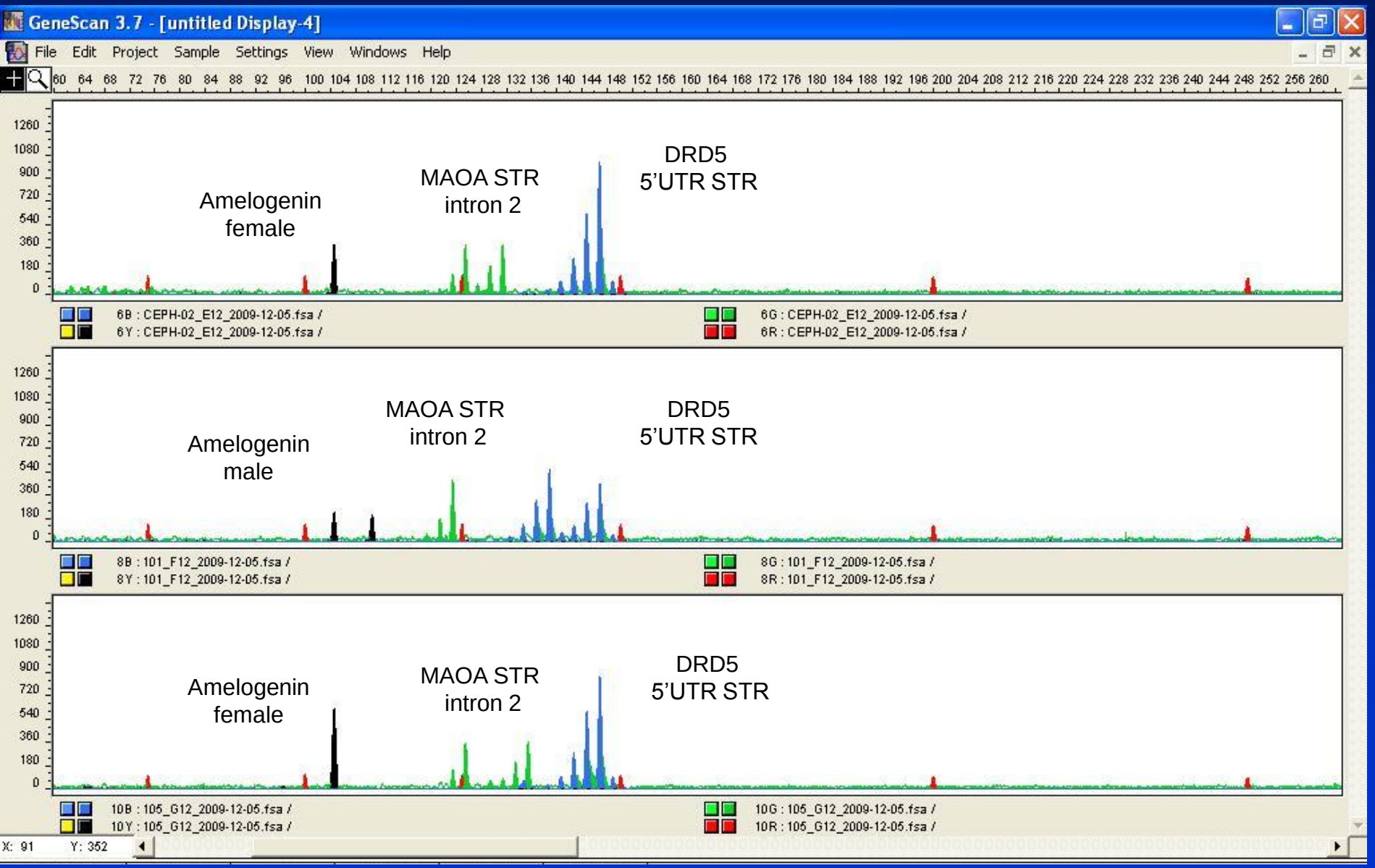
Untranslated Region

VNTR and Ins/del Genotyping on ABI 3130xl

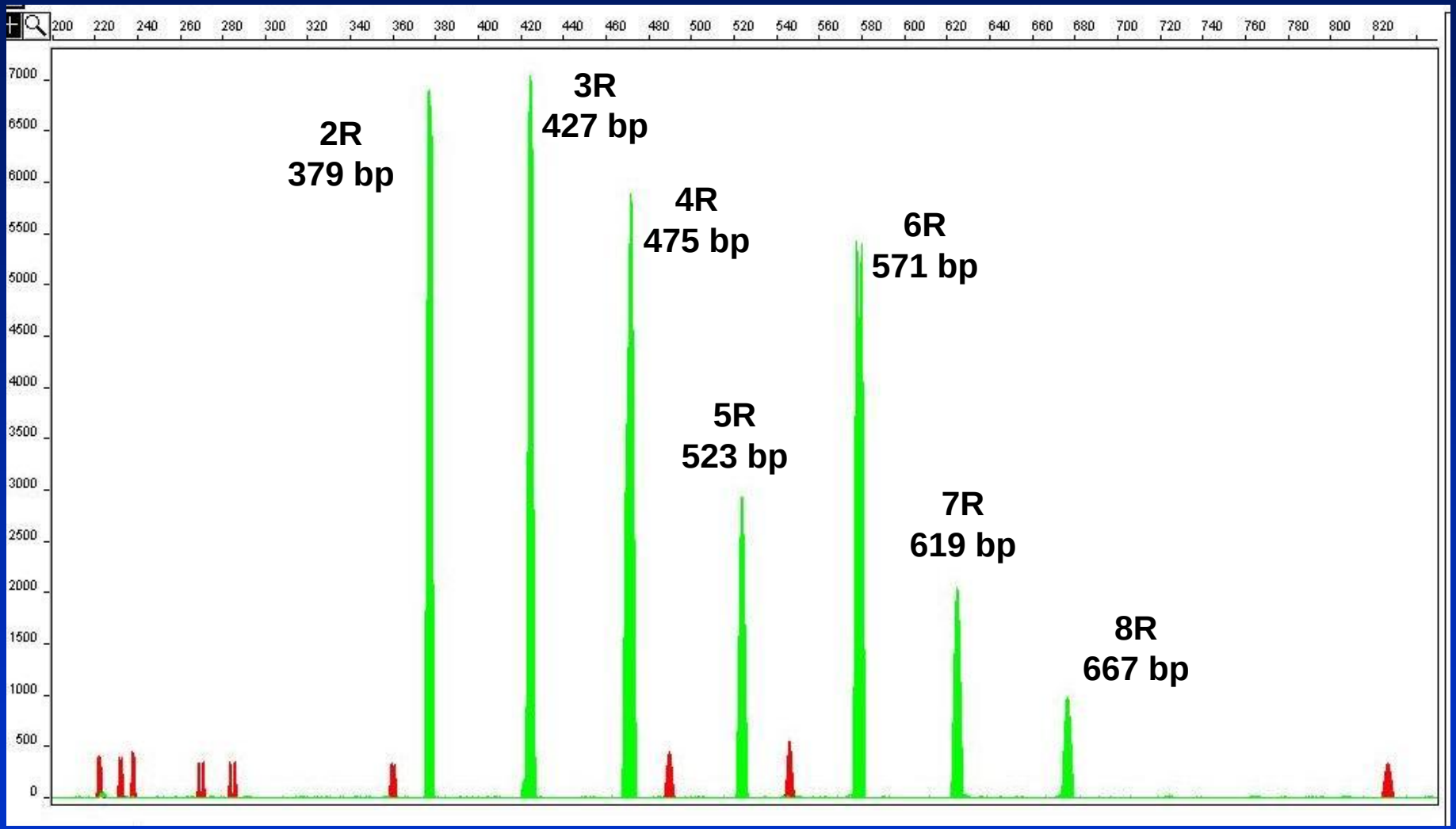


Genotyping four typical VNTR candidate genes

Dinucleotide Repeat Genotyping on ABI 3130xl



DRD4 Exon-3 48 bp VNTR



5HTTLPR showing the repeat structure

I	CCCTAC	TGCA	GCCCCCCC	AGCAT
II	CCCCCC	TGCA	ACCTCCC	AGCA
III	ACTCCC	TGTA	CCCCTCCT	AGGAT
IV	CGCTCC	TGCA	TCCCCC	ATTATC
V	CCCCCC	TTCA	CCCCTCGC	GGCAT
VI	CCCCCC	TGCA	ccccc	agcat
VII	cccccc	tgca	gccccccc	agcat
VIII	ctcccc	tgca	CCCCC	AGCAT
IX	CCCCCC	TGCA	GCCCTTCC	AGCA
X	TCCCCC	TGCA	CCTCTCCC	AGGAT
XI	CTCCCC	TGCA	ACCCCC	ATTAT
XII	CCCCCC	TGCA	CCCCTCGC	AGTAT
XIII	CCCCCC	TGCA	CCCCCC	AGCATC
XIV	CCCCCA	TGCA	CCCCC	GGCAT
XV	CCCCCC	TGCA	CCCCTCC	AGCAT
XVI	TCTCCT	TGCA	CCCTACC	AGTAT

Sequence of the 5HTTLPR

```
TCTCCCGCCTGGCGTTGCCGCTCTGAATGCCAGCACCTAACCCCTAATGT
                          Forward primer

I     CCCTACTGCAGCCCTCCCAGCAT
II    CCCCCCTGCAACCTCCCAGCA
III   ACTCCCTGTACCCCTCCTAGGAT
IV    CGCTCCTGCATCCCCCATTATC
V     CCCCCCTTCACCCCTCGCGGCAT
VI    CCCCCCTGCACCCCCRGCAT
VII   CCCCCCTGCAGCCCCCCCAGCAT
VIII  CTCCCCTGCACCCCCAGCAT
IX    CCCCCCTGCAGCCCTTCCAGCA
X     TCCCCCTGCACCTCTCCCAGGAT
XI    CTCCCCTGCAACCCCCATTAT
XII   CCCCCCTGCACCCCTCGCAGTAT
XIII  CCCCCCTGCACCCCCCCAGCATC
XIV   CCCCCATGCACCCCCGGCAT
XV    CCCCCCTGCACCCCTCCAGCAT
XVI   TCTCCTTGCACCCTACCAGTAT
      TCCCCC
GCATCCCGGCCTCCAAGCCTCCCGCCCACCTTGCGGTCCCCGCCCTGGCGTCTAGGT
                   Reverse 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

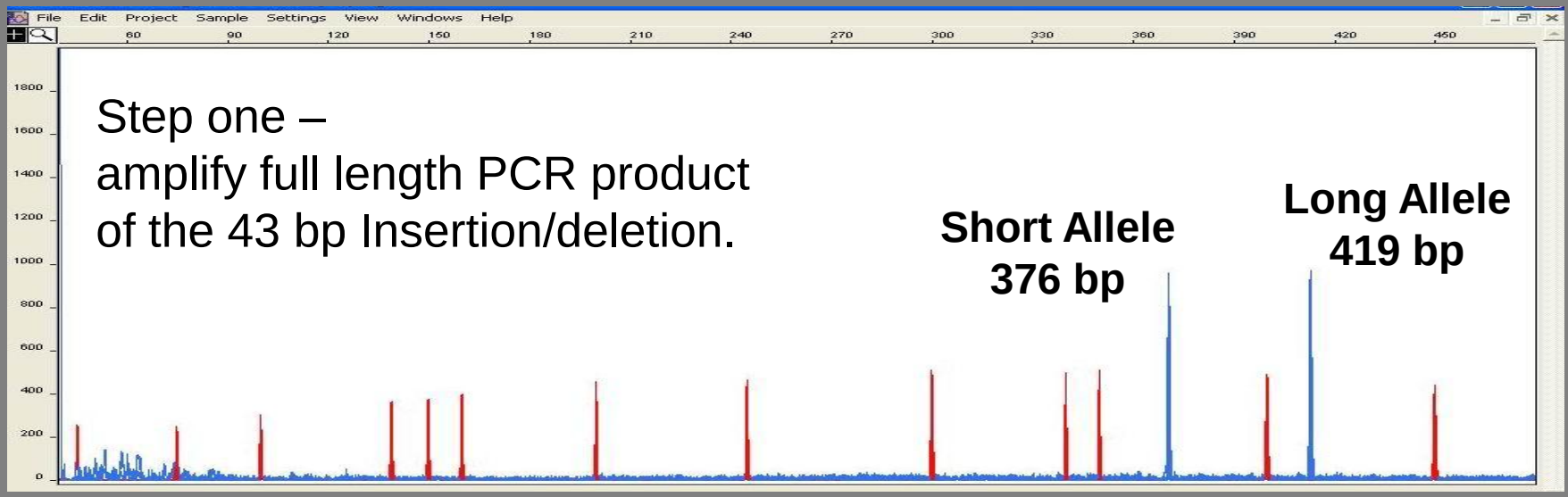
Error in original sequence - showed 6 C's, not 5
basis of the incorrect 44 bp deletion

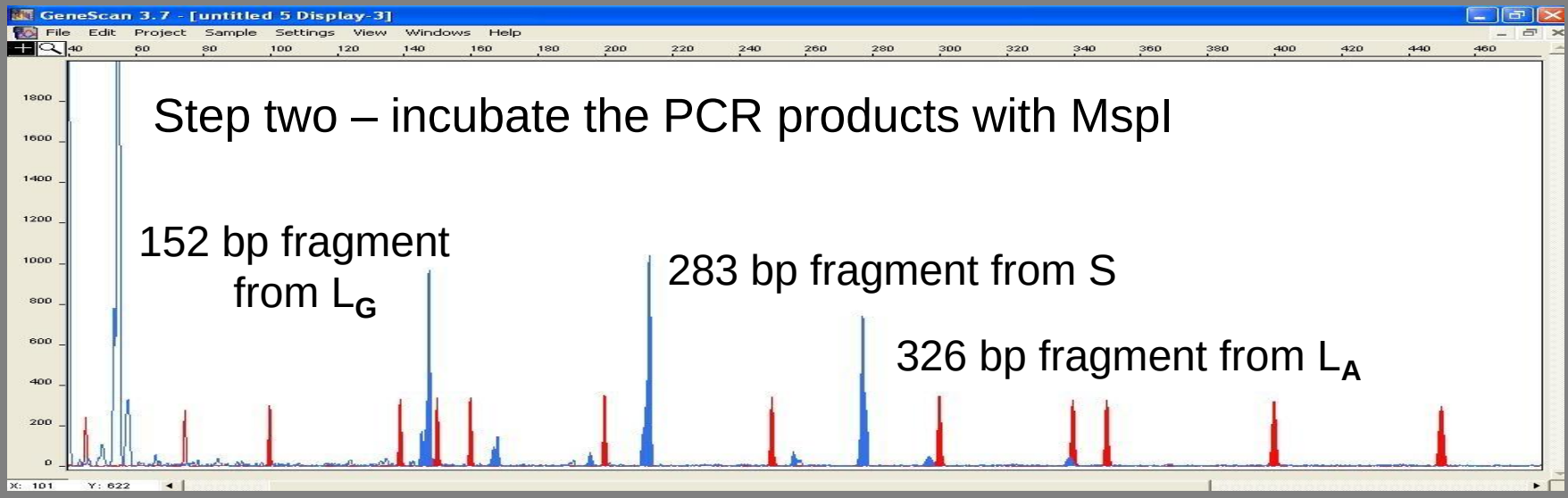
Restriction site = 283 or 326 bp

Full length = 376 or 419 bp

Insertion/Deletion

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





Genotyping methods: STR/VNTRs, summary

- Short tandem repeats → length differences

(Forensics)

(Mapping, DRD5, MAOA)

- 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

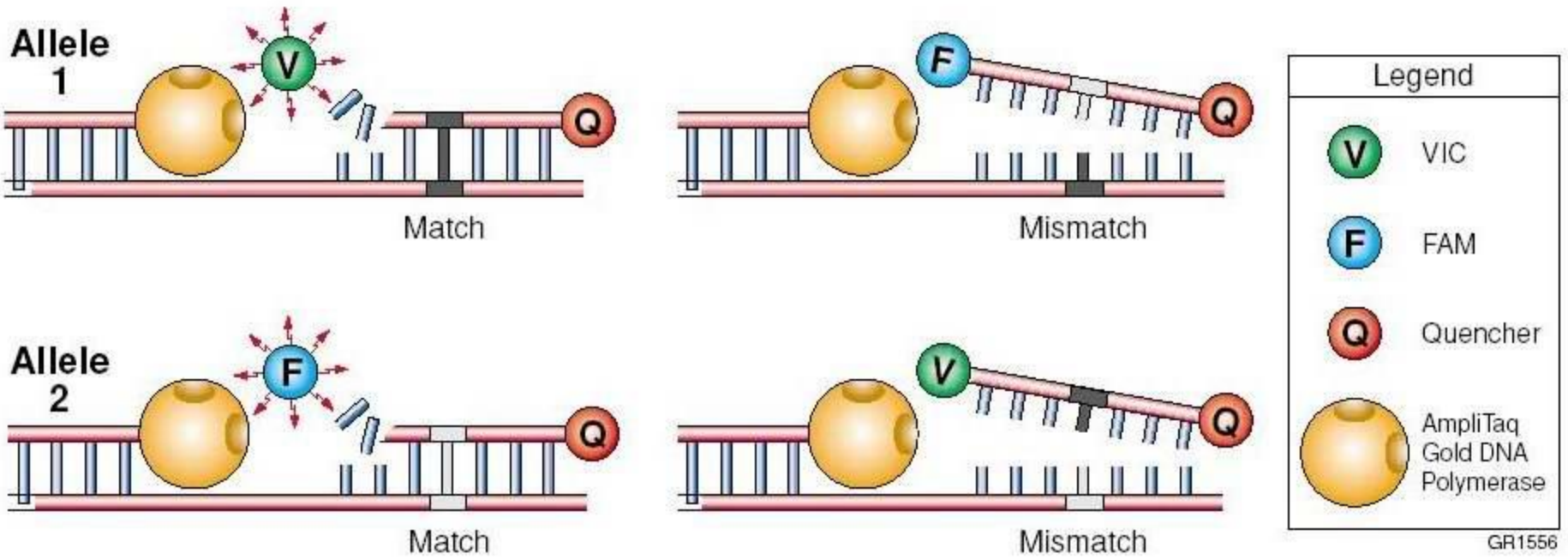
Three Methods

Restriction Endonuclease

Taqman Assays

Illumina Golden Gate

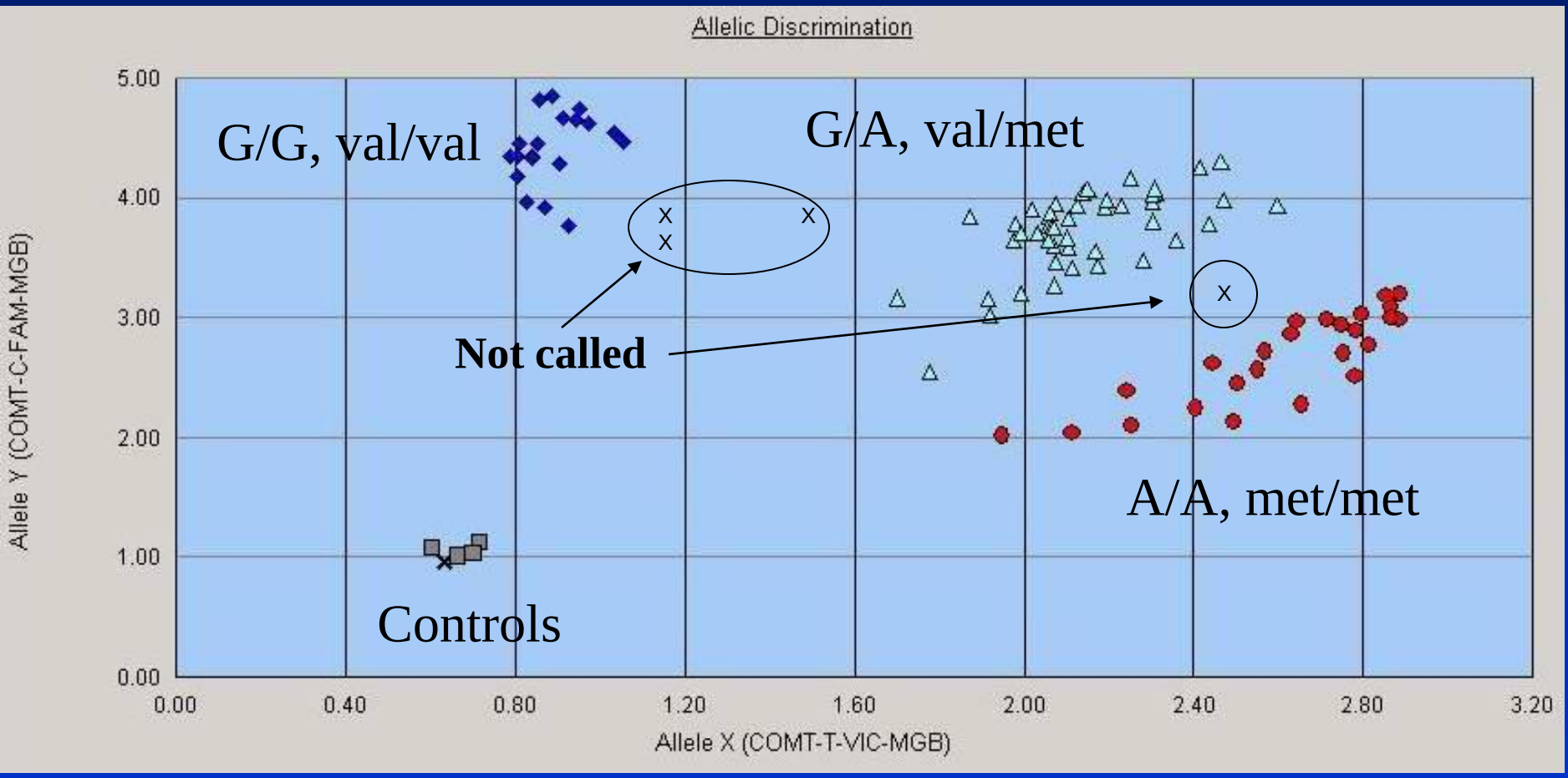
Genotyping SNPs with the ABI Taqman® Assay



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

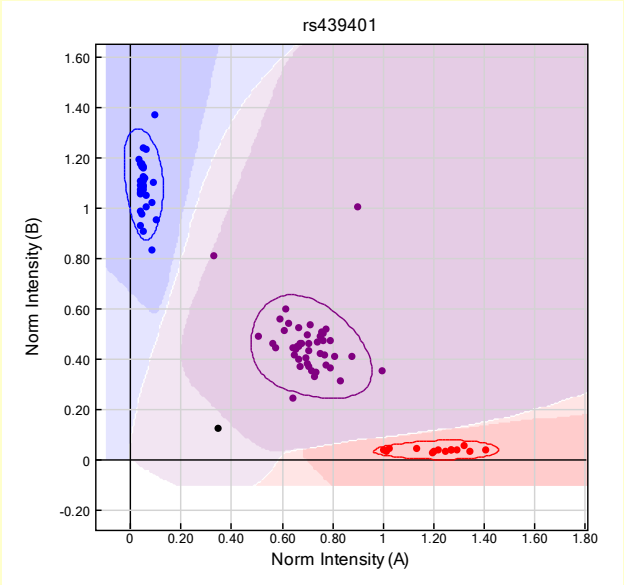
A substantial increase in…	**Indicates…**
VIC fluorescence only	homozygosity for Allele 1.
FAM fluorescence only	homozygosity for Allele 2.
both fluorescent signals	heterozygosity.

Allelic Discrimination assay for COMT val^{158}met SNP

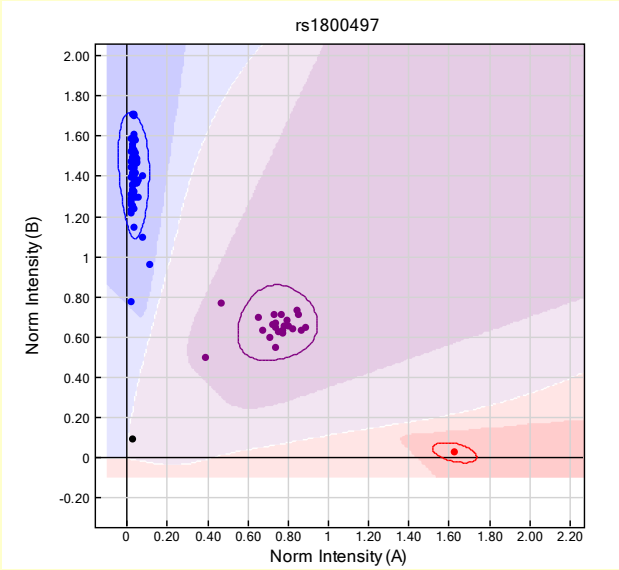


Four SNPs from the current Illumina Panel

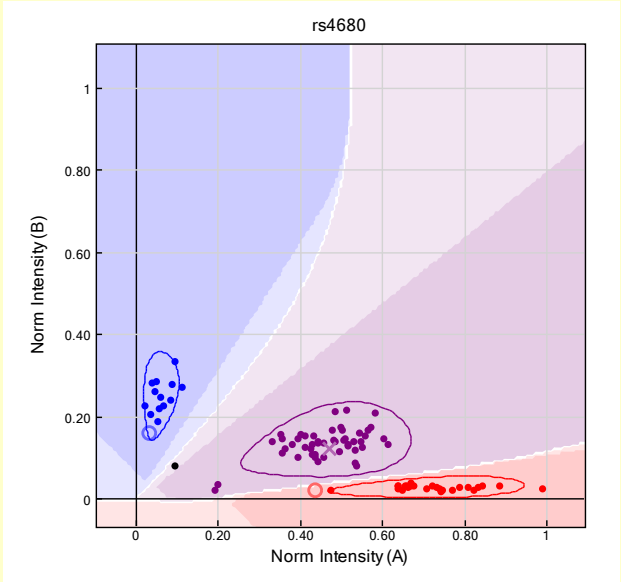
BDNF Val66Met



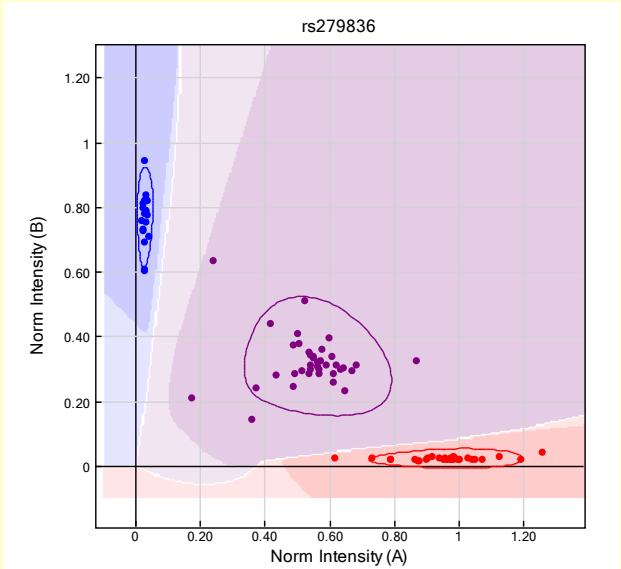
DRD2 TaqIA



COMT Val158Met



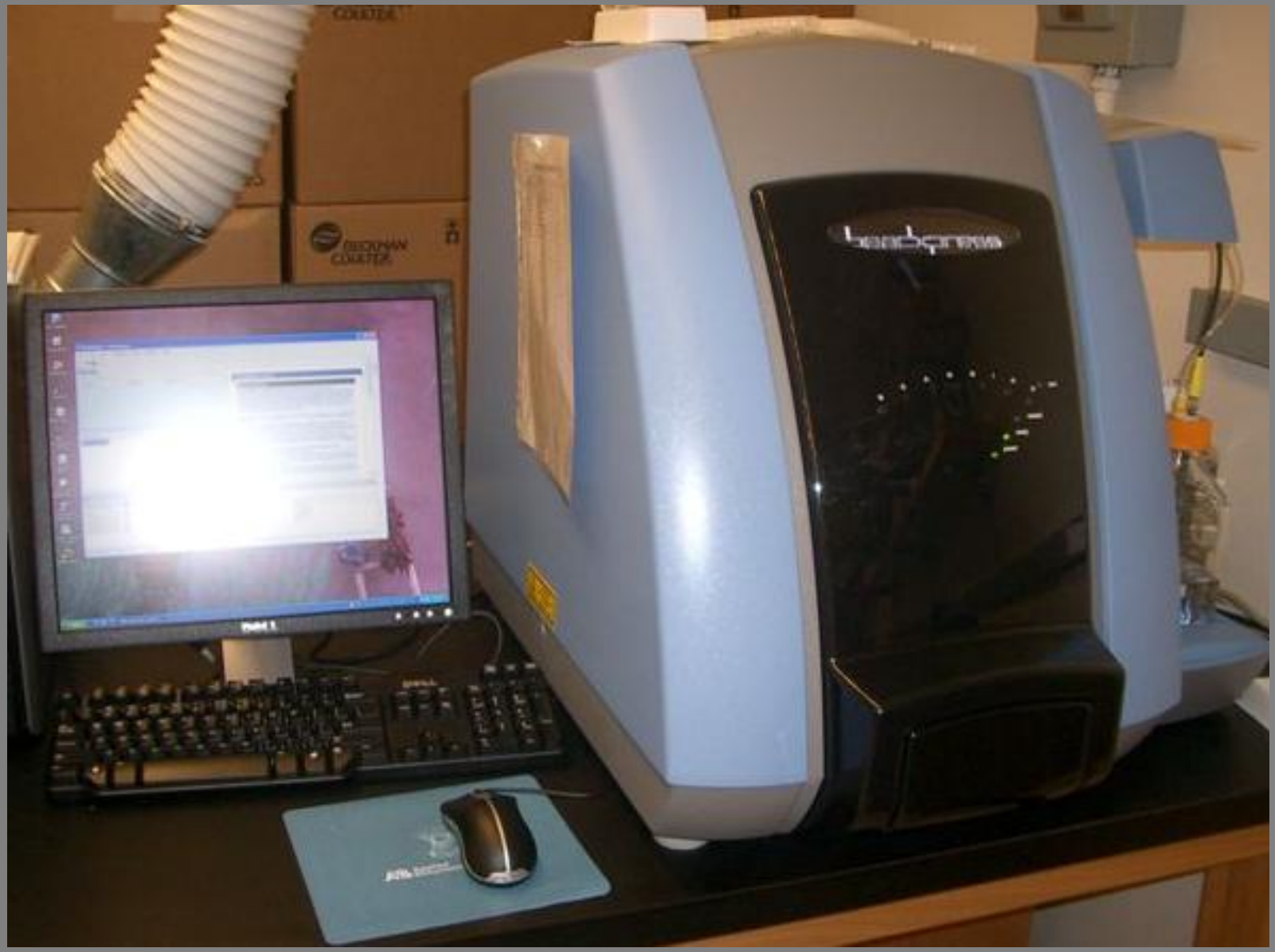
GABRA2



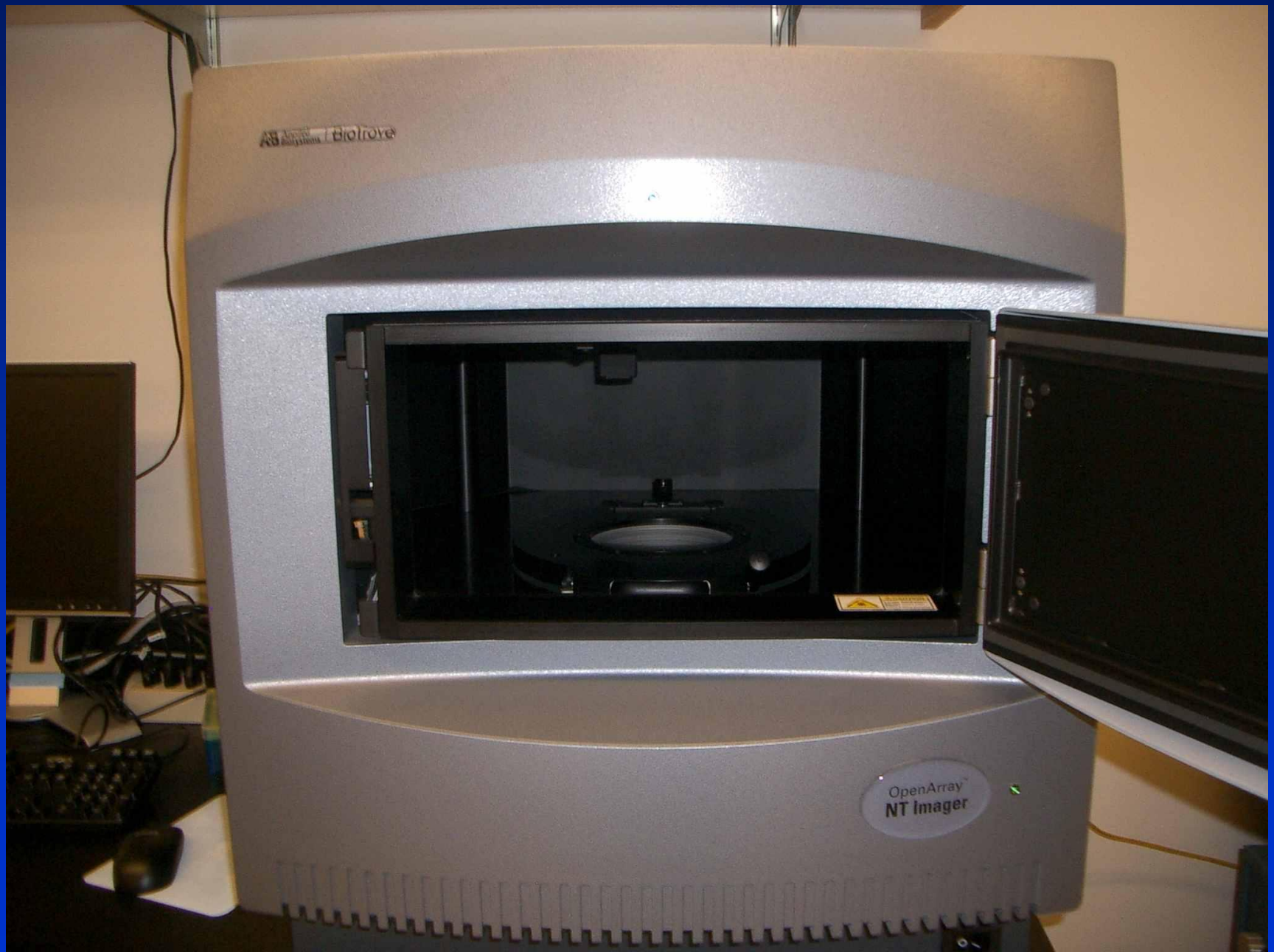
ABI 7000 – Taqman Assay Instrument external view

ABI 7000 – Taqman Assay Instrument internal view

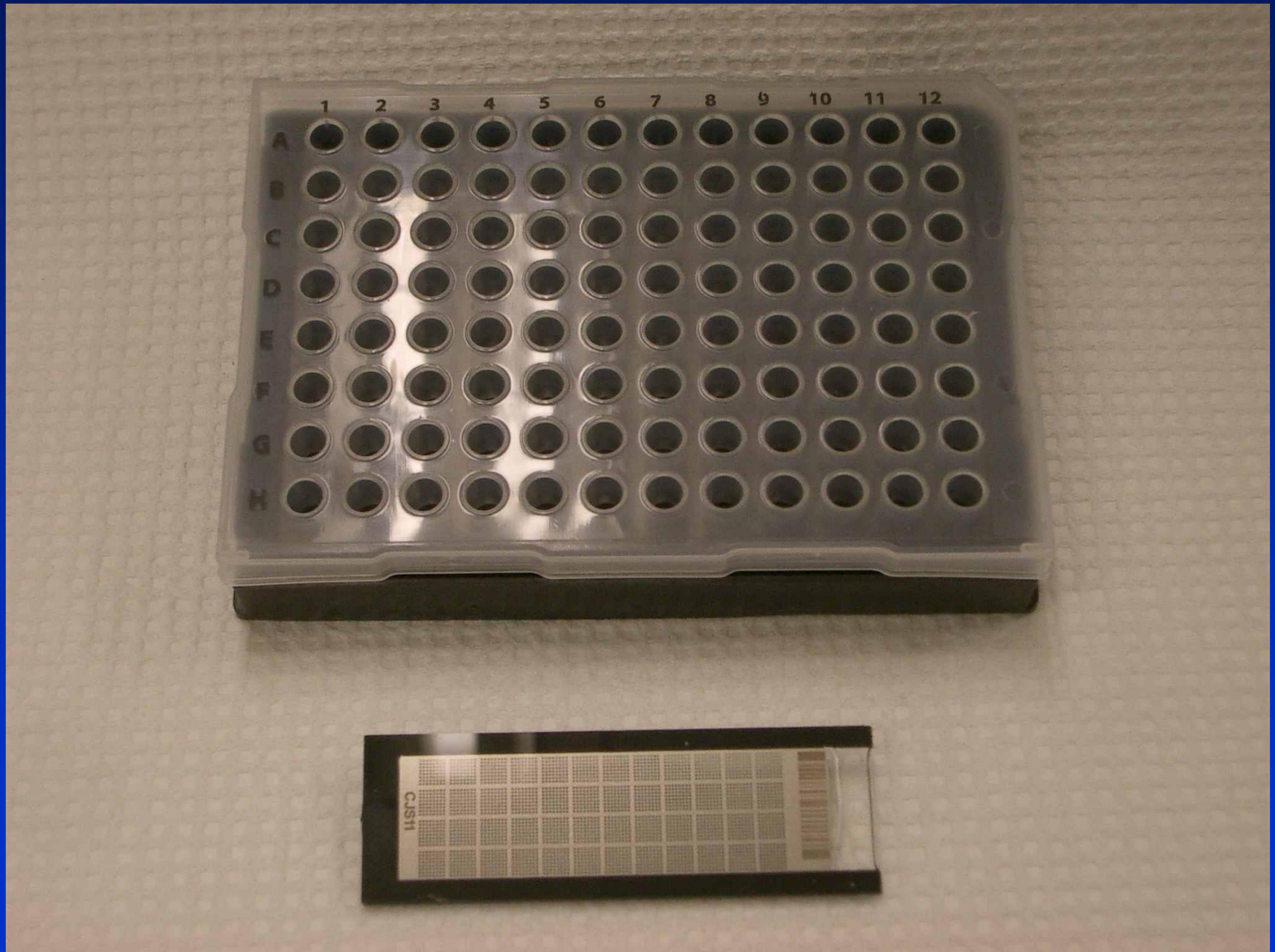
Illumina Bead Xpress Reader



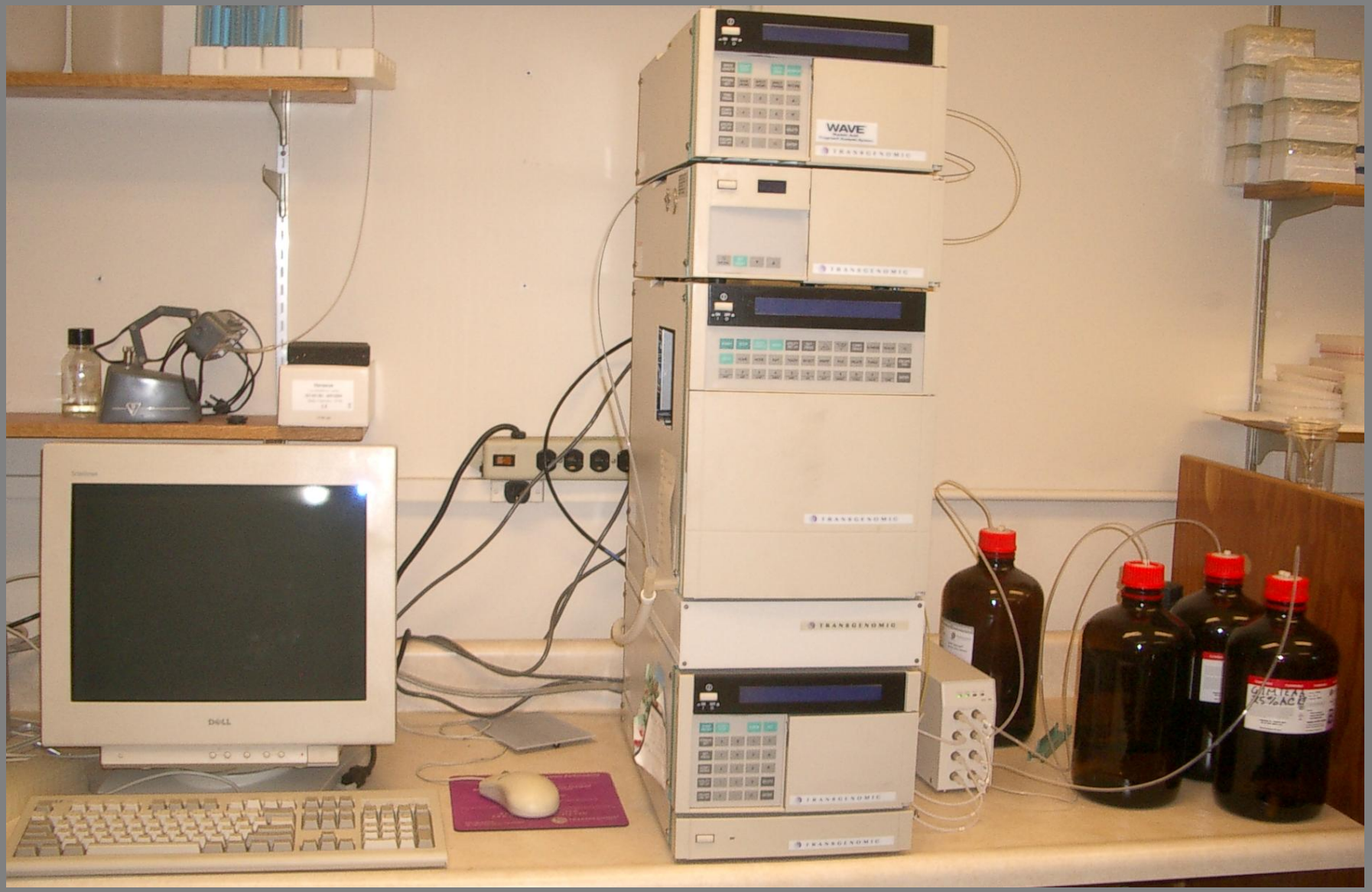
ABI Biotrove Open Array Reader



Biotrove Open Array Plate

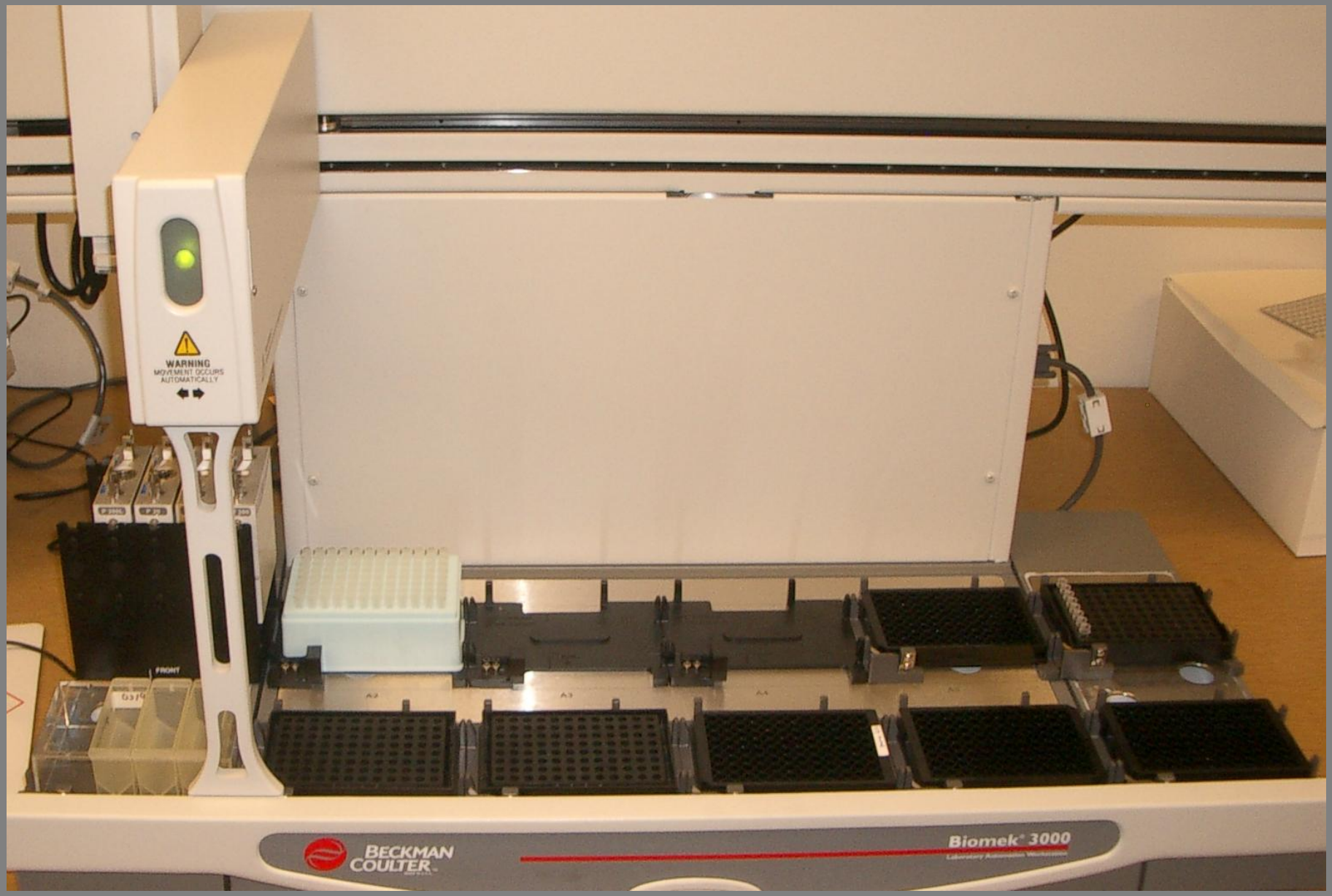


Transgenomic WAVE HPLC



Beckman-Coulter Biomek 3000 Robot

Beckman-Coulter Biomek 3000 Robot



Beckman-Coulter Biomek FX Robot

Beckman-Coulter Biomek FX Robot

Genotyping for Wave IV

DAT1	40 bp VNTR	+10 SNPs
DRD4	48 bp VNTR	+10 SNPs
SERT	43 bp ins/del	+10 SNPs
DRD2	TaqIA SNP	+10 SNPs
5HT2A	-1438 G/A	+10 SNPs
MAOA	30 bp VNTR promoter	
MAOA	STR intron 2	
DRD5	5'UTR STR	
COMT	val^{158}met SNP	

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

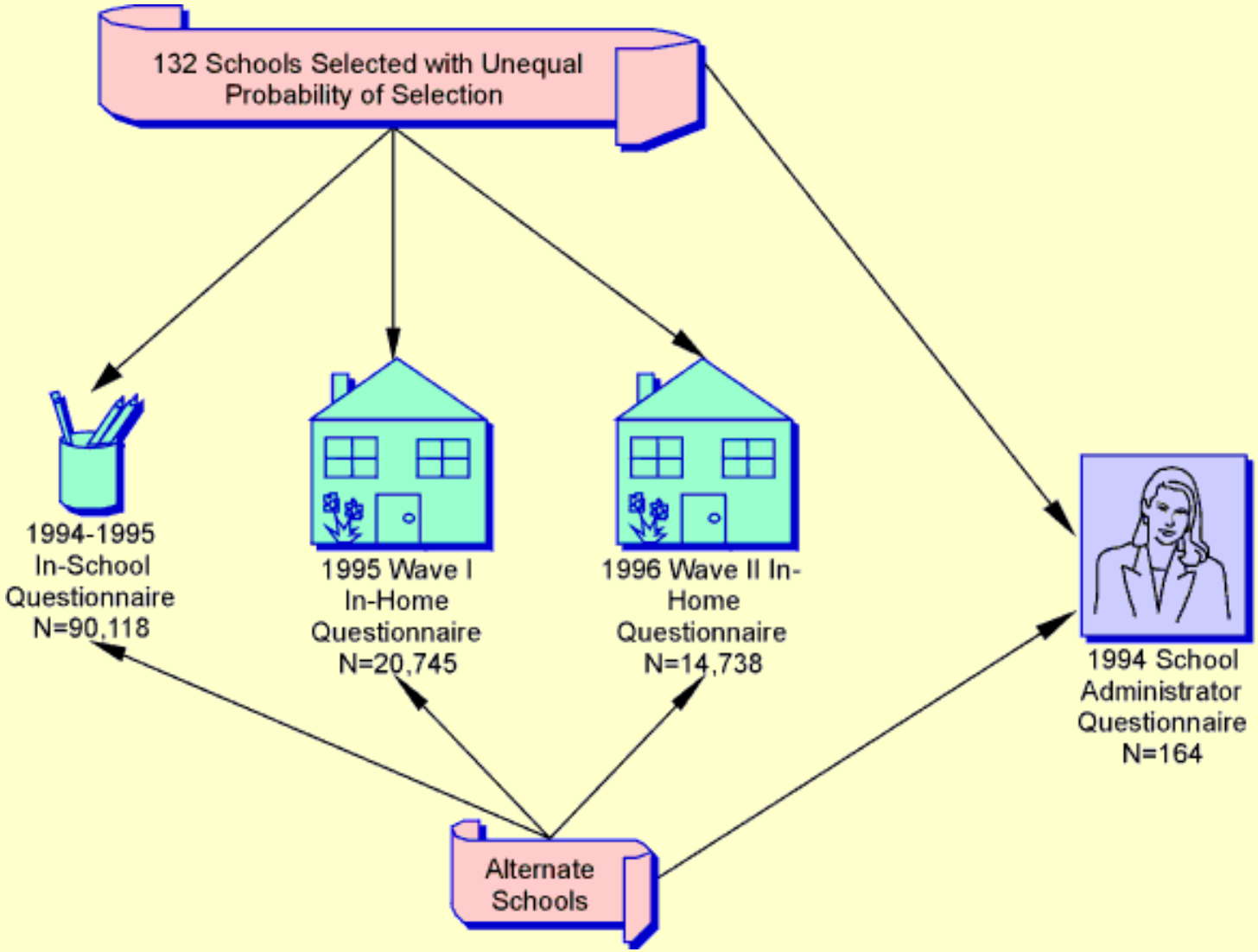
Name	Symbol	Type
Dopamine Transporter	DAT1	VNTR
Dopamine Receptor D4	DRD4	VNTR
Monoamine Oxidase A	MAOA	VNTR
Dopamine Receptor D2	DRD2	SNP
Cytochrome P450 - 2A6	CYP2A6	SNP
Serotonin Transporter	SLC6A4	VNTR

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

Symbol	Previous behavioral Associations
DAT1	Drug use/misuse, hyperactivity
DRD4	Novelty seeking, risk taking behaviors
MAOA	Aggression, depression, suicide, tobacco use, stress
DRD2	Drug use/misuse, inattention
CYP2A6	Metabolizes nicotine
SLC6A4	Depression, suicide, anxiety, violence, stress

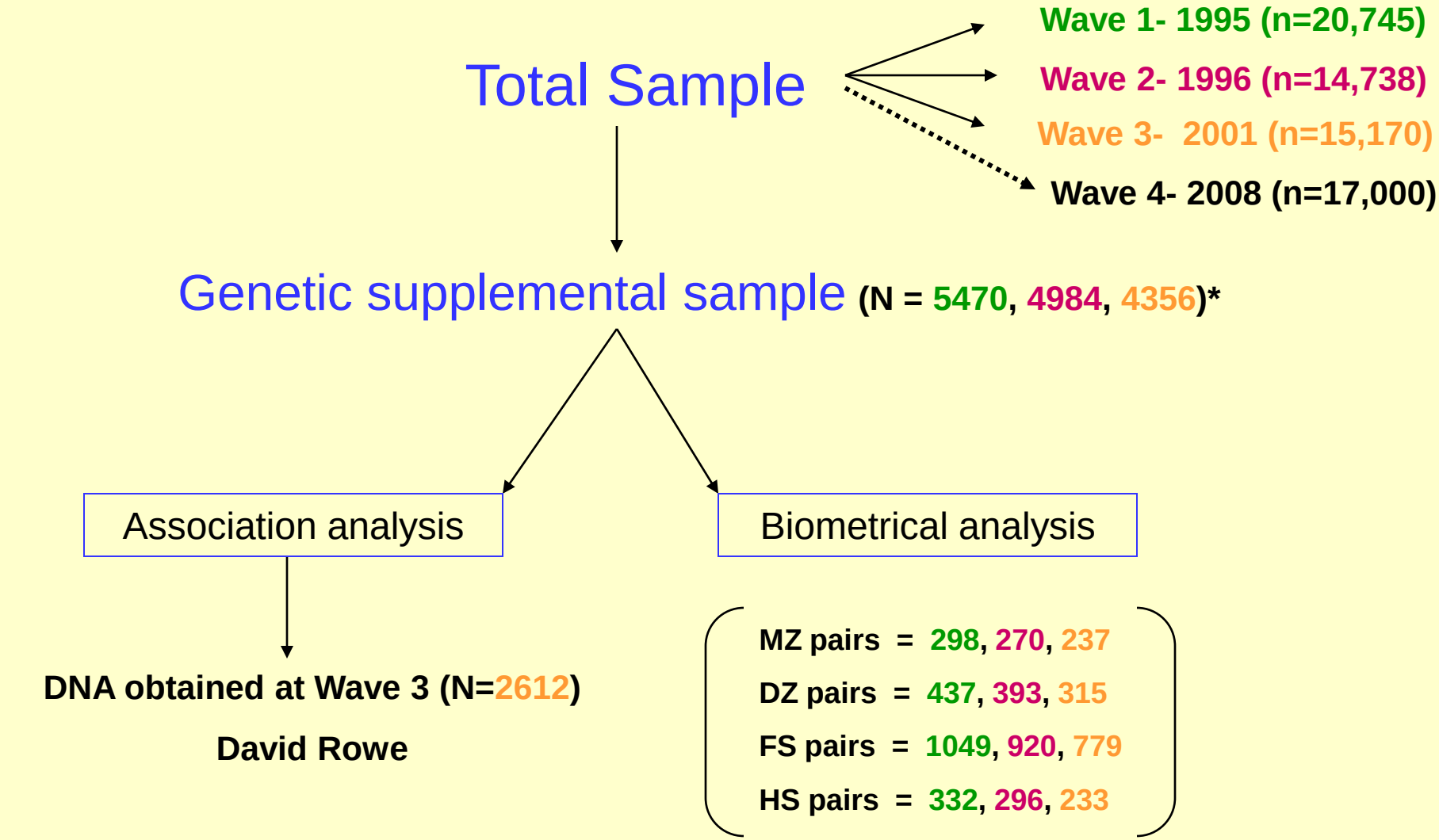
Sampling Structure for the National Longitudinal Study of Adolescent Health



Chantala, K & Tabor J, 1999

Carolina Population Center

National Longitudinal Study of Adolescent Health



Courtesy of David Timberlake

Twin Research and Human Genetics Volume 9 Number 6 pp. 988–997, 2006

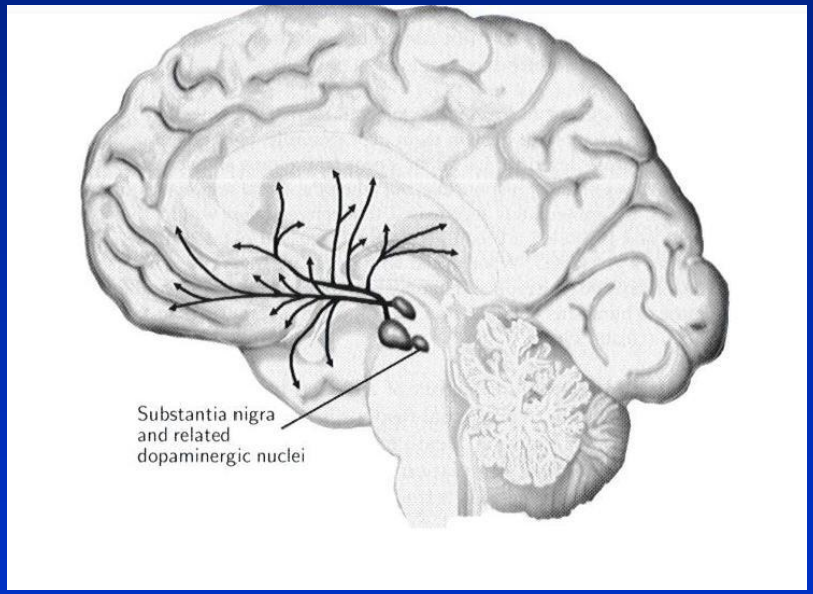
The National Longitudinal Study of Adolescent Health (Add Health) Twin Data

Kathleen Mullan Harris,[1,2] Carolyn Tucker Halpern,[1,3] Andrew Smolen,[4] and Brett C. Haberstick[4]

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

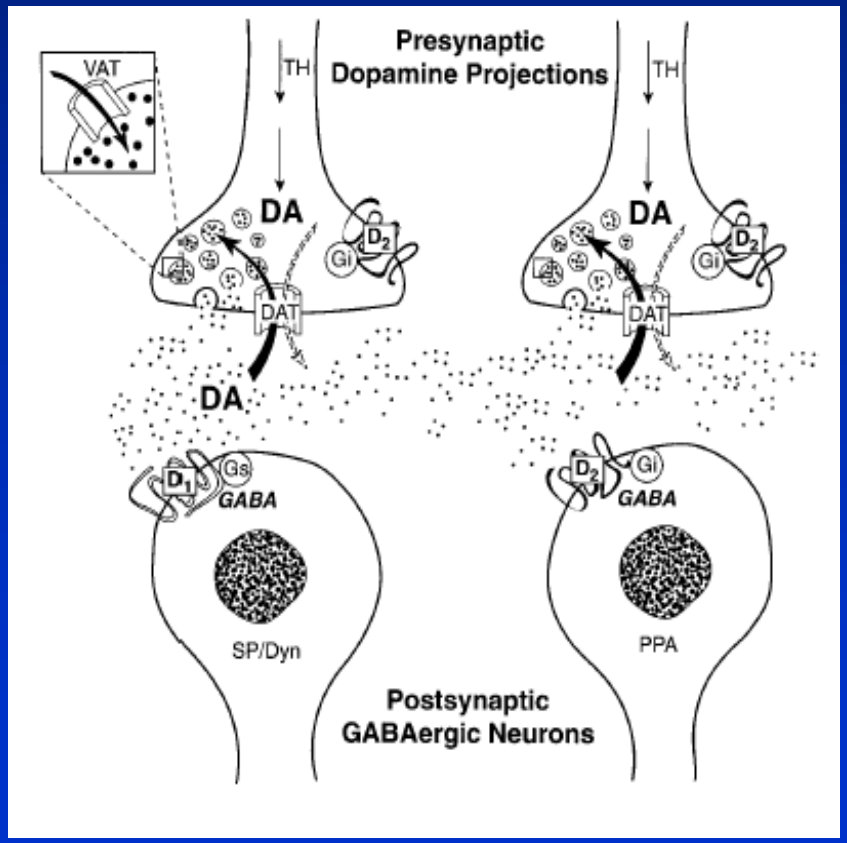
- Dopamine found in neurons of nigrostriatal, mesocortical and mesolimbic systems.
- Role in motor function, reward, reinforcement, emotional expression, neuroendocrine release and behavioural homoeostasis.



from Dr. Ursula. M. D'Souza

Dopaminergic Receptor System

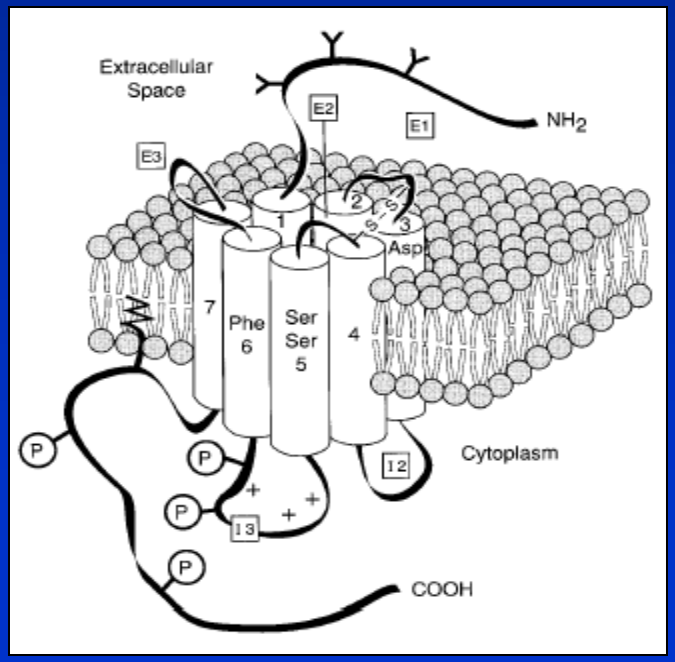
Transporters & Auto-transporters



Receptors

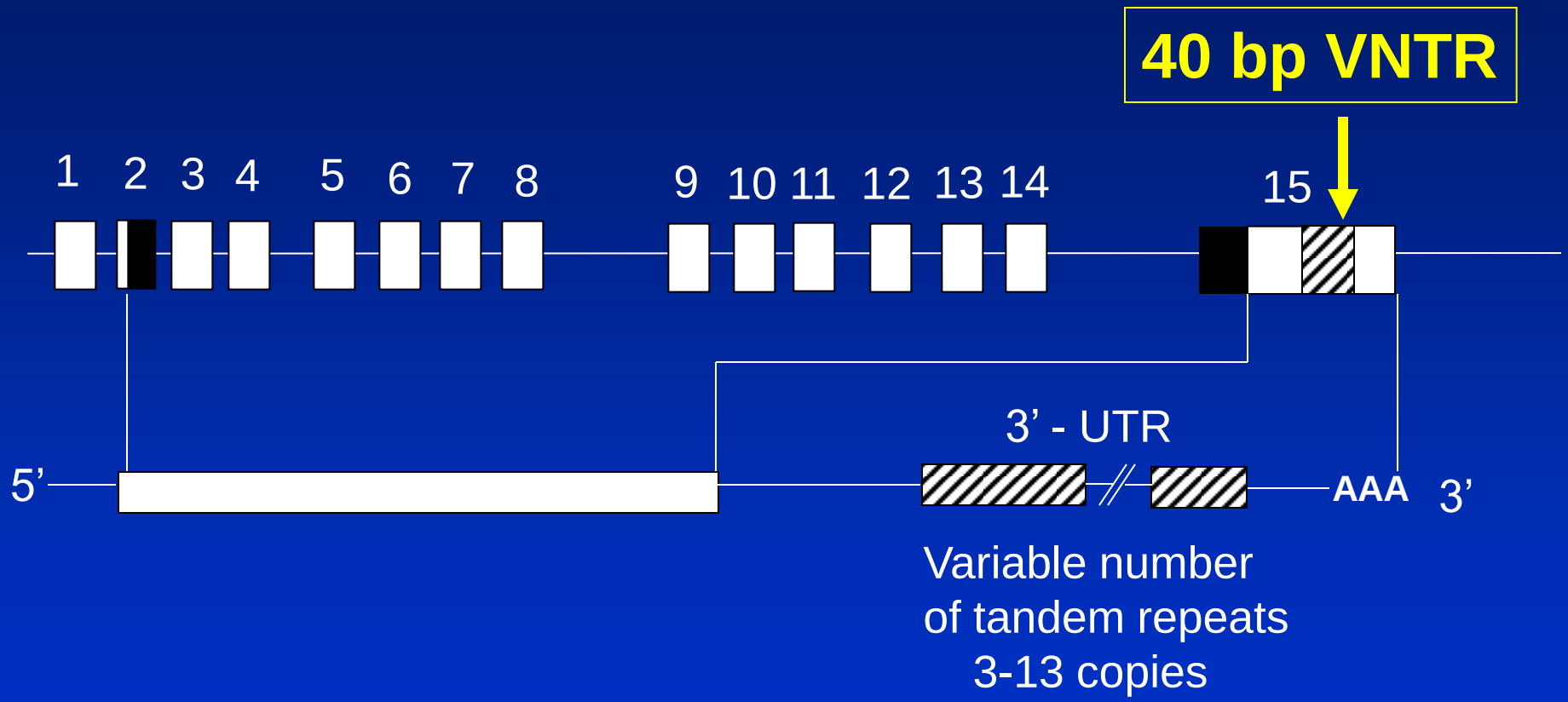
D_1-like (D1, D5)

D_2-like (D2, D3 and D4)



Missale et al., 1998: Physiology Reviews 78(1): 189-225

VNTR polymorphism of Dopamine Transporter

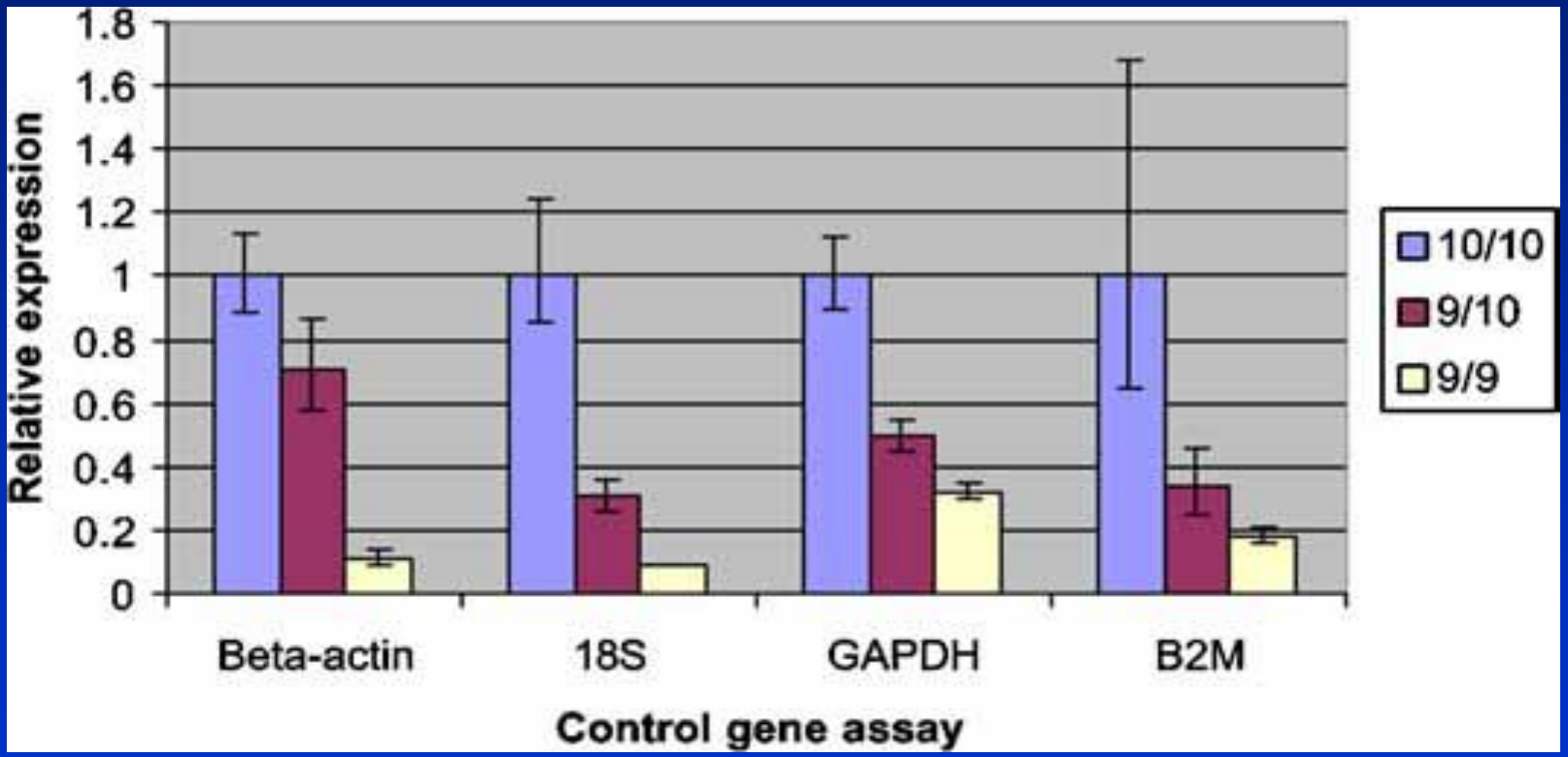


Adapted from Miller & Madras, 2002; Fuke et al., (2001), Vandenberg et al., 1992

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 – increased density of DAT in ADHD patients. Krause et al., (2000).
    - 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)



Mill et al., (2002), Am. J. Med. Genet. 114: 975-979

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

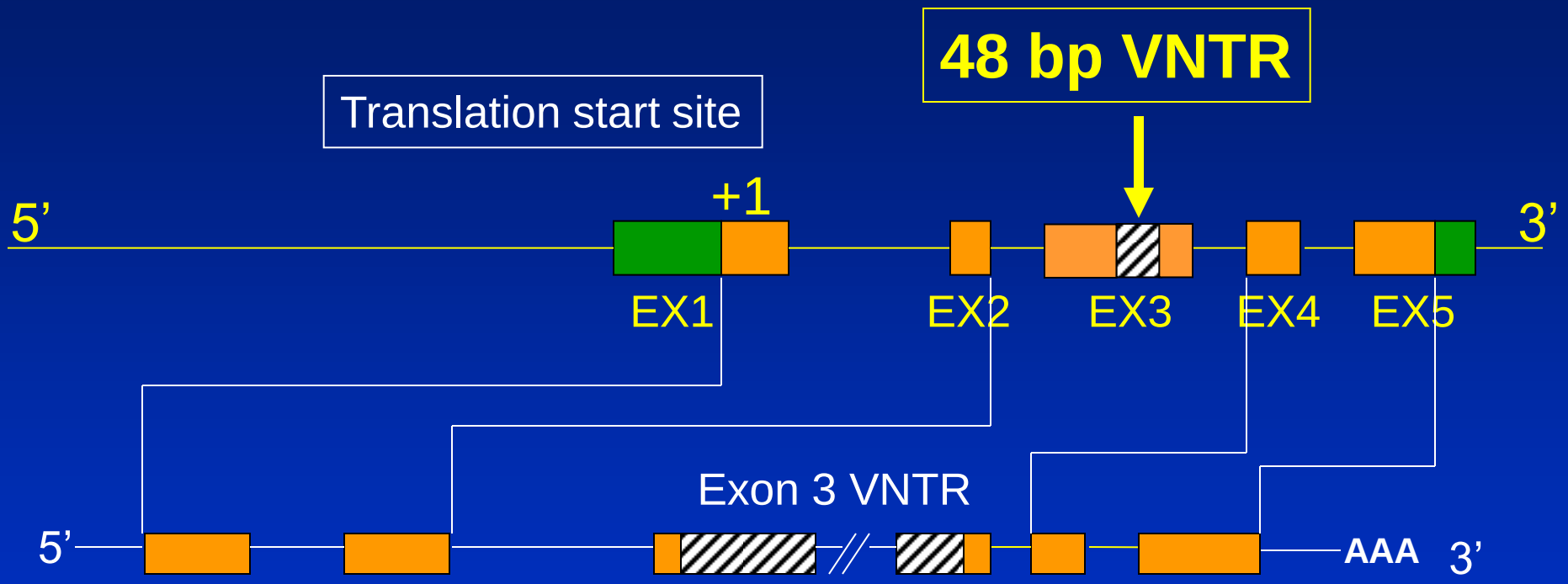
- DRD4 is A D_2-like receptor
- Fragment lengths vary between 379 bp - 811 bp
- *4R* (475 bp) & *7R* (619 bp) alleles are most common
  (64.3% and 20.6%, respectively)
- Results in variation in the 3rd cytoplasmic loop of the receptor affecting G-protein binding

- DRD4 is activated by dopmamine
- Inhibits adenylate cyclase and reduces cAMP levels

- *7R* allele exhibits blunted ability to reduce cAMP levels

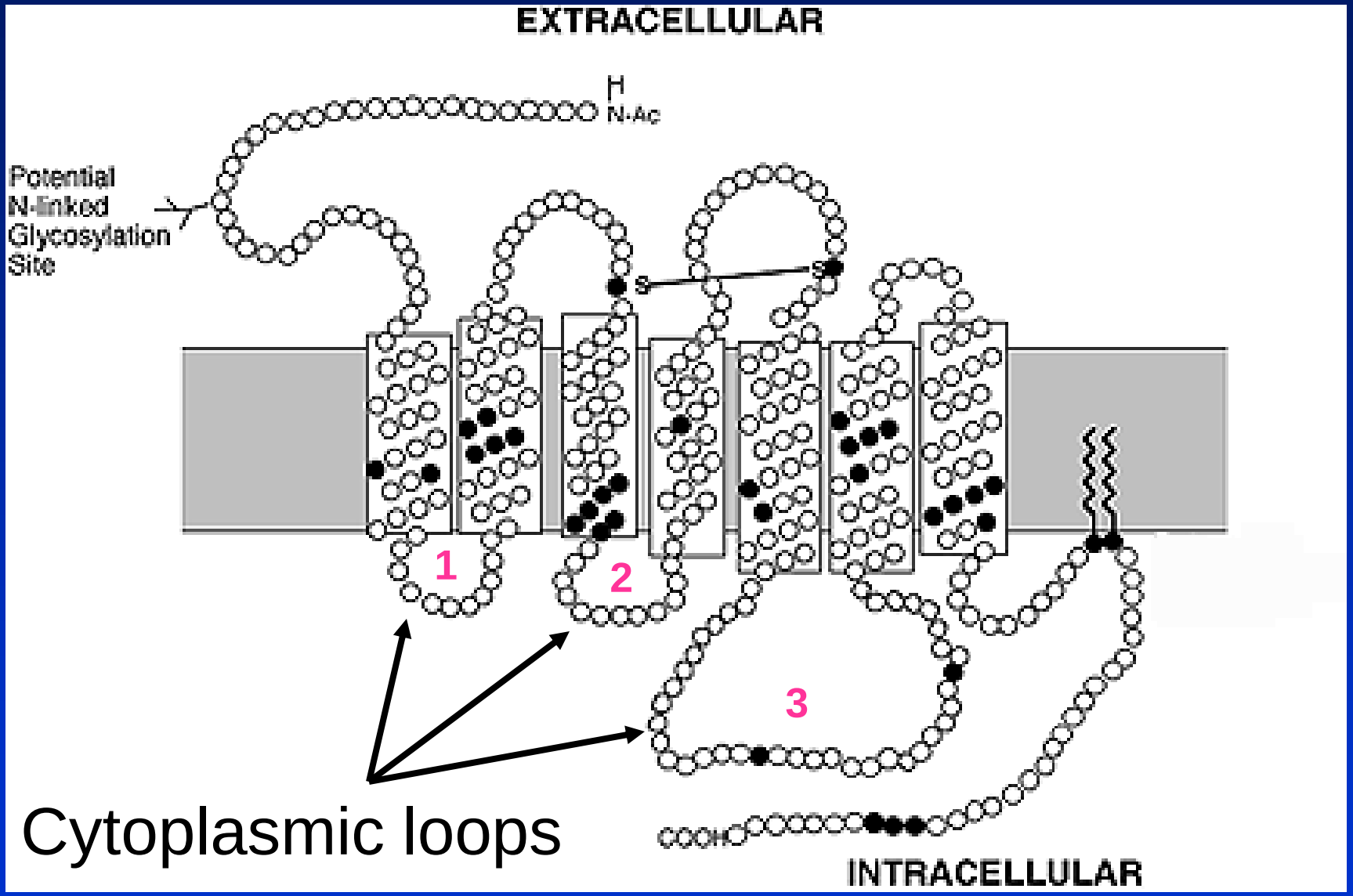
Wang et al., (2004); DiMaio et al., (2003); Anchordoquy et al., (2003)

DRD4 VNTR Polymorphism in Exon 3



Adapted from: D’Souza; van Tol et al., (1992)

Model of the DRD4 Receptor Protein

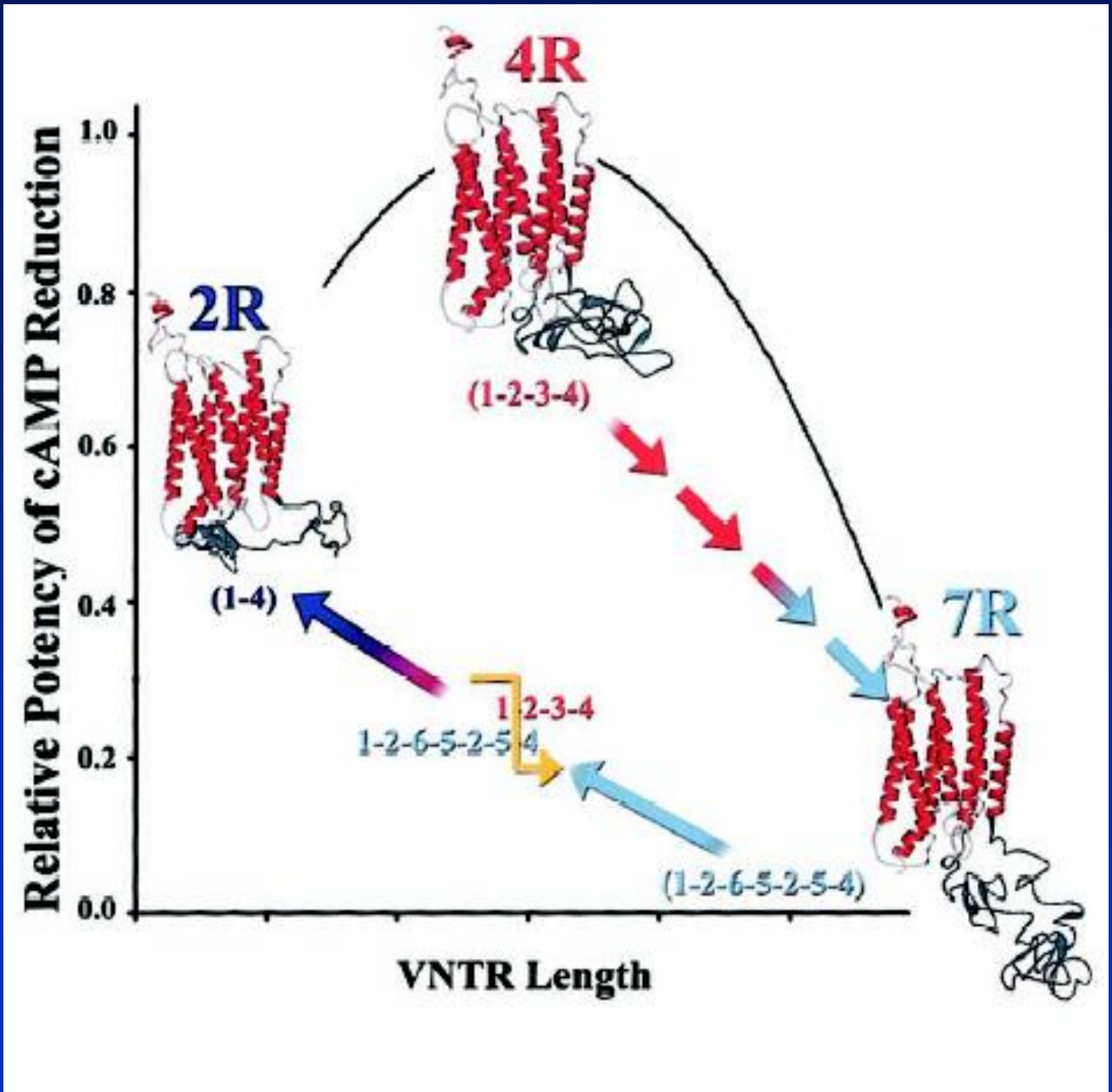


Cytoplasmic loops

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 3rd 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)



Wang et al., (2004), Am. J. Hum. Genet. 74: 931-944

Health Psychology
2006, Vol. 25, No. 2, 190–197

0278-6133/06/$12.00 DOI: 10.1037/0278-6133.25.2.190

An Association Between the DAT1 Polymorphism and Smoking Behavior in Young Adults From the National Longitudinal Study of Adolescent Health

David S. Timberlake, Brett C. Haberstick, Jeffrey M. Lessem, Andrew Smolen, Marissa Ehringer, and John K. Hewitt
University of Colorado at Boulder

Christian Hopfer
University of Colorado Health Sciences Center

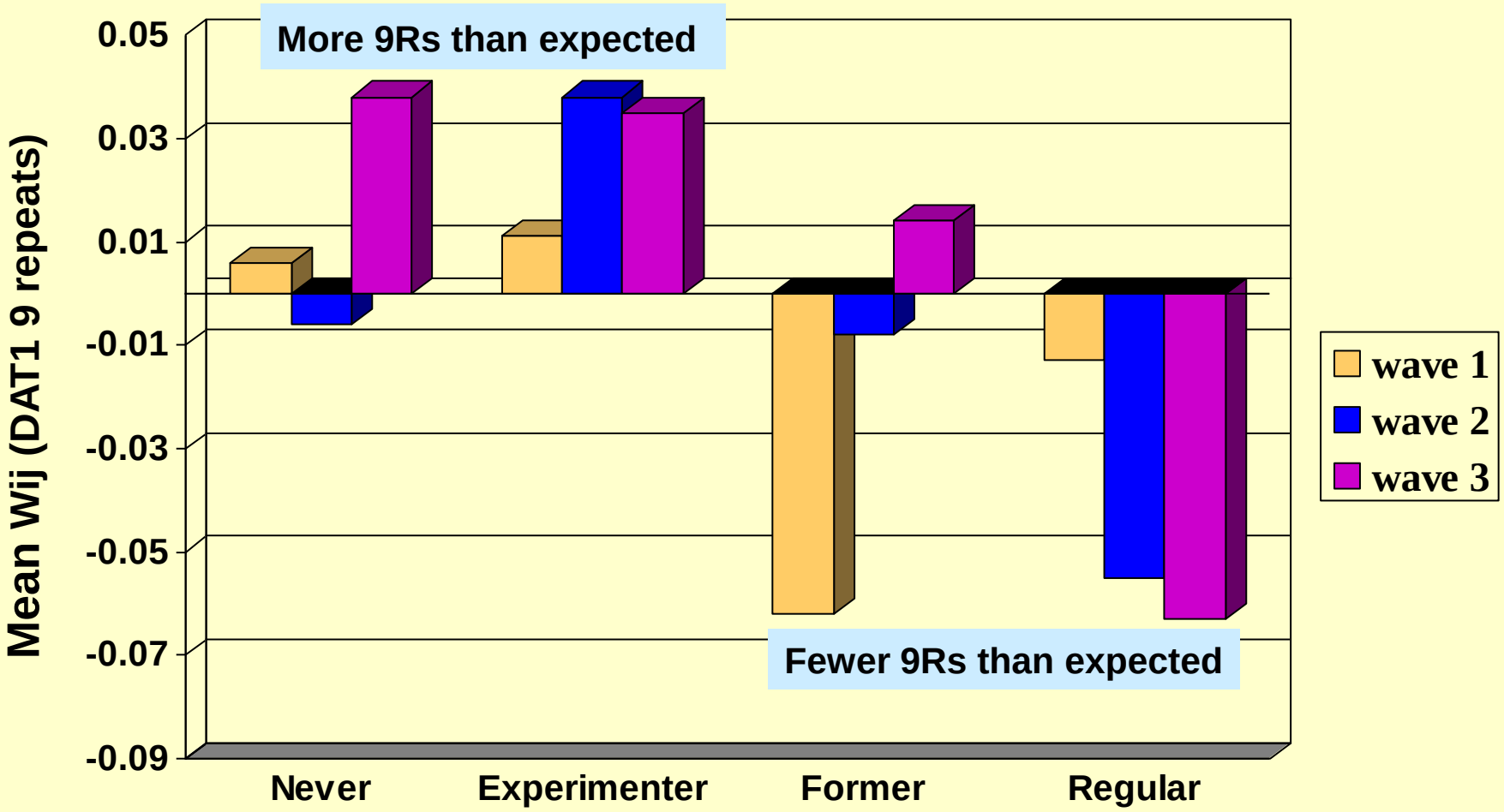
Question:

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

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Drug and Alcohol Dependence 78 (2005) 187–193
DRUG and ALCOHOL DEPENDENCE
www.elsevier.com/locate/drugalcdep

Genetic influences on quantity of alcohol consumed by adolescents and young adults☆

Christian J. Hopfer[a,*], David Timberlake[b], Brett Haberstick[b], Jeffrey M. Lessem[b], Marissa A. Ehringer[b], Andrew Smolen[b], John K. Hewitt[b]

[a] *Department of Psychiatry, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Box C-268-35, Denver, CO 80262, USA*
[b] *Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309, USA*

Question:

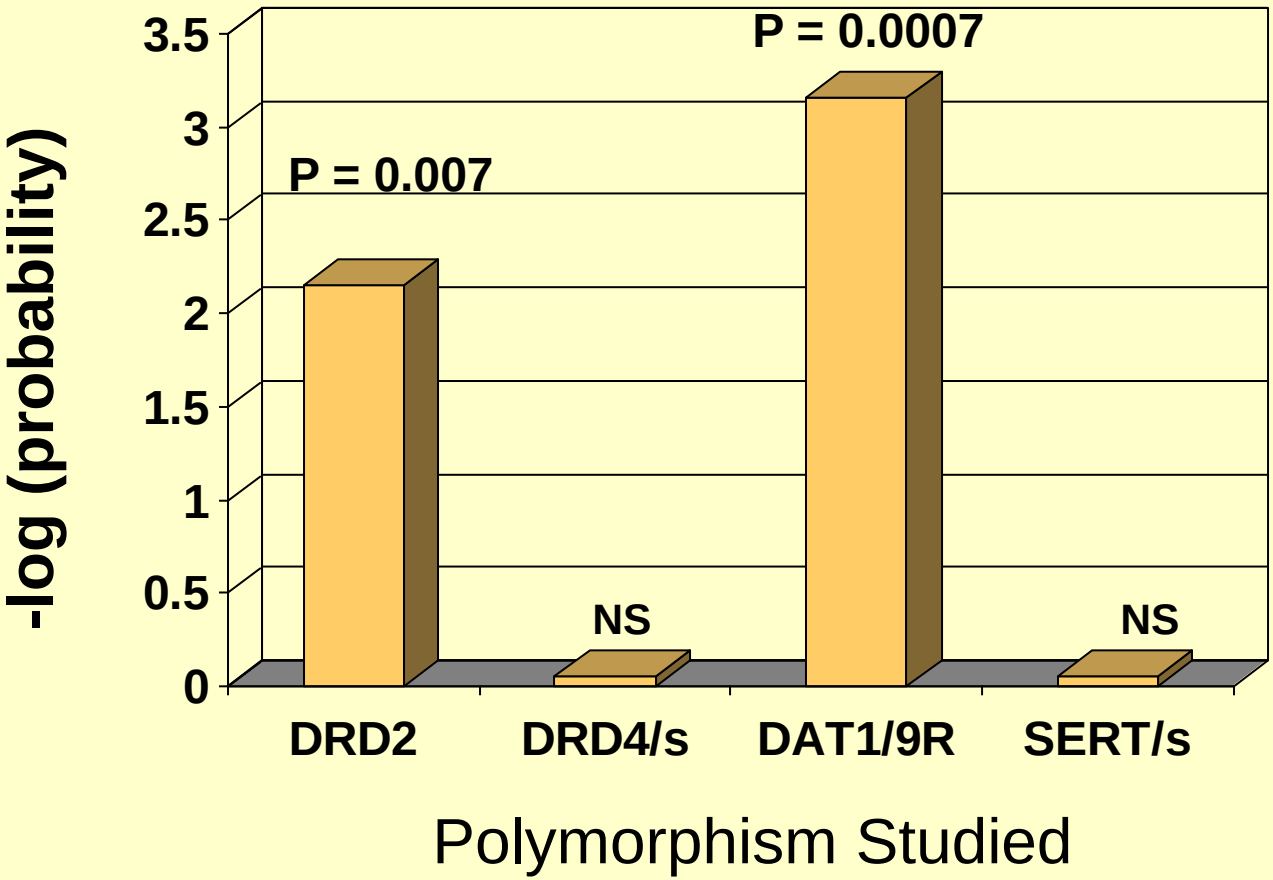
Do the DRD2 TaqI, DRD4, dopamine transporter or serotonin transporter polymorphisms affect alcohol consumption?

Answer:

Yes. For DRD2 and DAT1 9 repeat.

No. For DRD4 and SERT.

Association of Four Genes with Alcohol Consumption



The DRD2 TaqI and DAT1 polymorphisms are associated with average number of drinks per drinking episode

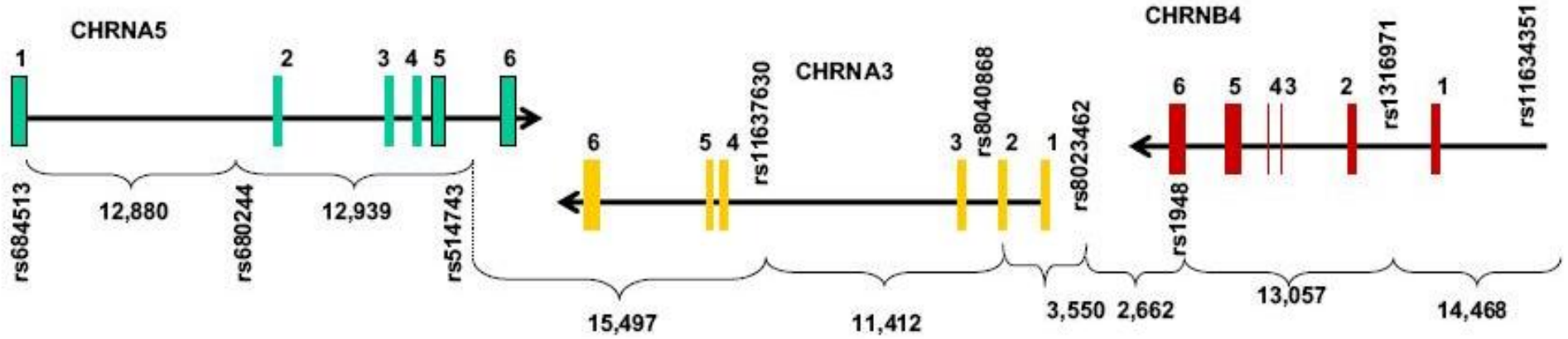
Human Molecular Genetics, 2008, Vol. 17, No. 5 724–734
doi:10.1093/hmg/ddm344
Advance Access published on November 30, 2007

The neuronal nicotinic receptor subunit genes (CHRNA6 and CHRNB3) are associated with subjective responses to tobacco

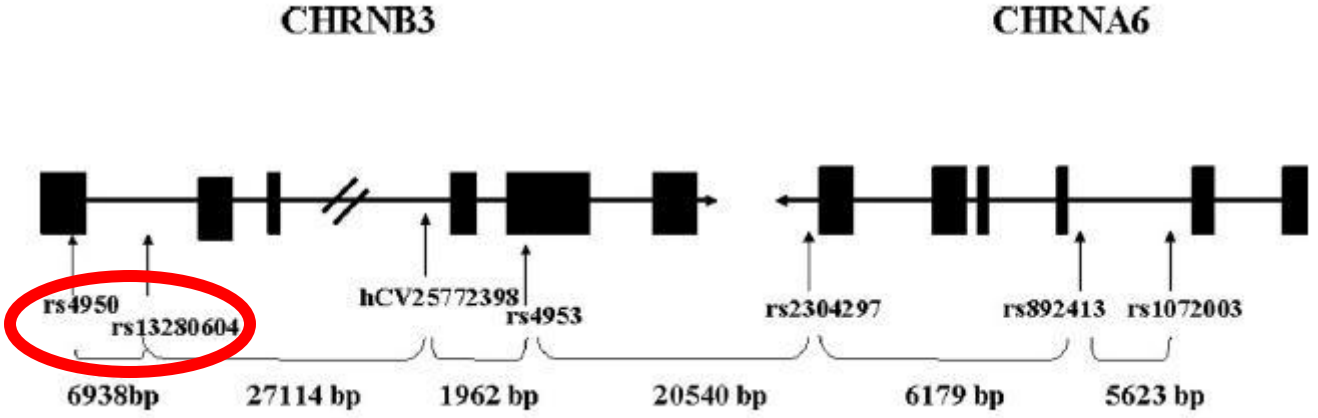
Joanna S. Zeiger[1,†], Brett C. Haberstick[1,4,†], Isabel Schlaepfer[1,2], Allan C. Collins[1,3], Robin P. Corley[1], Thomas J. Crowley[4], John K. Hewitt[1,3], Christian J. Hopfer[4], Jeffrey Lessem[1], Matthew B. McQueen[1,3], Soo Hyun Rhee[1,3] and Marissa A. Ehringer[1,2,*]

The Add Health sample was used to confirm an association between subjective effects to nicotine and the CHRNB3 gene.

CHRNA5/A3/B4 Gene Cluster on chromosome 15q25.1



CHRNB3 and CHRNA6 genes on chromosome 8p11.2 – 8p11



“the Caspi hypothesis….”

Science. 2002 Aug 2;297(5582):851-4.

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

  Dodge et al. (1990). Mechanisms in the cycle of violence. *Science* 250: 1678- 1683.

- Substance misuse

  Windsom, CS et al. Alcohol abuse in abused and neglect children followed-up: Are they at increased risk? *Journal of Studies on Alcohol:,* 58: 207-217.

- Depression

  Brown, J. et al. (1999). Childhood abuse and neglect: Specificity of effects on adolescent and young adult depression and sucidallity. *J. Am Acad Child Adolesc Psychiatry,* 38: 1490 - 1496.

- Poor academic performance

  Shonk SM et al. Maltreatment, compentency deficits, and risk for academic and behavioral development. *Dev. Psychopathology,* 37: 3 -17.

Neurological consequences:

- Promotes adaptation in particular brain structures.

  DeBillis, MD. (2001). Developmental traumatology: The psychobiological development of children and its implication.. *Dev. Psychopathology,* 13: 539- 564.

- Increased levels of neurotransmitters

  Glaser, D. (2003). Child abuse and neglect and the brain: A review. *J. Chilc Psychol Psychiatry*, 41: 91 - 116.

MAOA: Behavior and neurological effects

Prior evidence:

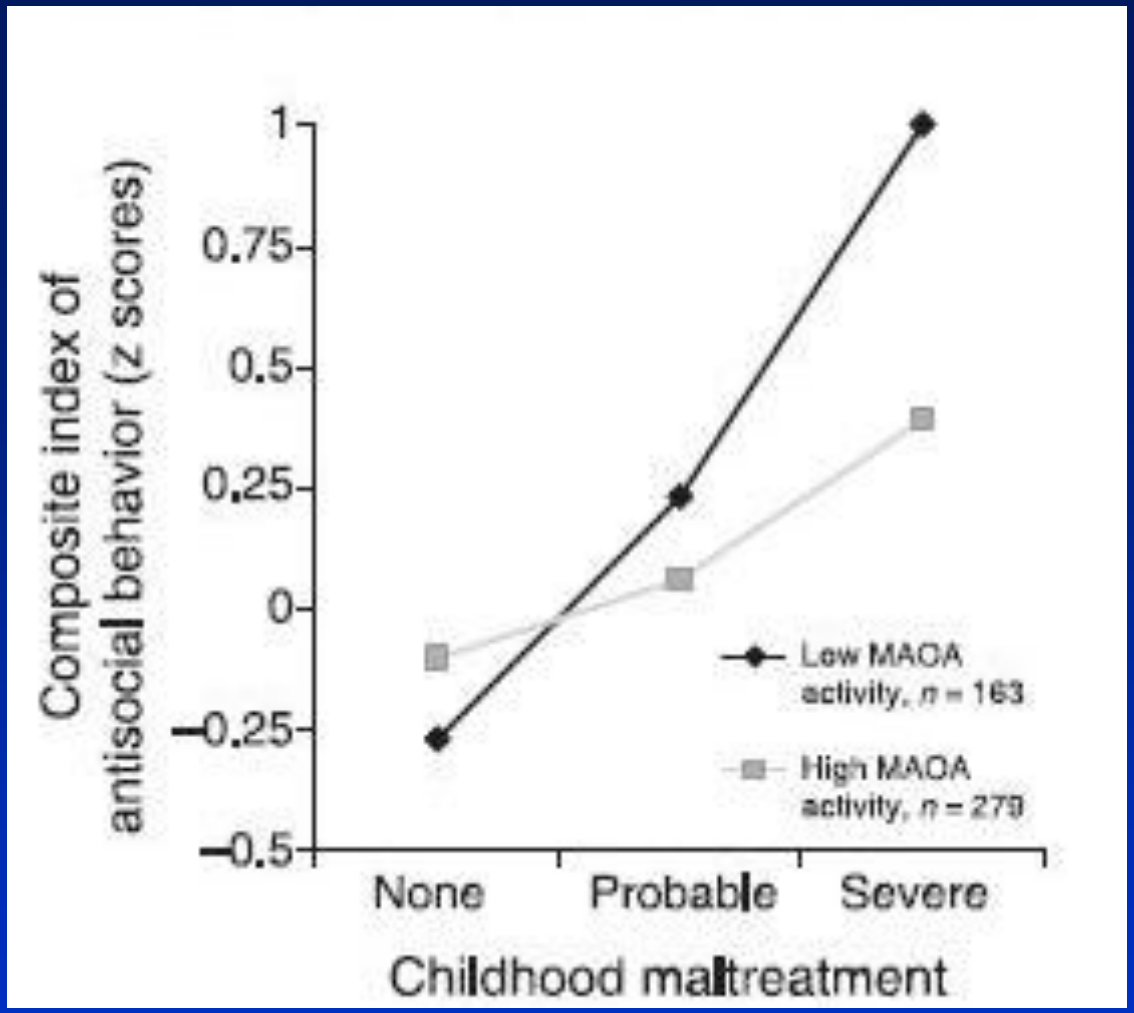
- **Aggression**
  1. **Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA. (1993)** Abnormal behavior associated with a point mutation in the structural gene encoding monoamine oxidase A. *Science,* 262: 578-580.
  2. **Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Poumin, S., Muller, U., Aguet, M., Babinet, C., Shih, JC., et al. (1995)** Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. *Science,* 268: 1763-1766.

- **Increased neurotransmitter levels**
  1. **Deckert J, Catalano M, Syagailo YV, Bosi M, Okladnova O, Di Bella D, Nothen MM, Maffei P, Franke P, Fritze J, Maier W, Propping P, Beckmann H, Bellodi L, Lesch KP. (1999)** Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. *Human Molecular Genetics,* Apr: 8(4):621-4.
  2. **Sabol, SZ, Hu, S, Hammer, D. (1998)** Functional polymorphism in the monoamine oxidase A promoter. *Human Genetics,* 103(3) 273 - 279.

Dunedin Sample

2R and 3R = low activity

3.5R and 4R = high activity



Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R. Role of genotype in the cycle of violence in maltreated children. Science. 297: 851-854, 2002.

"…Maltreated children with a genotype conferring high levels of MAOA expression were less likely to develop antisocial problems…"

American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 135B:59–64 (2005)

Monoamine Oxidase A (MAOA) and Antisocial Behaviors in the Presence of Childhood and Adolescent Maltreatment

Brett C. Haberstick,[1]* Jeffrey M. Lessem,[1] Christian J. Hopfer,[2] Andrew Smolen,[1] Marissa A. Ehringer,[1] David Timberlake,[1] and John K. Hewitt[1]

[1]*Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado*

[2]*Department of Psychiatry, University of Colorado Health Sciences Center, Denver, Colorado*

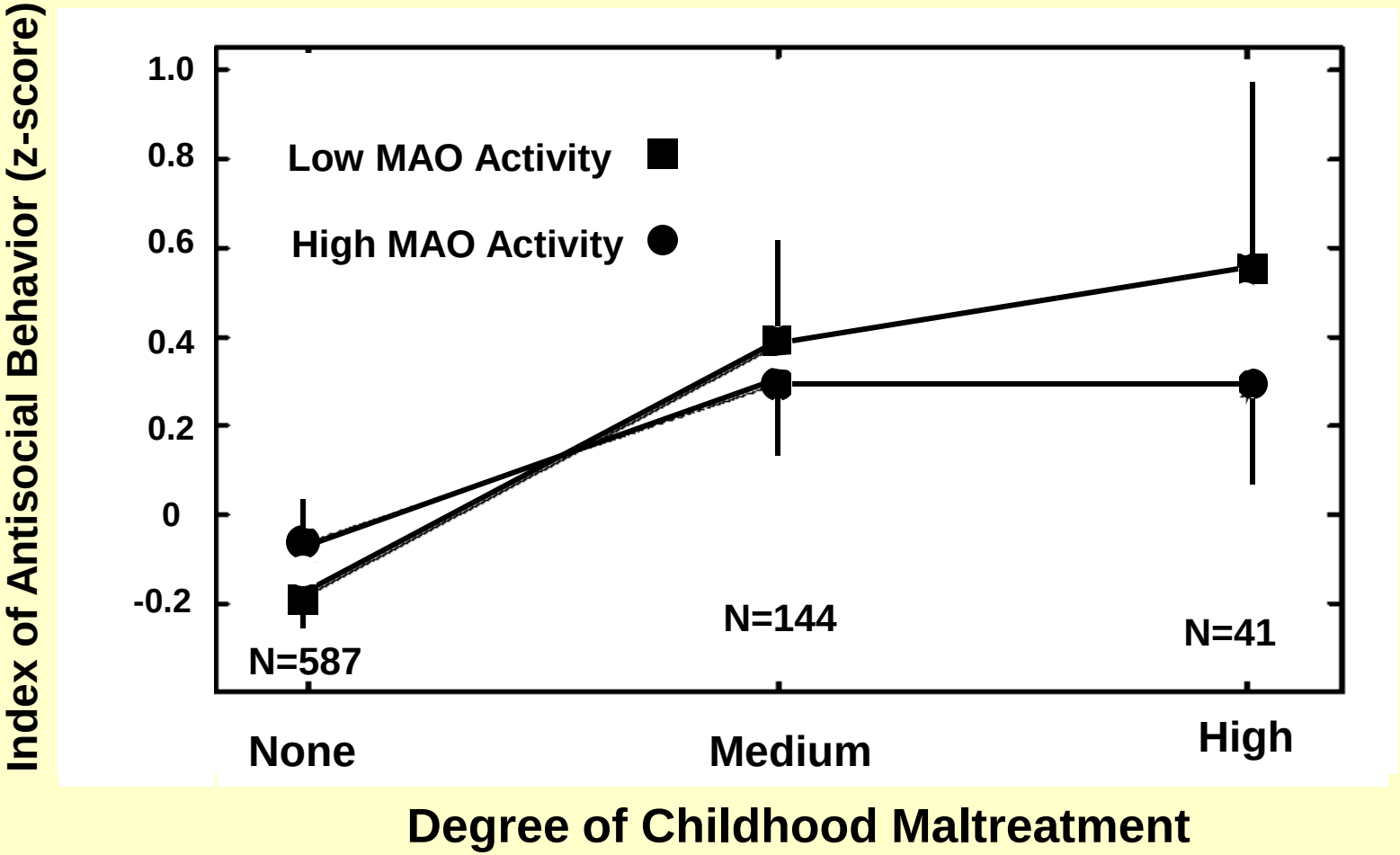
Question:

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



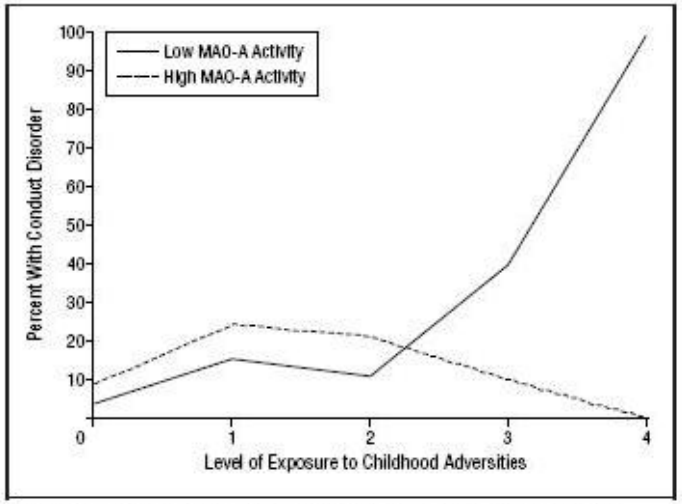
Higher early maltreatment leads to higher antisocial behavior

We found no effect of MAOA activity

ARCH GEN PSYCHIATRY/VOL 61, JULY 2004, 738-744

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.

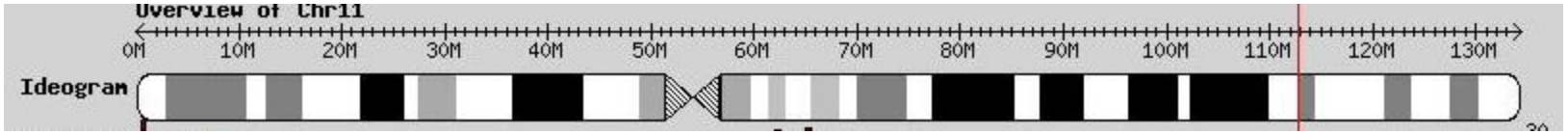
Table 2. Prevalence of Conduct Disorder by MAO-A Genotype and Level of Exposure to Childhood Adversity*

	Level of Exposure to Childhood Adversity, No. (%)				
	0	1	2	3	4
Low MAO-A	4/99 (4.04)	3/19 (15.79)	3/27 (11.11)	2/5 (40)	1/1 (100)
High MAO-A	22/249 (8.84)	13/53 (24.53)	10/47 (21.28)	1/10 (10)	0/4 (0)
P Value, 2-tailed Fisher exact test	*P* = .17	*P* = .53	*P* = .35	*P* = .24	*P* = .20

Dopamine D2 Receptor, DRD2

11q22-q23

minus strand



[112,690,461 ▶ ◀ [113,149,635

TTC12 → **DRD2** ← ZW10 ←

ANKK1 → TMPRSS5 ←

rs1801028
Ser311Cys

rs17601612 **TaqI B** **rs1079597** **rs6275** **NcoI** **rs6277** **HpaII** **rs1800497T** **aqI A**

rs427422

rs4350392

5’ **3’**

1 2 3 4 5 6 7 8

rs7122454 **rs4587762** **rs12363125** **rs1124492** **rs6277** **C957T**

Coding Region

J Neural Transm (2010) 117:827–830
DOI 10.1007/s00702-010-0421-8

BASIC NEUROSCIENCES, GENETICS AND IMMUNOLOGY - ORIGINAL ARTICLE

Association between the A1 allele of the DRD2 gene and reduced verbal abilities in adolescence and early adulthood

Kevin M. Beaver · Matt DeLisi · Michael G. Vaughn · John Paul Wright

Received: 28 January 2010 / Accepted: 12 May 2010 / Published online: 9 June 2010

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,[1] John Paul Wright,[2] and Kevin M. Beaver[3]

International Journal of Offender Therapy and Comparative Criminology
XX(X) 1–17

DOI: 10.1177/0306624X10361583
http://ijo.sagepub.com
SAGE

"…offenders who are violently victimized are more likely to carry the DRD2 (A1) risk allele than offenders who have not been violently victimized."

International Journal of
Offender Therapy and
Comparative Criminology
Volume 51 Number 6
December 2007 620-645

10.1177/0306624X07304157
http://ijo.sagepub.com
hosted at
http://online.sagepub.com

Evidence of a Gene X Environment Interaction in the Creation of Victimization

Results From a Longitudinal Sample of Adolescents

Kevin M. Beaver
Florida State University, Tallahassee
John Paul Wright
University of Cincinnati, Ohio
Matt DeLisi
Iowa State University, Ames
Leah E. Daigle
Georgia Southern University, Statesboro
Marc L. Swatt
Northeastern University, Boston, MA
Chris L. Gibson
University of Florida, Gainesville

"*DRD2 (TaqIA genotype)* interacted with delinquent peers to predict victimization (in causcasian males)."

Psychiatry Research 175 (2010) 160–164

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Genetic risk, parent–child relations, and antisocial phenotypes in a sample of African-American males

Kevin M. Beaver [a,*], Ashley Sak [a], Jamie Vaske [b], Jessica Nilsson [a]

[a] College of Criminology and Criminal Justice, Florida State University, Tallahassee, FL 32306, United States
[b] Division of Criminal Justice, University of Cincinnati, Cincinnati, OH 45221-0389, United States

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."

Psychiatric Genetics 2009, **19**:14-26

Trajectories of depressive symptoms, dopamine D2 and D4 receptors, family socioeconomic status and social support in adolescence and young adulthood

Guang Guo[a] and Kathryn Harker Tillman[b]

Males heterozygous for DRD2 (A1/A2) displayed more depressive symptoms than either homozygous males. Heterosis.

Interaction with DRD4 2R/2R.

Arch Sex Behav (2007) 36:543–554
DOI 10.1007/s10508-006-9084-9

ORIGINAL PAPER

Gene-Environment Contributions to Young Adult Sexual Partnering

Carolyn T. Halpern · Christine E. Kaestle · Guang Guo · Denise D. Hallfors

"Contrary to hypothesis, presence of the A1 *DRD2* allele was associated with having had fewer sex partners in the past year."

Hum Genet (2007) 121:125–136
DOI 10.1007/s00439-006-0244-8

ORIGINAL INVESTIGATION

Contributions of the *DAT1* and *DRD2* genes to serious and violent delinquency among adolescents and young adults

Guang Guo · Michael E. Roettger · Jean C. Shih

"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."

Twin Research and Human Genetics April 2007, **10:** 285-298

Helping Relationships and Genetic Propensities: A Combinatoric Study of DRD2, Mentoring, and Educational Continuation

Michael J. Shanahan,[1] Lance D. Erickson,[2] Stephen Vaisey,[1] and Andrew Smolen[3]

[1] *University of North Carolina, Chapel Hill, United States of America*
[2] *Brigham Young University, Utah, United States of America*
[3] *University of Colorado, Boulder, United States of America*

"(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)

	N	p<.05	p<.01	p<.001
Candidate genes (10)	570	21	4	0
Zygosity panel (12)	684	64	24	2
Total (22)	1254	85	28	2

Hewitt

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.

SCIENCE 301:386-389 (2003)

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 Poulton[3]

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.

REVIEW

JAMA. 2009;301(23):2462-2471

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 Kovacs, 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 de-

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.

The NEW ENGLAND JOURNAL of MEDICINE

N Engl J Med 2003;348:1096-103.

ORIGINAL ARTICLE

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.*

Mutation	Change in Amino Acid Sequence	No. Affected		Allele Frequency	
		Obese Subjects	Controls	Obese Subjects	Controls
Known					
C728T	Thr112Met†‡	2	0	0.002	
C886T	Arg165Trp‡§	1	0	0.001	
A700G	Val103Ile†‡§¶‖	11	1	0.012	0.016
A1144C	Ile251Leu†‡‖	5	0	0.005	
Novel					
A424G	Thr11Ala¶	1	0	0.001	
T544C	Phe51Leu	1	0	0.001	
A991G	Met200Val	2	0	0.002	
C408T	Thr5Thr	1	0	0.001	
A1419G 3' UTR	—	1	0	0.001	

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 *a priori* replication.
- More scientific skepticism.

Add Health Wave 4 provides a real opportunity to achieve all of these goals.

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Ms. Natalie Meinerz
Ms. Alicia Pardo

Carolina Population Center & Add Health Study

Dr. Kathleen Mullan Harris
Dr. Carylon Tucker Halpern
Ms. Joyce Tabor
Ms. Dana Creighton

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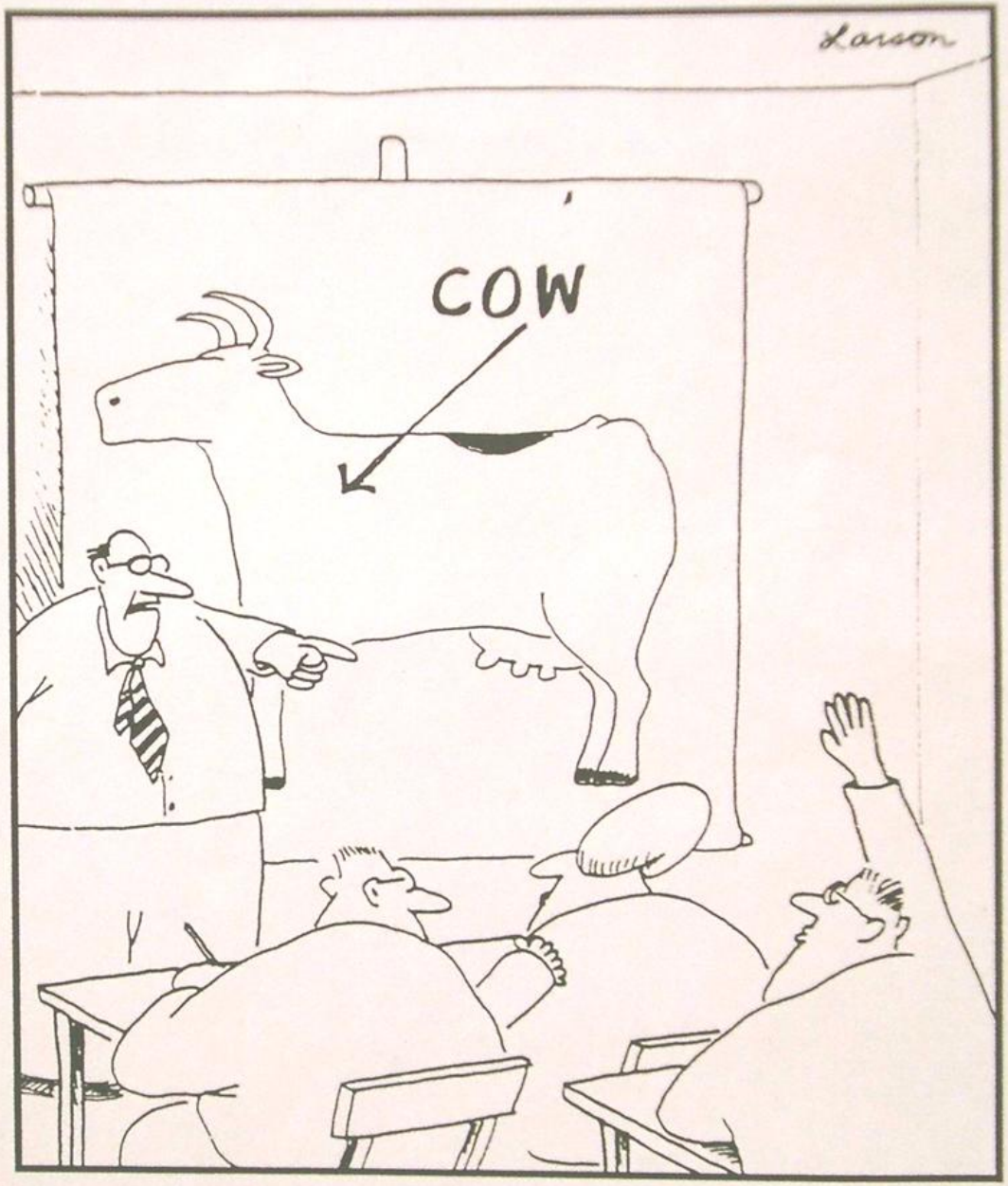
The National Longitudinal Study of Adolescent Health
HD31921 **PI: Kathleen Mullan Harris**

Add Health Co-Funders

- National Institute of Child Health and Human Development*
- National Cancer Institute*
- National Center for Health Statistics, Centers for Disease Control and Prevention, DHHS
- National Center for Injury Prevention and Control, Centers for Disease Control and Prevention, DHHS*
- National Center for Minority Health and Health Disparities*
- National Institute of Allergy and Infectious Diseases*
- National Institute of Deafness and Other Communication Disorders*
- National Institute of General Medical Sciences
- National Institute of Mental Health
- National Institute of Nursing Research*
- National Institute on Aging*
- National Institute on Alcohol Abuse and Alcoholism*
- National Institute on Drug Abuse*
- National Science Foundation*
- Office of AIDS Research, NIH*
- Office of the Assistant Secretary for Planning and Evaluation, DHHS*
- Office of Behavioral and Social Sciences Research, NIH*
- Office of the Director, NIH
- Office of Minority Health, Centers for Disease Control and Prevention, DHHS
- Office of Minority Health, Office of Public Health and Science, DHHS
- Office of Population Affairs, DHHS*
- Office of Research on Women's Health, NIH*

*Wave 4 co-funders



"Yes … I believe there's a question there in the back."