# SESSION 4: NEUROGENETICS AND HEALTH

**Dr. Rahul Kushwah**

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AnantLife

# REVIEW FROM LECTURES 1-3

- Folate and MTHFR
- CYP2D6 and codeine
- Vitamin A deficiency diet planning
- Gluten Intolerance
- BPA and Cancer
- Detoxification (Phase 1, 2)
- Detox – cancer
- CYP2A6 and lung cancer
- Estrogen Metabolism
- Strength training genetics
- Sedentary lifestyle hypertension
- **CYP2C9-Warfarin-Vitamin K**
- **ROS and exercise**
- **Type 1 diabetes genetics**
- **Type 2 diabetes genetics**
- CYP3A4 and Graperfruit juice (implications)
- COMT and Dopamine
- NAT, Red Meat and Cancer Risk

# SESSIONS 1 - 8

| Session | Topic | Evaluations |
|---|---|---|
| **1** | INTRODUCTION TO MOLECULAR GENETICS, MOLECULAR BIOLOGY AND HUMAN GENETICS | Discussion – Participation |
| 2 | NUTRITIONAL AND DIETARY GENETICS: HOW DO OUR GENES REGULATE OUR NUTRITION AND NUTRITIONAL HEALTH? | Discussion - Participation |
| **3.** | FITNESS GENETICS AND GENETICS OF CHRONIC DISEASES: HOW DO OUR GENES REGULATE OUR RESPONSE TO EXERCISE AND HOW DO GENES REGULATE THE RISK OF CHRONIC METABOLIC DISORDERS? | Discussion - Participation |
| **4.** | DETOXIFICATION GENETICS: HOW DO OUR GENES REGULATE DETOXIFICATION WHICH INDIRECTLY IMPACTS OVERALL HEALTH AND DISEASE RISK? | Discussion - Participation |
| **5.** | NEUROGENETICS: HOW DO OUR GENES REGULATE THE SYNTHESIS AND BREAKDOWN OF NEUROTRANSMITTERS AND ITS IMPACT ON OUR HEALTH ? | Take home exam on sections 1-5, due during session 6 |
| **6.** | GENETICS OF ENDOCANNABINOID PATHWAYS: HOW DO OUR GENES REGULATE THE RESPONSE TO CANNABIS? | Discussion - Participation |
| **7.** | SKIN GENETICS: HOW DO OUR GENES REGULATE OUR SKIN HEALTH ? | Take home assignment – due during session 8 |
| **8.** | DISCUSSION AND PRACTICAL APPLICATIONS OF GENETIC TESTS DISCUSSED IN SESSIONS 2-7 | Discussion - Participation |

# SESSION OBJECTIVES:

- Genetics of Autism and Dyslexia
- Genetics of Schizophrenia and psychosis
- Genetics of Depression
- Genetics of ADHD, OCD, aggression and addiction
- Genetics and cognition

# TERMINOLOGY

Peripheral and Central Nervous system - The PNS consists of the nerves and ganglia outside the brain and spinal cord.

Autonomic nervous system the part of the nervous system responsible for control of the bodily functions not consciously directed, such as breathing, the heartbeat, and digestive processes.

Somatic nervous system - peripheral nervous system associated with the voluntary control of body movements via skeletal muscles.

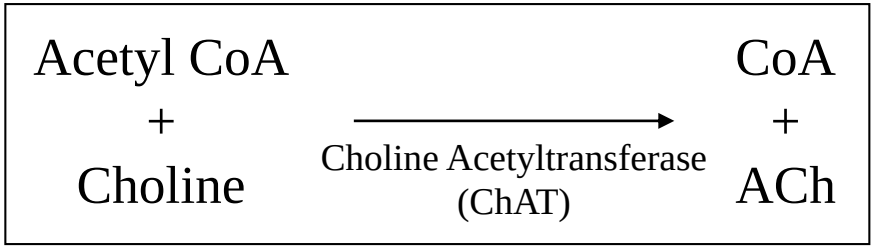
Excitatory and Inhibitory neurons - An excitatory neurons is defined as a neuron that triggers a positive change in the membrane of a post synaptic neuron it connects to. An inhibitory neuron triggers a negative change in the membrane of a post synaptic neuron it connects to.

Neurotransmitters - It is a type of chemical messenger which transmits signals across a chemical synapse, such as a neuromuscular junction, from one neuron (nerve cell) to another "target" neuron, muscle cell, or gland cell.

# Acetylcholine

- Neurotransmitter used at muscle junctions
- Mostly excitatory effects

**Synthesis**

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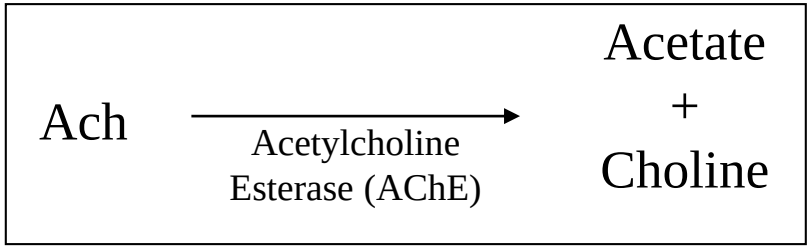
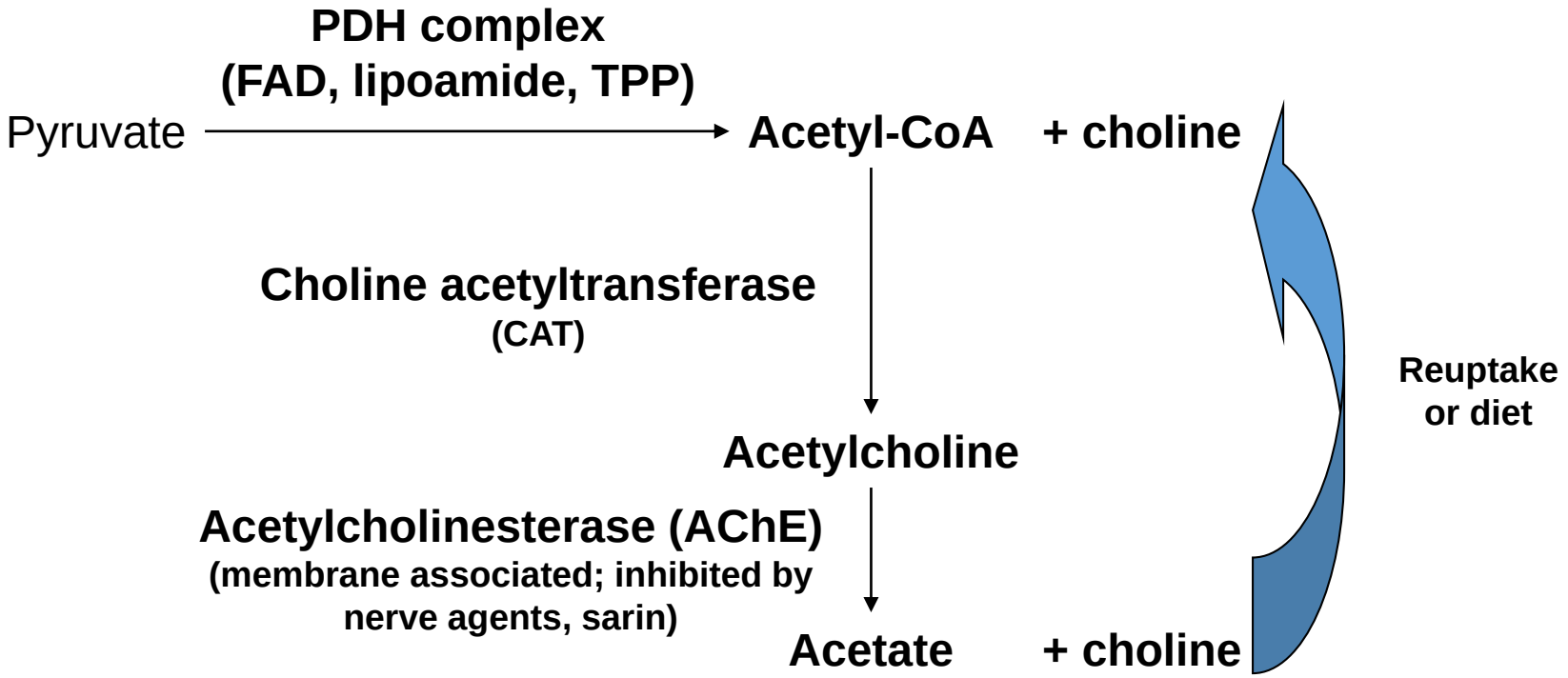


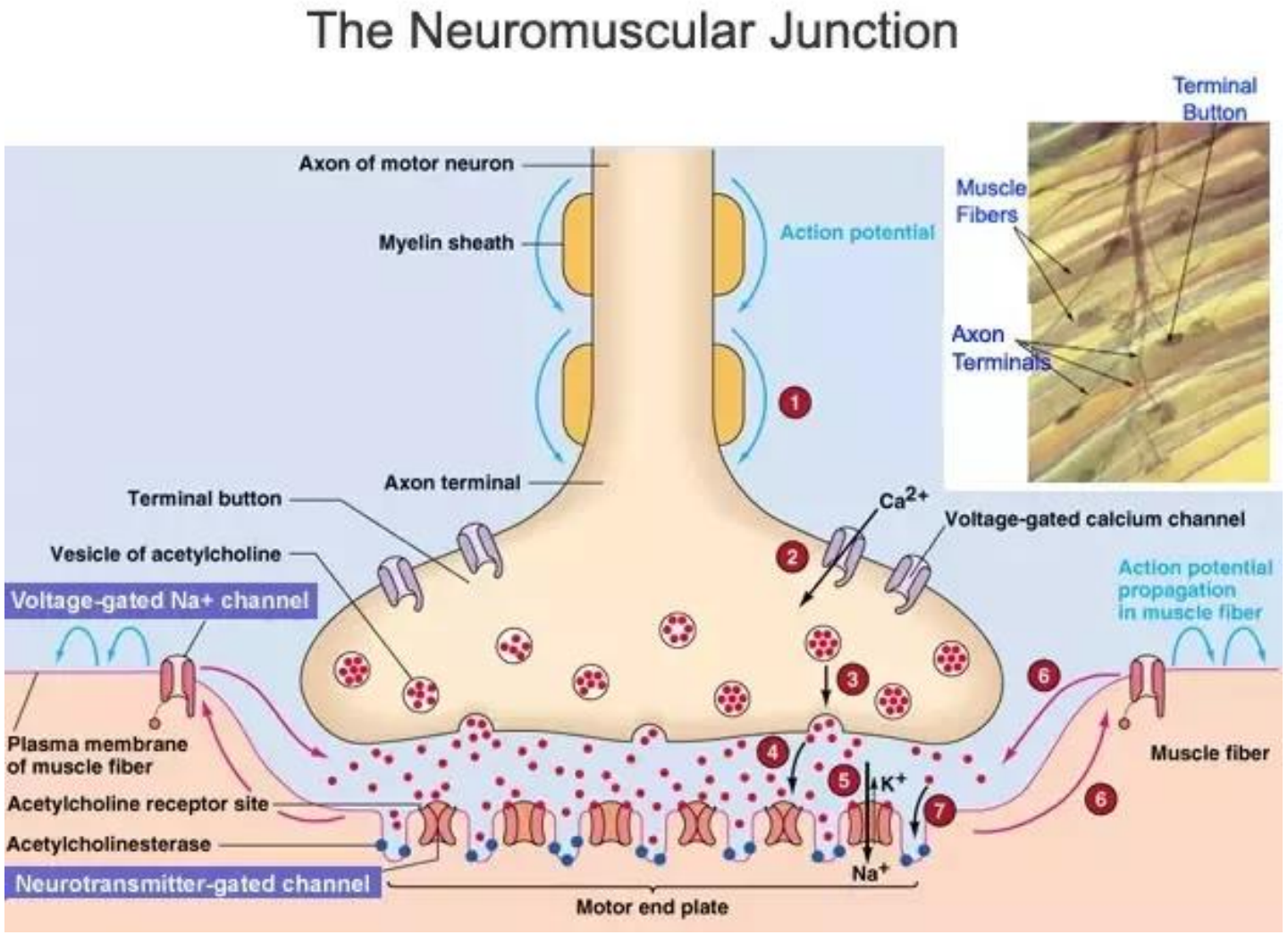

- 2 receptor types
  - Nicotinic (ionotropic- ion)
  - Muscarinic (metabotropic – G protein)

# Acetylcholine Synthesis and Degradation



# Neurotransmitters and Neuromodulators

## Acetylcholine: Neuromuscular junction



# Functions of Acetylcholine

- Basal Forebrain: arousal and attention
  - Learning and memory
  - Alzheimer Disease
- Parabrachial nucleus: REM sleep
  - Continue to fire during REM
  - # cells correlate with REM sleep
- Reward and Addiction
- Pain and other sensory input
- PNS function – muscle activity

# Acetylcholine synthesis Alzheimers

- CHAT gene
- This gene encodes for choline acetyl transferase
- ChAT catalyzes the transfer of an acetyl group from the coenzyme acetyl-CoA to choline, yielding acetylcholine (ACh).
- In Alzheimers – reduced cholinergic transmission
- Variation associated with response to acetylcholinesterases (break down Ach) inhibitors
- rs733722, T allele associated with response

# Acetylcholine synthesis Alzheimers

- CHAT gene and Alzheimer's risk
- The missense transition of G → A in rs3810950 polymorphism results in a change of alanine to threonine in the amino acid sequence – reduced CHAT levels

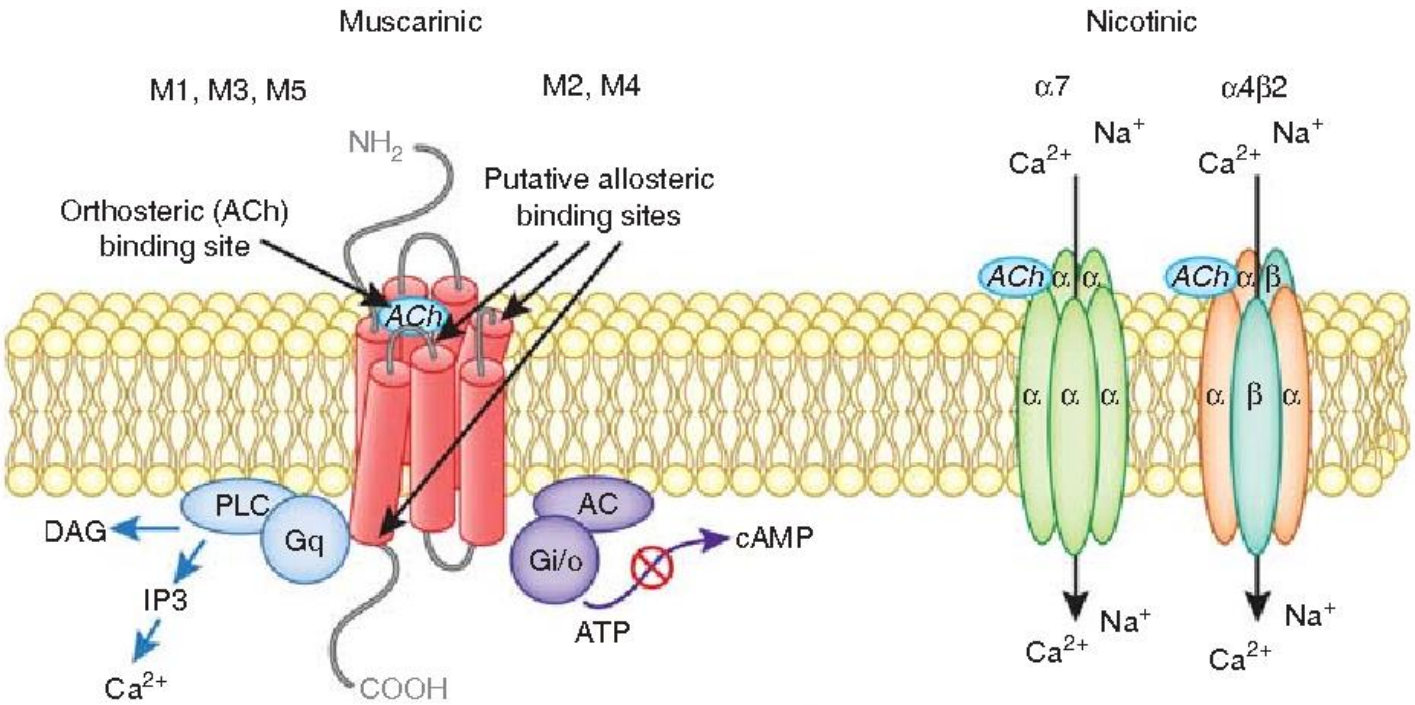
Table 3

Subgroup analyses of the association between CHAT rs3810950 polymorphism and Alzheimer's disease risk.

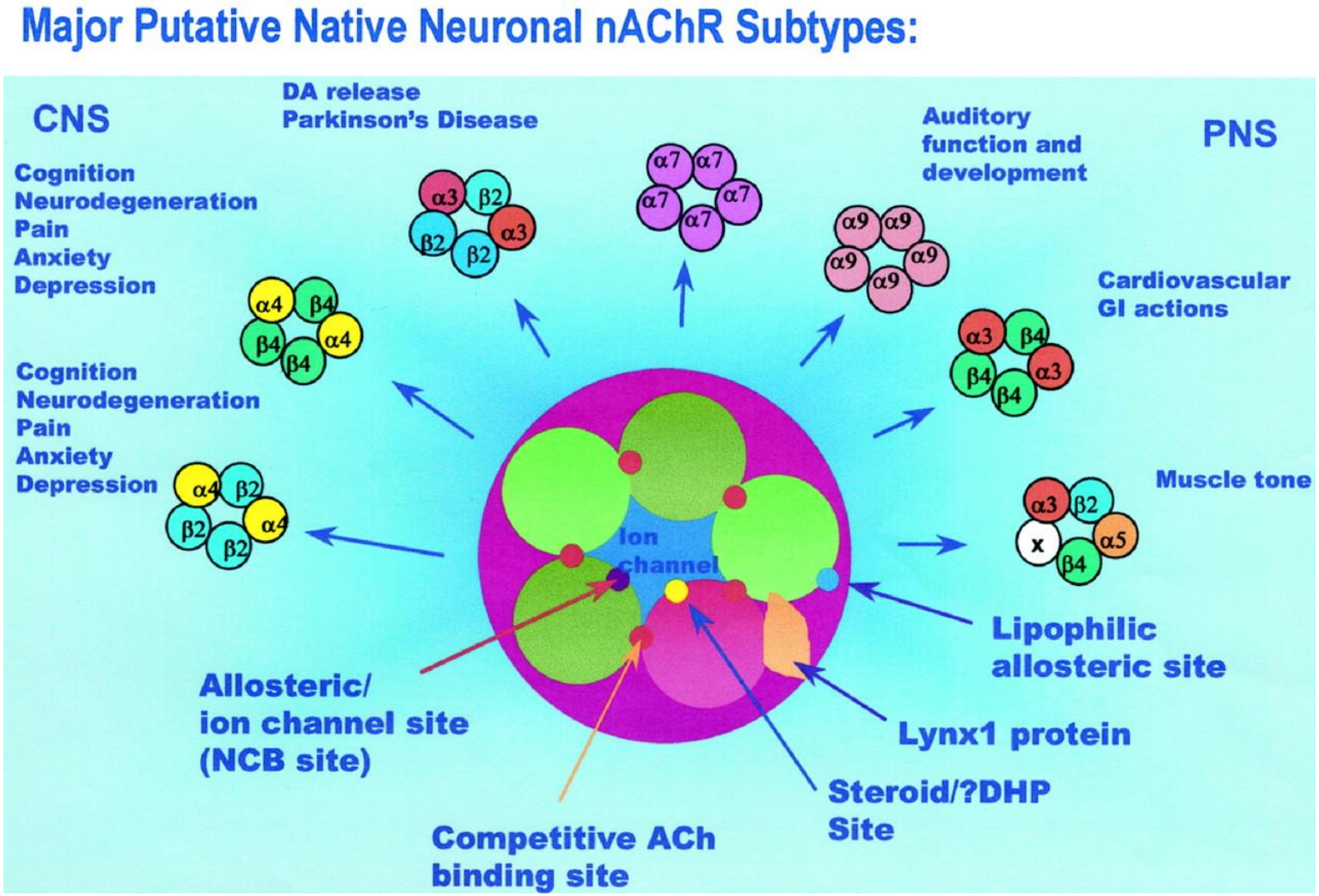
| | Genetic comparison | $I^2$ (%) | Effect model | OR [95% CI] | $P_{OR}$ | Statistical power |
|---|---|---|---|---|---|---|
| Overall | A versus G | 74 | Random | 1.18 [1.01, 1.37] | 0.03 | NA |
| | AA versus GG | 72 | Random | 1.63 [1.09, 2.42] | 0.02 | NA |
| | AG versus GG | 37 | Fixed | 0.99 [0.90, 1.10] | 0.87 | NA |
| | AA + GA versus GG | 62 | Random | 1.08 [0.92, 1.28] | 0.34 | 46.23% |
| | AA versus GG + GA | 66 | Random | 1.65 [1.20, 2.26] | <0.01 | 99.99% |

- CHAT gene therapy to increase acetylcholine levels in Alzheimer's disease models

# ACETYLCHOLINE RECEPTORS



# NICOTINIC ACETYLCHOLINE RECEPTORS



# CHRNA3

- Member of the nicotinic acetylcholine receptor family of proteins. Members of this family of proteins form pentameric complexes comprised of both alpha and beta subunits.
- This locus encodes an alpha-type subunit, as it contains characteristic adjacent cysteine residues. The encoded protein is a ligand-gated ion channel that likely plays a role in neurotransmission.
- Polymorphisms in this gene have been associated with an increased risk of smoking initiation and an increased susceptibility to lung cancer.

# CHRNA3 – A allele

- A allele rs1051730 and smoking behaviour

| Smoking measure | Case subjects n | Case subjects Allele prevalence | Case subjects *P** | Control Subjects n | Control Subjects Allele prevalence | Control Subjects *P** |
|---|---|---|---|---|---|---|
| No. of cigarettes/d† | | | | | | |
| 1–10 | 133 | 0.35 | | 139 | 0.28 | |
| 11–20 | 546 | 0.37 | | 457 | 0.32 | |
| 21–30 | 367 | 0.44 | | 234 | 0.36 | |
| 31+ | 423 | 0.41 | .004 | 326 | 0.37 | <.001 |
| Duration of cessation (y) | | | | | | |
| 23+ | 175 | 0.37 | | 145 | 0.30 | |
| 13–22 | 211 | 0.39 | | 179 | 0.35 | |
| <13 | 620 | 0.40 | .197 | 317 | 0.33 | .430 |
| Time to first cigarette (min) | | | | | | |
| Former smokers | | | | | | |
| >1 h | 154 | 0.39 | | 138 | 0.29 | |
| 31–60 | 191 | 0.39 | | 119 | 0.33 | |
| 6–30 | 258 | 0.40 | | 167 | 0.31 | |
| ≤5 | 251 | 0.39 | .935 | 188 | 0.38 | .006 |
| Current smokers | | | | | | |
| >1 h | 44 | 0.39 | | 85 | 0.30 | |
| 31–60 | 84 | 0.44 | | 119 | 0.32 | |
| 6–30 | 166 | 0.40 | | 180 | 0.36 | |
| ≤5 | 129 | 0.41 | 1.000 | 130 | 0.39 | .011 |

* *P* values calculated using Cochran–Armitage test.

† Thorgeirsson et al. (6) reported allele prevalences in 13945 Icelandic smokers by number of cigarettes per day to be 0.31, 0.35, 0.38, and 0.39, respectively.

# CHRNA3 – A allele – cigarettes and alcohol

| | Case subjects | | | | | | Control subjects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | n | Cig/d | *P* value† | n | FTND score | *P* value† | n | Cig/d | *P* value† | n | FTND score | *P* value† |
| GG | 685 | 27.16 | | 583 | 4.68 | | 764 | 25.06 | | 729 | 4.13 | |
| AG | 869 | 27.09 | | 733 | 4.63 | | 770 | 25.99 | | 733 | 4.27 | |
| AA | 300 | 29.80 | .004 | 259 | 5.13 | .012 | 193 | 29.36 | <.001 | 187 | 5.10 | <.001 |

* N = number of case or control subjects used to analyze a given smoking behavior; Cig = cigarettes; FTND = Fagerstrom Test for Nicotine Dependence.

† From Kruskall–Wallis test.

Increased addiction to smoking cigarettes in smokers and increased tobacco dependence.

Decreased levels of prepulse inhibition PPI (AA). PPI is a neurological phenomenon in which a weaker prestimulus (prepulse) inhibits the reaction of an organism to a subsequent strong startling stimulus (pulse).

Decreased alcohol sensitivity (AA), less likely to feel buzzed. This can result in higher alcohol consumption and AA may increase the risk of alcohol abuse.

Increased inability to quit smoking during pregnancy (AG and AA)

# CHRNA4

- Member of the nicotinic acetylcholine receptor family of proteins. Members of this family of proteins form pentameric complexes comprised of both alpha and beta subunits.
- This locus encodes an alpha-4 type subunit.
- Polymorphisms in this gene have been associated with an increased addiction risk

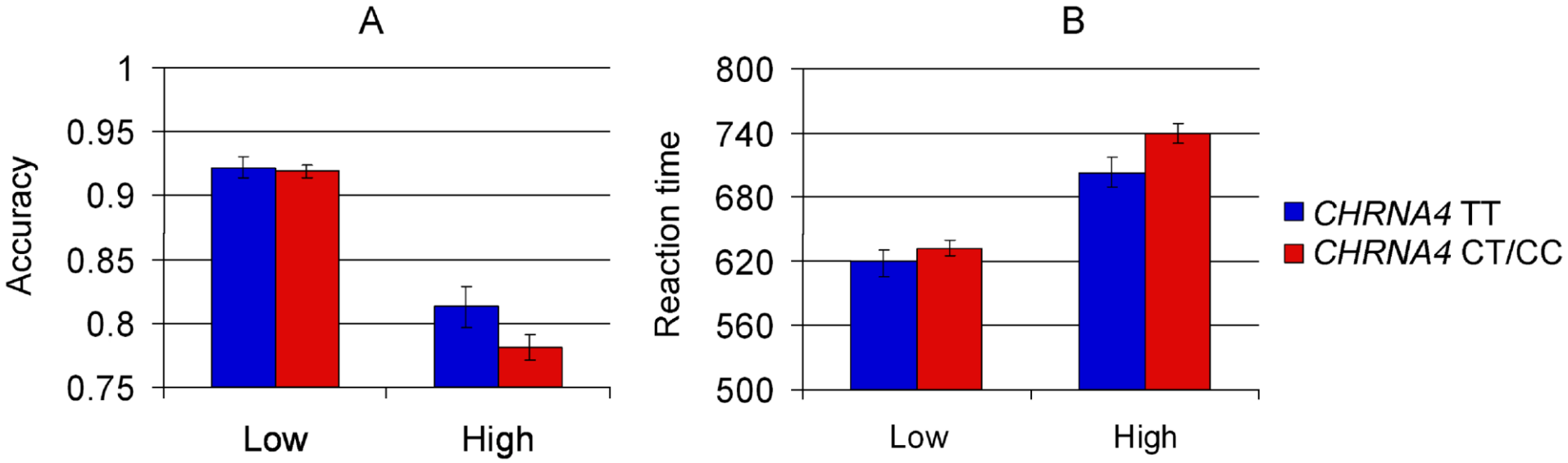
# CHRNA4 – smoking cessation

| Patient group | Success rate (%) | | |
|---|---|---|---|
| | CC | CT or TT | *p*-value |
| Overall ($n = 483$) | 37.0 | 46.4 | 0.05 |
| Varenicline ($n = 167$) | 29.5 | 50.9 | 0.007 |
| Varenicline plus bupropion ($n = 79$) | 40.0 | 50.0 | 0.41 |
| Bupropion plus NRT ($n = 237$) | 42.1 | 42.2 | 0.98 |

*NRT, nicotine replacement therapy (patch and/or gum).*

- Varenicline is a medication to treat smoking addiction – agonist of acetylcholine receptor
- C allele associated with nicotine addiction and lower success rate of intervention for treating smoking addiction (rs1044396)

# CHRNA4 AND COGNITION




- T allele associated with higher accuracy in settings of multiple stimuli provided to brain
- T allele associated with faster reaction time

# CHRNA4 AND INTERNET GAMING DISORDER

- Preoccupation with gaming
- Withdrawal symptoms when gaming is taken away or not possible (sadness, anxiety, irritability)
- Tolerance, the need to spend more time gaming to satisfy the urge
- Inability to reduce playing, unsuccessful attempts to quit gaming
- Giving up other activities, loss of interest in previously enjoyed activities due to gaming
- Continuing to game despite problems
- Deceiving family members or others about the amount of time spent on gaming
- The use of gaming to relieve negative moods, such as guilt or hopelessness
- Risk, having jeopardized or lost a job or relationship due to gaming

# CHRNA4 – Internet Gaming Disorder

**Characteristics and results of the allelic association analysis of the SNP showing a statistically significant difference in MAF between the IGD and NC groups.**

| SNP | Gene | Chromosome No. | Region | Major allele | Minor allele | MAF (IGD) | MAF (NC) | $\chi 2$ | $p$ | $p'$ | Cramer's V |
|---|---|---|---|---|---|---|---|---|---|---|---|
| rs1044396* | *CHRNA4* | 20 | exon | C | T | 0.0833 | 0.3 | 9.09 | 0.003 | 0.043 | 0.275 |

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SNP, Single nucleotide polymorphism; MAF, Minor allele frequency; IGD, Internet gaming disorder patients; NC, Normal controls; No.,Number;

- C allele associated with Internet Gaming Disorder

# CHRNA5

- Member of the nicotinic acetylcholine receptor family of proteins. Members of this family of proteins form pentameric complexes comprised of both alpha and beta subunits.
- This locus encodes an alpha-5 type subunit.
- Polymorphisms in this gene have been associated with an increased addiction risk – nicotine and cocaine

# CHRNA5 – Nicotine and Cocaine addiction

- Rs16969968
- Risk allele for nicotine dependence (A)
- Protective allele for cocaine dependence

Table 3

Multivariate Allelic Association Between rs16969968 in *CHRNA5,* Cocaine Dependence and Nicotine Dependence

| | N | *OR* (95% CI) | *P* |
|---|---|---|---|
| Cocaine Dependence (Case) | 260 | 0.41 (0.22, 0.76) | 0.0045 |
| No Cocaine Dependence (Control) | 244 | 1.00 | |
| Nicotine Dependence | 196 | 2.14 (1.15, 4.01) | 0.0171 |
| Non-Smoker | 237 | 1.37 (0.77, 2.44) | 0.28 |
| Non-Nicotine Dependent Smoker | 71 | 1.00 | |

# CHRM3

- Muscarinic cholinergic receptors belong to a larger family of G protein-coupled receptors.
- Bind to muscarine (a molecular found in mushrooms)
- Muscarinic receptors influence many effects of acetylcholine in the central and peripheral nervous system.
- The muscarinic cholinergic receptor 3 controls smooth muscle contraction and its stimulation causes secretion of glandular tissue.

# CHRM3 – Post operative nausea

- Rs2165870
- A allele
- Post operative nausea (PNS effects)
- Anti-emetics needed
- RS7511970
- T allele
- Late onset Alzheimers (CNS effects)

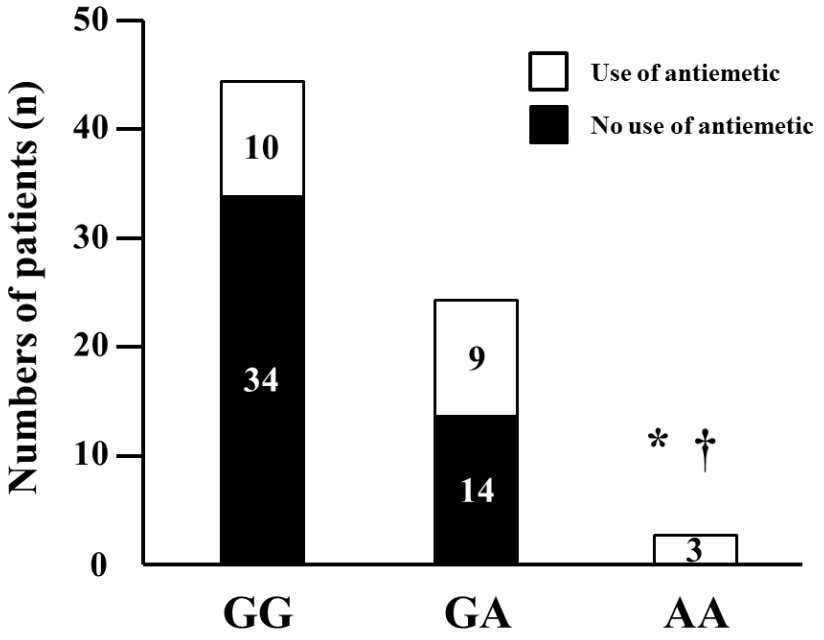



**Figure 1. Ratio of patients who needed to receive an antiemetic in AA genotype was higher than those in GA and GG genotypes. *: $p < 0.05$ vs. GA genotype, †: $p < 0.01$ vs. GG genotype. GG: wild-type, GA: heterozygous mutant, AA: homozygous mutant**

# WHAT TO DO IF SOMEONE IS AT INCREASED RISK OF ADDICTION ?

- Avoidance of addictive component (smoking)
- Monitoring of gaming activity (internet gaming disorder)
- Dietary intake to increase acetylcholine levels
- Increase intake of choline rich foods
- Animal fats such as egg yolks, cream, fatty cheeses, fatty fish, fatty meats, and liver. Non-animal sources include avocadoes and almonds.

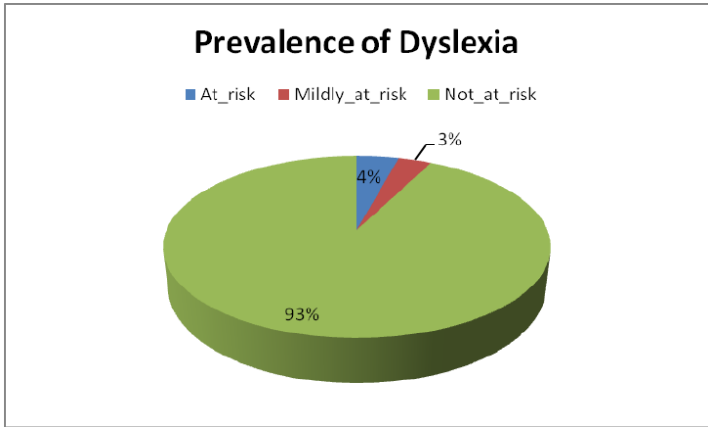
# DYSLEXIA

***DYS*** = TROUBLE

***LEXIA*** = WORDS

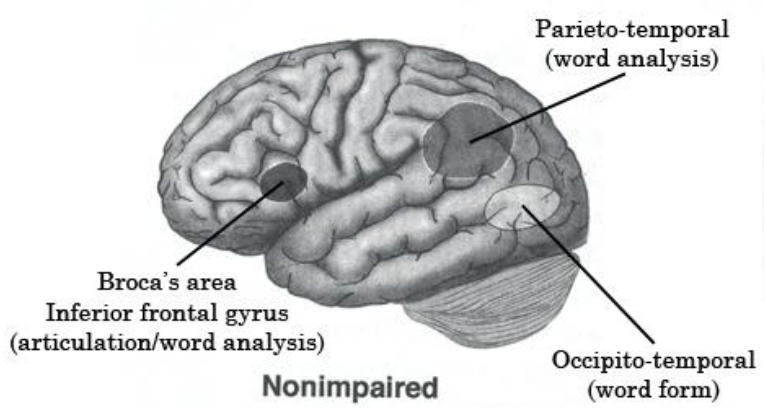
## *TROUBLE WITH WORDS*

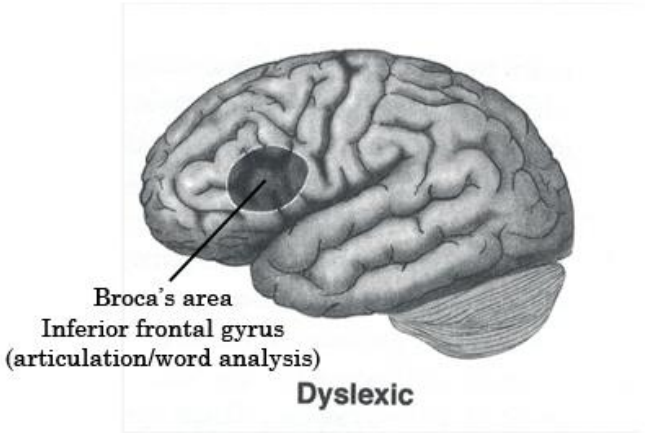
- NEUROLOGIC IN ORIGIN - GENETIC
- LIFELONG – ENVIRONMENT MAY ALTER COURSE
- CORE DEFICIT IN PHONOLOGICAL COMPONENT OF LANGUAGE
- READING COMPREHENSION > WORD READING
- ACCOMPANYING CHALLENGES ( 50% )
  - ADHD
  - SENSORY MOTOR DIFFICULTY
  - BEHAVIORAL PROBLEMS
- MORE CHALLENGING TO REMEDIATE



# DYSLEXIA AND DCDC2

- Doublecortin domain-containing protein 2 is a protein that in humans is encoded by the DCDC2 gene
- The gene appears to have a strong linkage with the processing of speech information when writing.
- Reduced activity associated with dyslexia
- Impacts Broca's area - the frontal lobe of the dominant hemisphere, usually the left, of the brain with functions linked to speech production



# DYSLEXIA AND GENETICS: DCDC2

| | Controls ($n$=184) | All cases ($n$=72) | Severe cases ($n$=25) | Nonsevere cases ($n$=43) | Dysphonetic cases ($n$=34) | Nondysphonetic cases ($n$=34) |
|---|---|---|---|---|---|---|
| Deletion genotype del/del | 0% | 3%*** | 0% | 4.7%*** | 0% | 6%*** |
| Deletion alleles | 4% | 10%** | 8%* | 13%** | 7% | 15%*** |
| rs793862 genotype A/A | 5.6% | 13.9%* | 8% | 16.3% | 8.8% | 17.6%* |
| rs807701 genotype C/C | 8.2% | 19.4%* | 16% | 20.9% | 17.6% | 20.6%* |
| rs807724 genotype G/A | 38.4% | 23.6%* | 28% | 23.3% | 29.4% | 20.6% |

$p$ values for genotypes are according to the genotype relative risk (GRR) of shown genotype vs all other genotypes (Lathrop, 1983). $p$ values for the deletion are for allelic differences between cases and controls.

$n$ number of individuals

*$p$<0.05; **$p$<0.01; ***$p$<0.005

# DYSLEXIA AND GENETICS: KIAA0319

- KIAA0319 encoded protein may play a role in the development of the cerebral cortex (the outer layer of the cerebrum (the cerebral cortex ), composed of folded gray matter and playing an important role in consciousness) by regulating neuronal migration and cell adhesion.
- Involved in neuronal migration during development of the cerebral neocortex.
- May function in a cell autonomous and a non-cell autonomous manner and play a role in appropriate adhesion between migrating neurons and radial glial fibers.
- May also regulate growth and differentiation of dendrites.
- Mutations associated with dyslexia

# DYSLEXIA AND GENETICS: KIAA0319

- Mutations that reduce gene expression associated with dyslexia
- Triple haplotype
- rs4504469-rs2038137-rs2143340
- C allele associated with dyslexia risk
- 40 percent reduction in enzymatic activity
- Impact in neuronal signaling

- IF RISK IDENTIFIED EARLY
- Educational plan / speech therapy
- Read aloud to child starting at 6 months

# AUTISM

Autism spectrum disorder (ASD) is a developmental disorder that affects communication and behavior.
Although autism can be diagnosed at any age, it is said to be a "developmental disorder" because symptoms generally appear in the first two years of life.

People with ASD have:
Difficulty with communication and interaction with other people
Restricted interests and repetitive behaviors
Symptoms that hurt the person's ability to function properly in school, work, and other areas of life

**1 in 42 males**
**were diagnosed with ASD**

**1 in 165 females**
**were diagnosed with ASD**

# What causes autism?

- Many pathways, with most not yet well defined or understood
  - Small percentage of children with ASD have a genetic or chromosomal disorder of **known** significance (10-20%)
    - 2-6% of ASD → Fragile X
    - Advanced parental age is possible
  - Other biological / environmental factors under investigation; few effects are well-established
  - Pollutants, prenatal stress, other health risk factors, etc.

# Risk factors

- Children born to older parents are at a higher risk for having ASD
- ASD tends to occur more often in people who have certain genetic or chromosomal conditions. 10% have been found to have:
  - Down Syndrome
  - Fragile X Syndrome (X-linked inheritance – males worse)
  - Tuberous Sclerosis (benign tumors in brain and other organs)
  - Other genetic disorder

# Risk Factors

- Males are 4-5 times more likely to have ASD than females

- Family risk factors
  - Families with one child with ASD have about 18.7% increased risk of having a second child with ASD

  - Twin studies
    - 36-95% of identical (monozygotic) twin pairs
    - 0-31% of non-identical (dizygotic) twin pairs
  - 20-40% of siblings of a child with autism have language and/or social deficits

# AUTISM GENETICS

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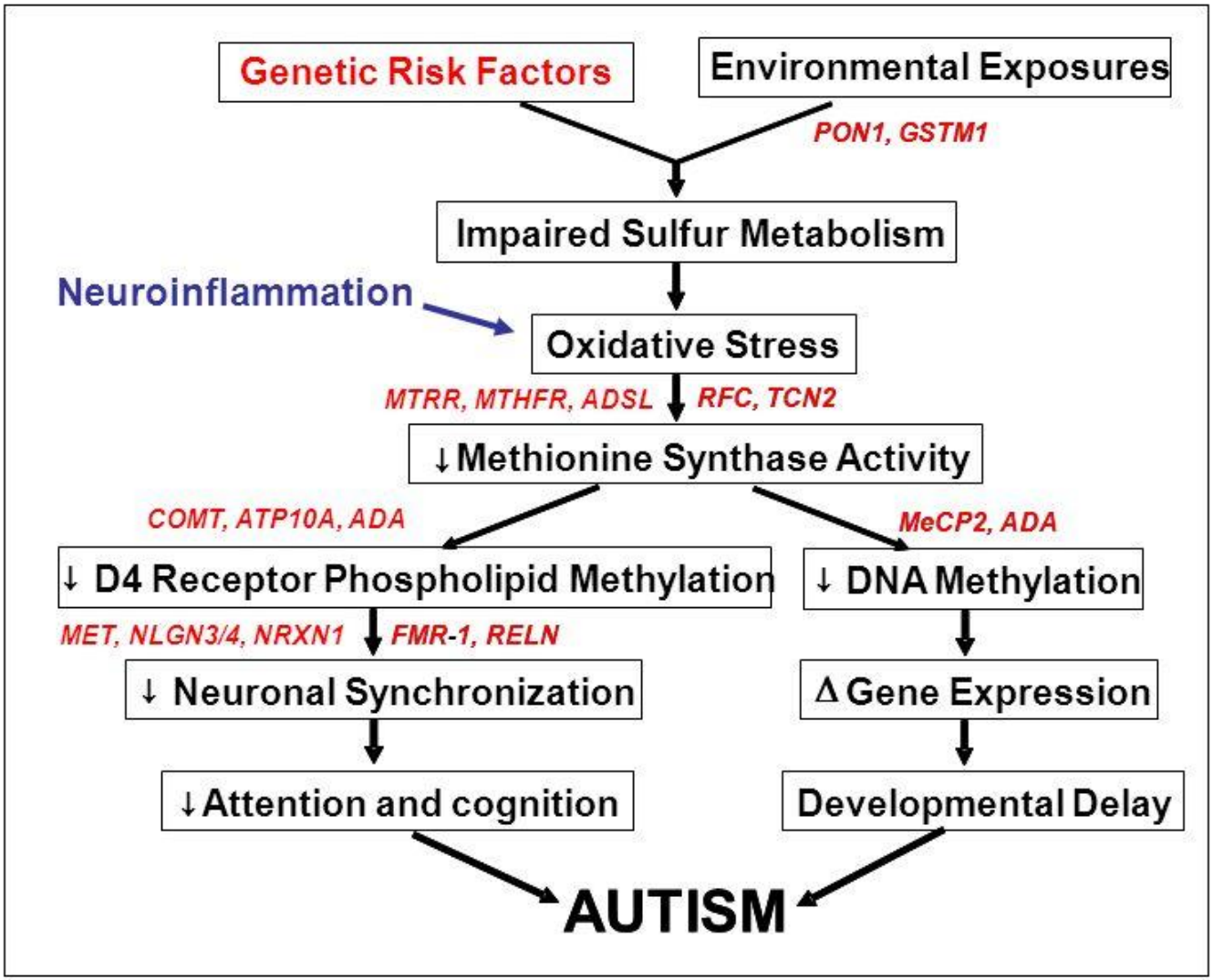
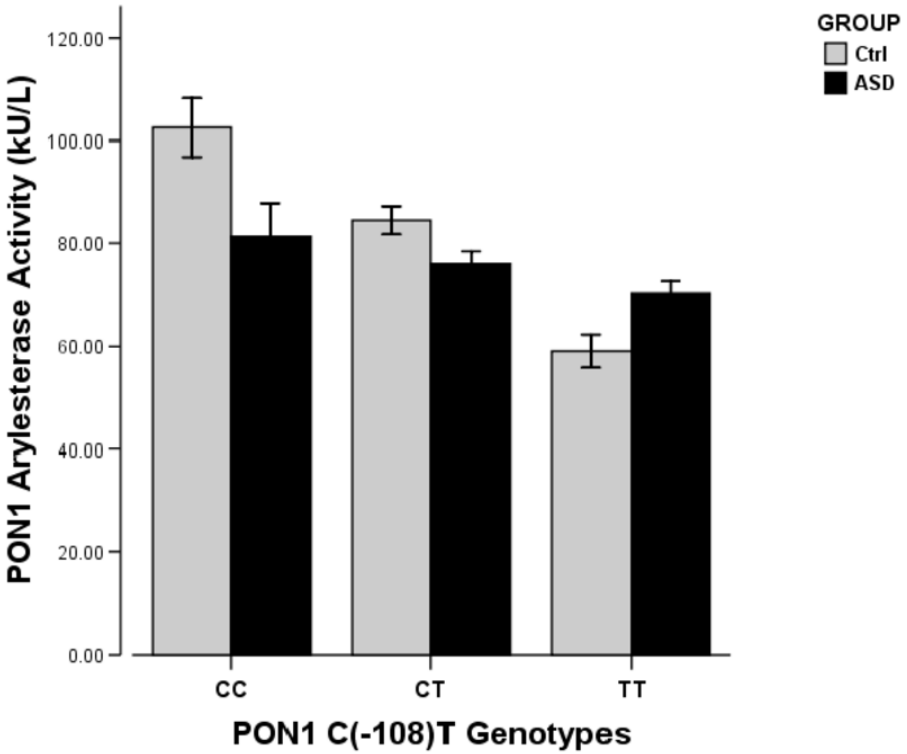
**Genetic and Environmental Factors Can Combine to Cause Autism**

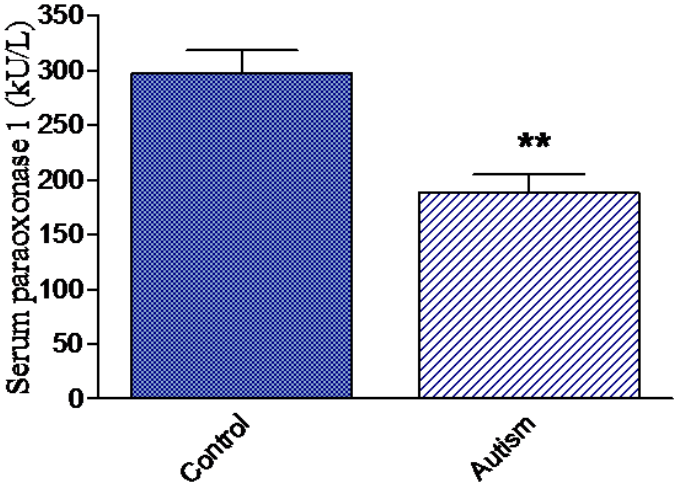


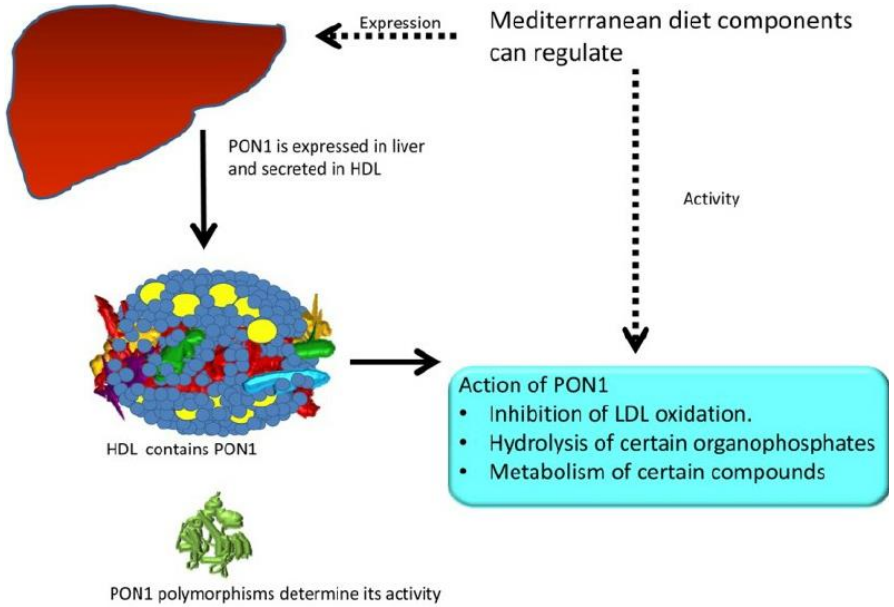
# PON-1 AND AUTISM

- Paraoxonase 1
- Following synthesis in the kidney and liver, the enzyme is secreted into the circulation, where it binds to high density lipoprotein (HDL) particles and hydrolyzes thiolactones and xenobiotics, including paraoxon, a metabolite of the insecticide parathion.
- Metabolism of compounds found in pesticides and insecticides
- Levels increase 3.5x times between birth and age 7
- Low levels associated with autism

# LOW PON1 AND AUTISM



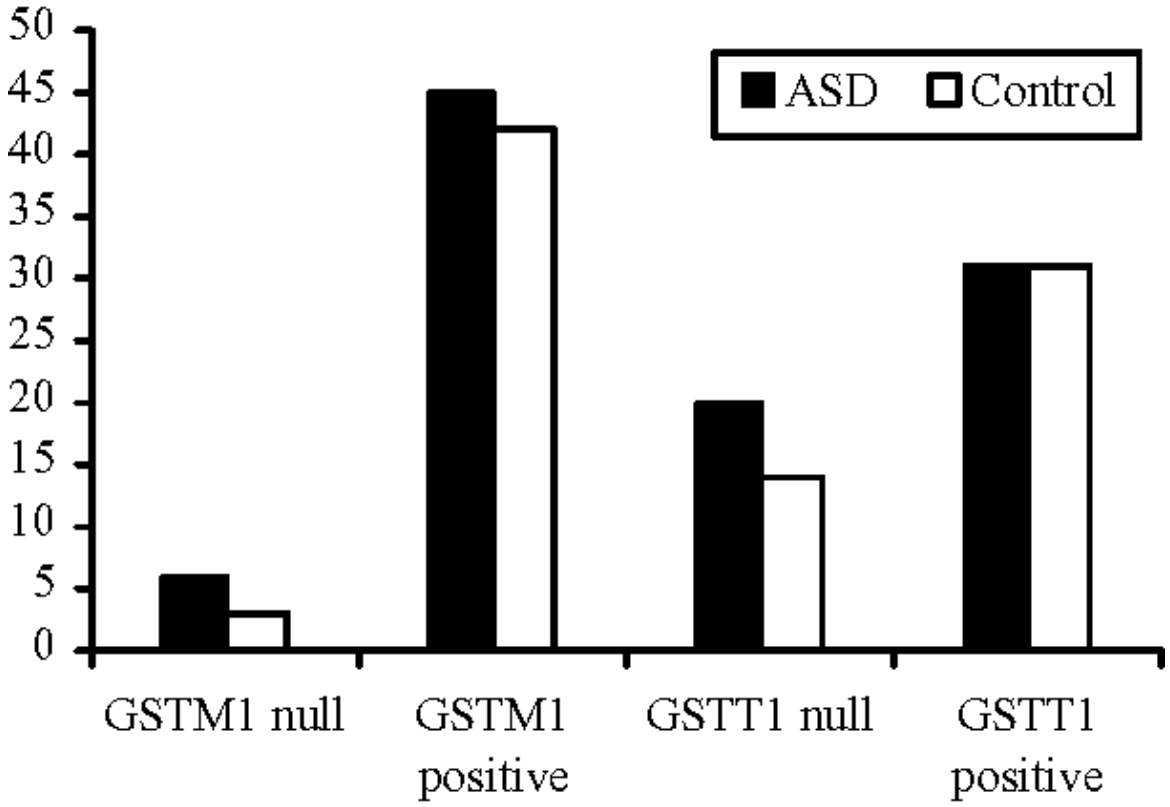



# GSTM1 AND AUTISM

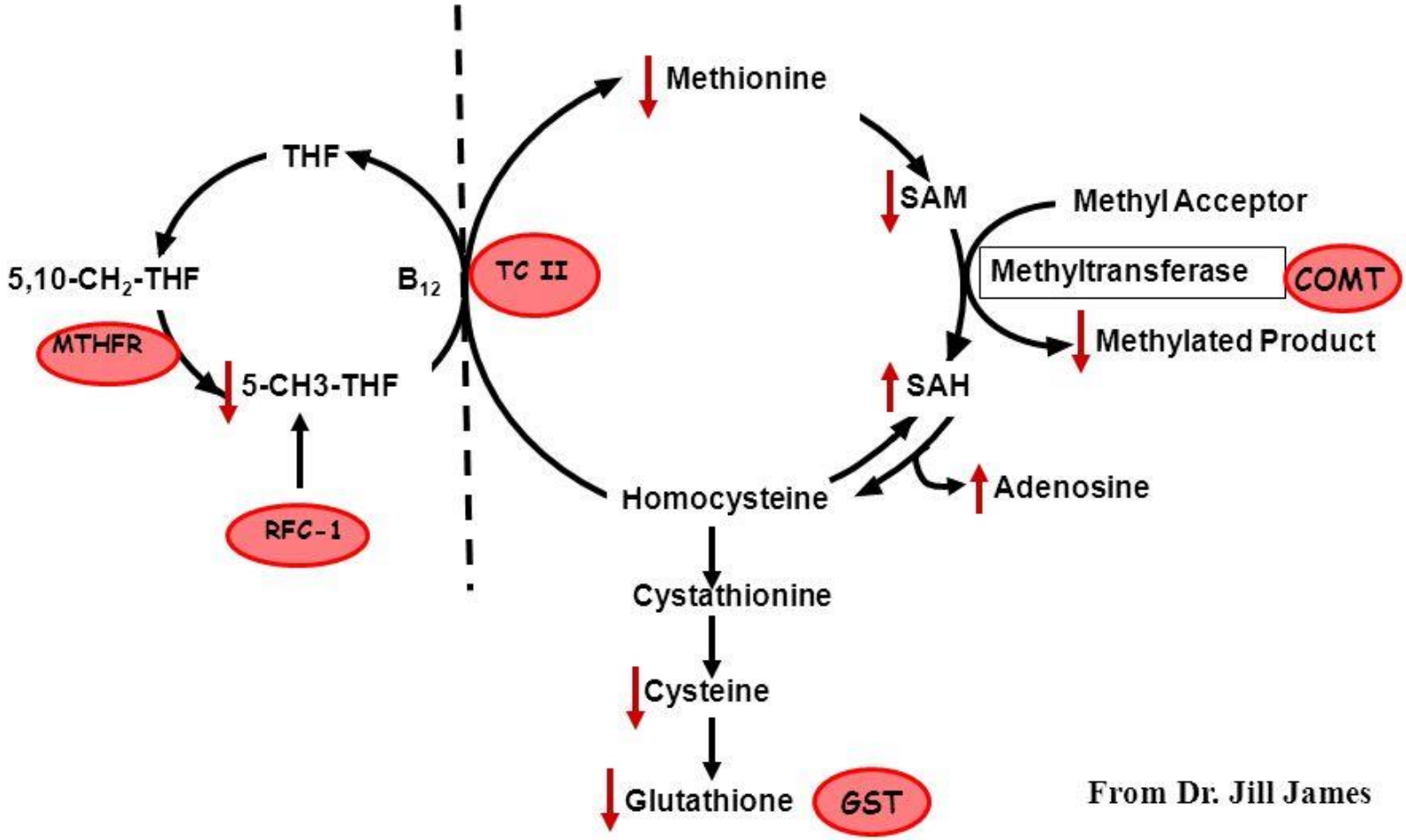
- Glutathione S transferase mu1
- Detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress, by conjugation with glutathione
- Impairment in detoxification may increase exposure to toxins which can impact neuronal development

# LOW GSTM1 AND AUTISM




- Low activity associated with autism
- Toxin exposure during fetal development and reduced/lack of GSMT1 activity

# Autism-associated Genetic Polymorphisms in the Methionine Cycle

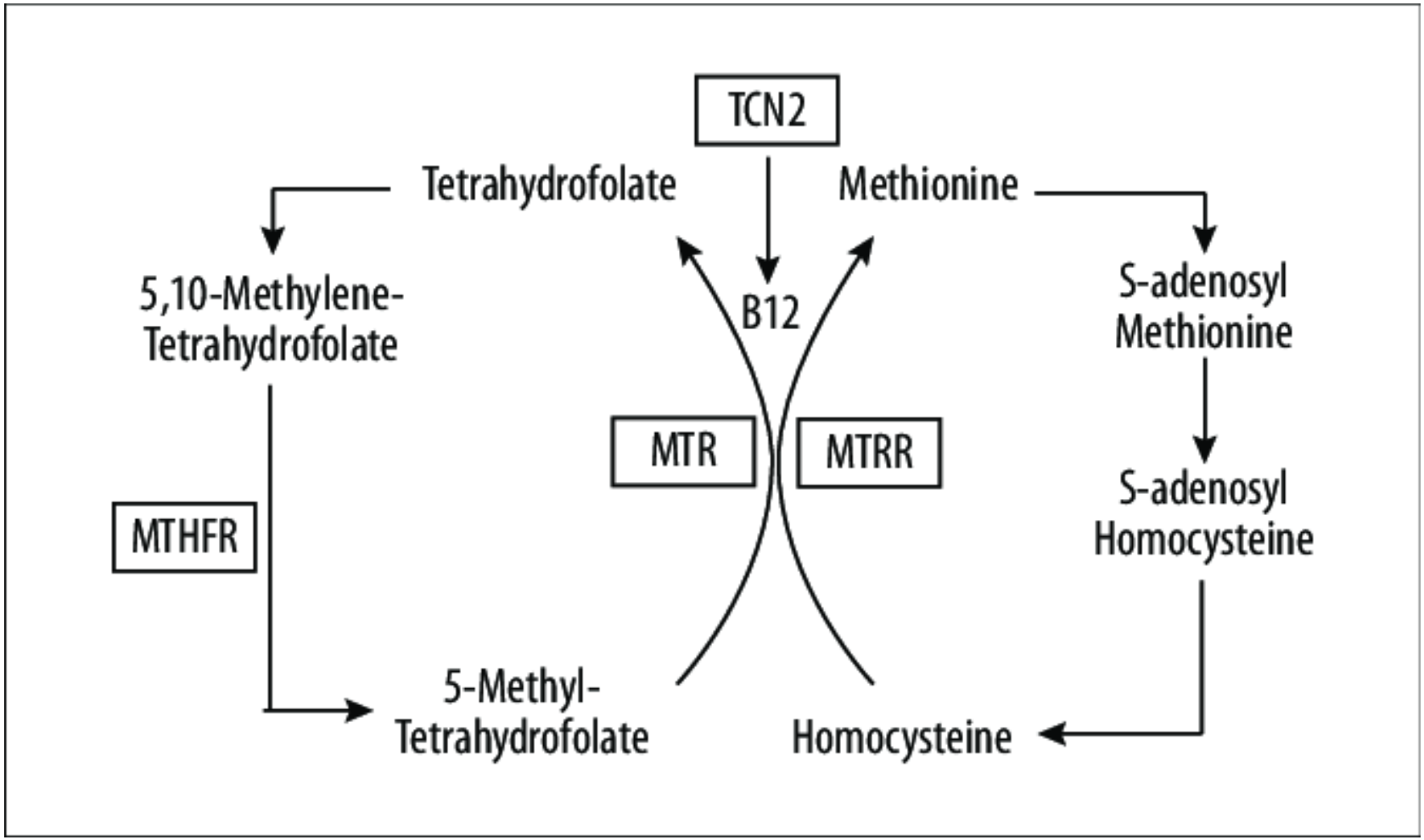



From Dr. Jill James

# MTRR AND AUTISM

- The MTRR gene provides instructions for making an enzyme called methionine synthase reductase.
- This enzyme is required for the proper function of another enzyme called methionine synthase. MTRR interacts with methionine synthase, to ensure the continued production of the essential amino acid methionine
- Methionine synthase helps process amino acids, which are the building blocks of proteins.
- Involved in folic acid cycle and homocysteine cycle

# MTRR AND AUTISM



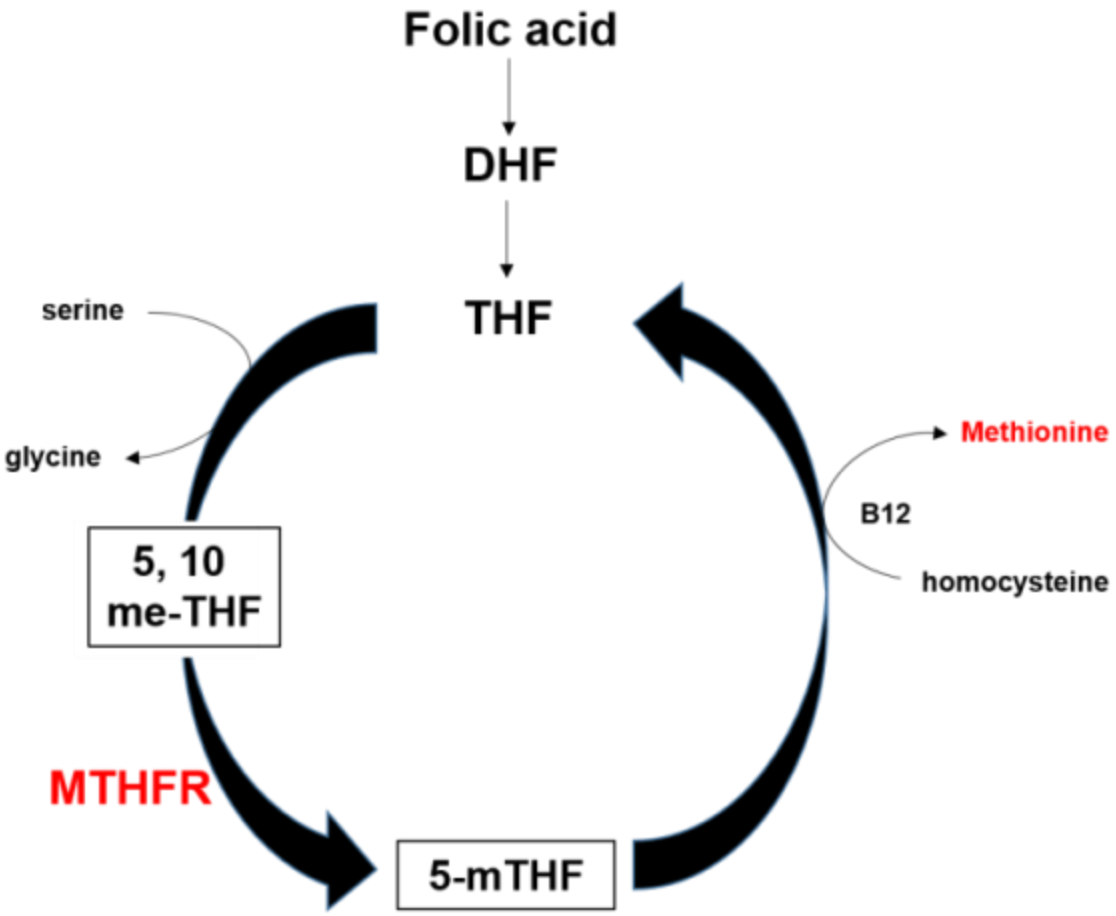
# MTRR AND AUTISM

| | Cases | Control | Odds Ratio (95% CI) | $P^a$ | $P^b$ |
|---|---|---|---|---|---|
| **rs1801394** | | | | | |
| Genotype | (n = 142) | (n = 214) | | | |
| A/A | 14 (9.9 %) | 28 (13.1 %) | Ref | <0.001 | - |
| A/G | 108 (76 %) | 184 (86 %) | 1.17 (0.6, 2.3) | - | 0.64 |
| G/G | 20 (14.1 %) | 2 (0.9 %) | 20 (4.1, 98) | - | **< 0.001** |
| Allele | (n = 284) | (n = 428) | | | |
| A | 136 (48 %) | 240 (56 %) | - | 0.04 | - |
| G | 148 (52%) | 188 (44%) | - | - | - |

[a] allele and genotype frequencies in cases and controls were compared using $\chi^2$ test.
[b] significance level for allele and genotype frequencies in cases and controls

- Reduced MTRR levels associated with autism (G allele) -> Reduced methionine
- Supplementation with L-methionine

# FOLATE AND AUTISM




- MTHFR key in producing bioactive 5-methylfolate
- Adequate 5-methylfolate needed to keep homocysteine levels in check
- MTHFR mutations associated with autism

# MTHFR AND AUTISM

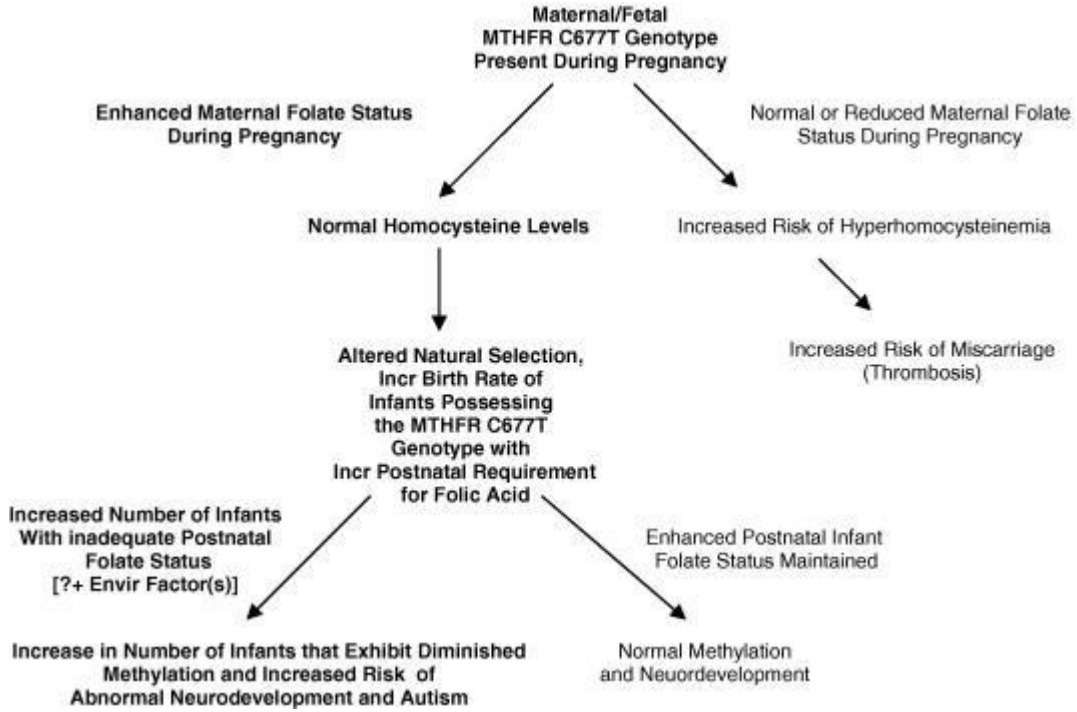



**Table 2.** Frequency of 677CT Genotypes in Autistic and Control Populations

| 677C→T | CC | CT | TT | T ALLELE FREQUENCY |
|---|---|---|---|---|
| Autistic | 35 (21%)* | 94 (56%)* | 39 (23%)* | 0.51* |
| Control | 2570 (48%) | 2213 (41%) | 606 (11%) | 0.32 |

*$P<0.0001$

# MTHFR AND AUTISM

**Table 4**. Frequency of Compound Heterozygous Genotypes in Autistic and Control Populations

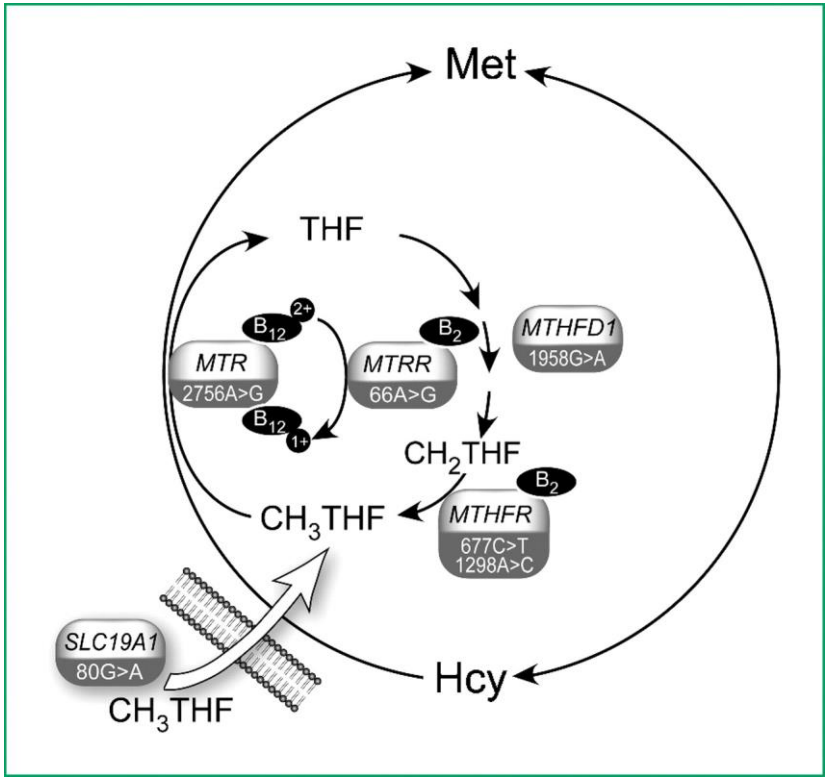
| | 677CT and 1298AC | NON 677CT and 1298AC |
|---|---|---|
| Autistic | 42(25% )* | 126 (75%) |
| Non-Autistic | 43(15%)* | 236(85%) |

*$P=0.01$

- Adequate folate supplementation during pregnancy
- Postnatal supplementation also needed

# RFC/SLC19A1 AND AUTISM

- Codes for folate transporter
- Plays a role in maintaining adequate cellular concentrations of folate



# RFC/SLC19A1 AND AUTISM

| *rs1023159* | KU | AGRE |
|---|---|---|
| Genotype | ($n$ = 144) | ($n$ = 191) |
| G/G | 72 (50.0%) | 63 (31.4%) |
| A/G | 63 (43.8%) | 104 (53.1%) |
| A/A | 9 (6.3%) | 30 (15.5%) |
| Allele | ($n$ = 288) | ($n$ = 394) |
| G | 207 (71.9%) | 230 (58.4%) |
| A | 81 (28.1%) | 164 (41.6%) |

KU, Kanazawa University; AGRE, Autism Genome Resources Exchange.

- G allele associated with reduced transporter activity
- Associated with autism

# TCN2 AND AUTISM

- The TCN2 gene provides instructions for making a protein called transcobalamin (formerly known as transcobalamin II).
- This protein transports cobalamin (also known as vitamin B12) from the bloodstream to cells throughout the body.
- Binds to vitamin b12 and the complex is then taken up by receptor mediated endocytosis.
- B12 is needed for folic acid cycle – Homocysteine to methionine

# TCN2 AND AUTISM

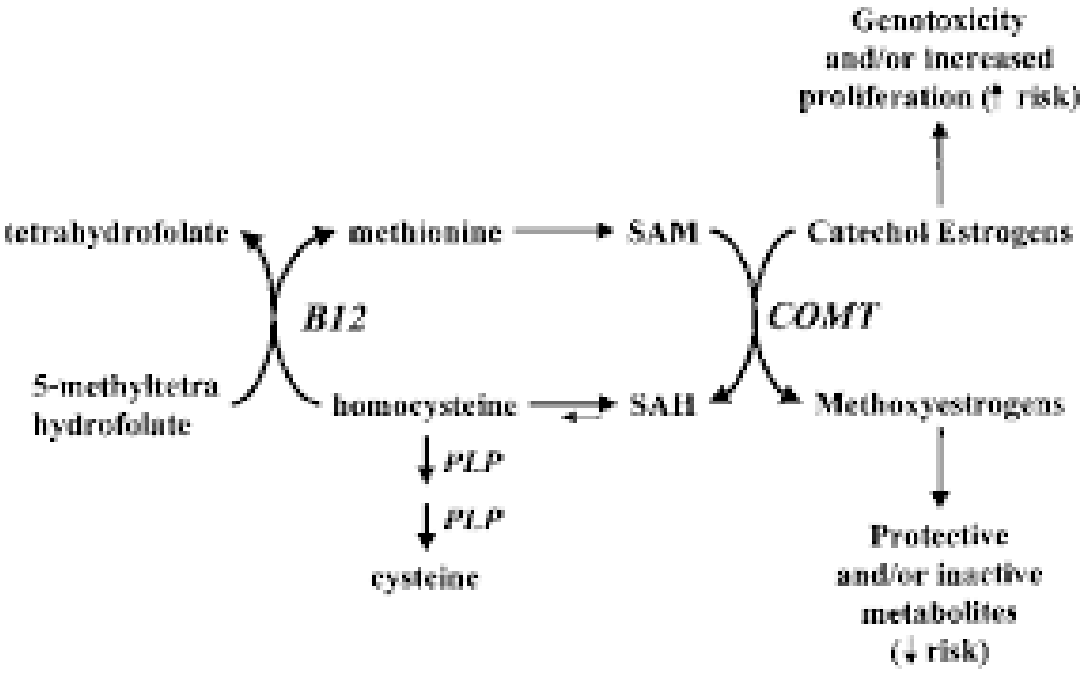
Allele frequencies, genotype distributions, odds ratios, and 95% confidence intervals (CI) in autistic cases and controls. Significant and borderline significant differences are in bold type.

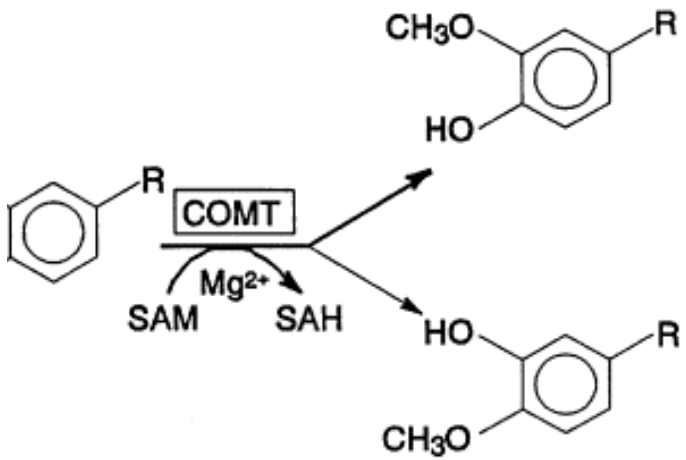
| | | | | |
|---|---|---|---|---|
| ***TCN2*** | C | 375 (52) | 231 (58) | Reference |
| **776 C>G** | **G** | **346 (48)** | **169 (42)** | **1.25 (0.97, 1.6)** |
| | CC | 108 (30) | 63 (32) | Reference |
| | CG | 159 (44) | 105 (52) | 0.88 (0.58, 1.3) |
| | **GG** | **93 (26)** | **32 (16)** | **1.70 (1.02, 2.8)** |
| | **CC+CG** | **268 (74)** | **168 (84)** | **0.55 (0.35, 0.8)** |

- Reduced TCN2 function associated with autism
- Impact on folate cycle
- Vitamin B12 supplementation ?

# COMT AND AUTISM

- Catechol O methyl transferase
- Breakdown of estrogen, neurotransmitters
- Role in methionine cycle and breakdown of toxins



# COMT AND AUTISM

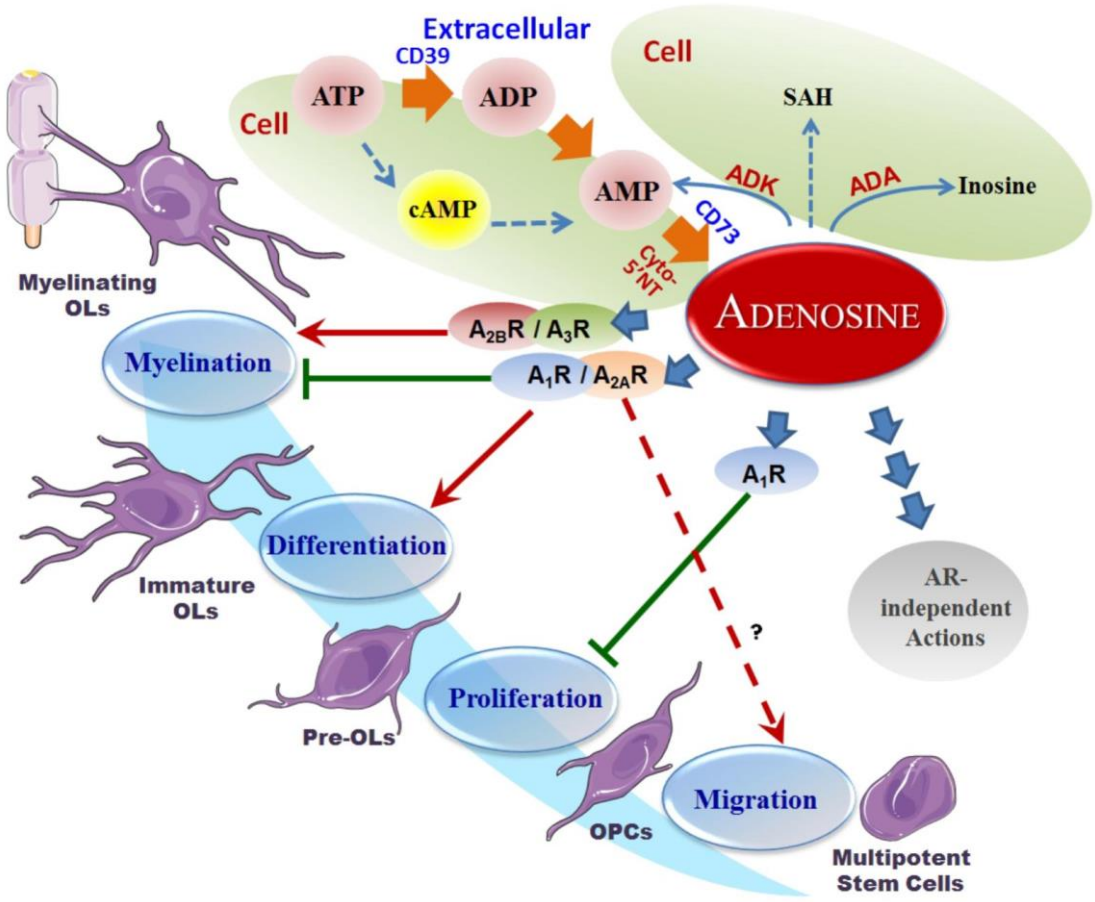
Allele frequencies, genotype distributions, odds ratios, and 95% confidence intervals (CI) in autistic cases and controls. Significant and borderline significant differences are in bold type.

| | | | | |
|---|---|---|---|---|
| ***COMT*** | A | 340 (47) | 215 (54%) | Reference |
| **472G>A** | **G** | **376 (53)** | **181 (46)** | **1.31 (1.02,1.7)** |
| | AA | 86 (24) | 57 (29) | Reference |
| | AG | 168 (47) | 101 (51) | 1.10 (0.7, 1.7) |
| | **GG** | **105 (29)** | **40 (20)** | **1.74 (1.02, 2.9)** |

- Reduced COMT associated with autism
- Increased toxin exposure
- Impact on methionine cycle

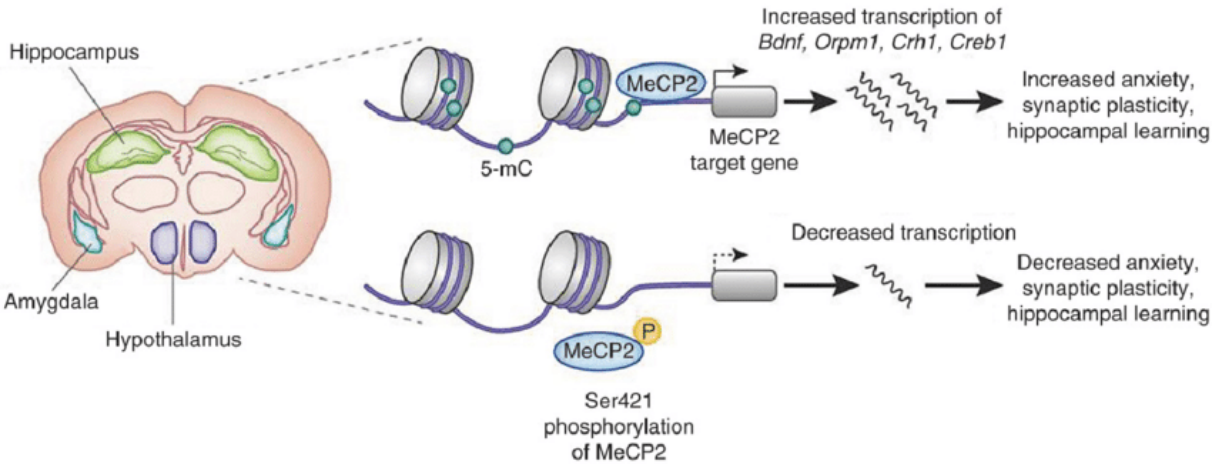
# ADA AND AUTISM

- Encodes of adenosine deaminase – involved in purine synthesis
- Needed for breakdown of adenosine from food and nuclear turnover of adenosine in tissues
- Adenosine important for neuronal development
- Rs73598374 - A allele Asp8Asn polymorphism - 35 % reduction



# MECP2 AND AUTISM

- The MeCP2 protein is present in cells throughout the body, although it is particularly abundant in brain cells.
- In the brain, the MeCP2 protein is important for the function of several types of cells, including nerve cells (neurons).
- Many of the genes that are known to be regulated by the MeCP2 protein play a role in normal brain function, particularly the maintenance of synapses.



# MECP2 AND AUTISM

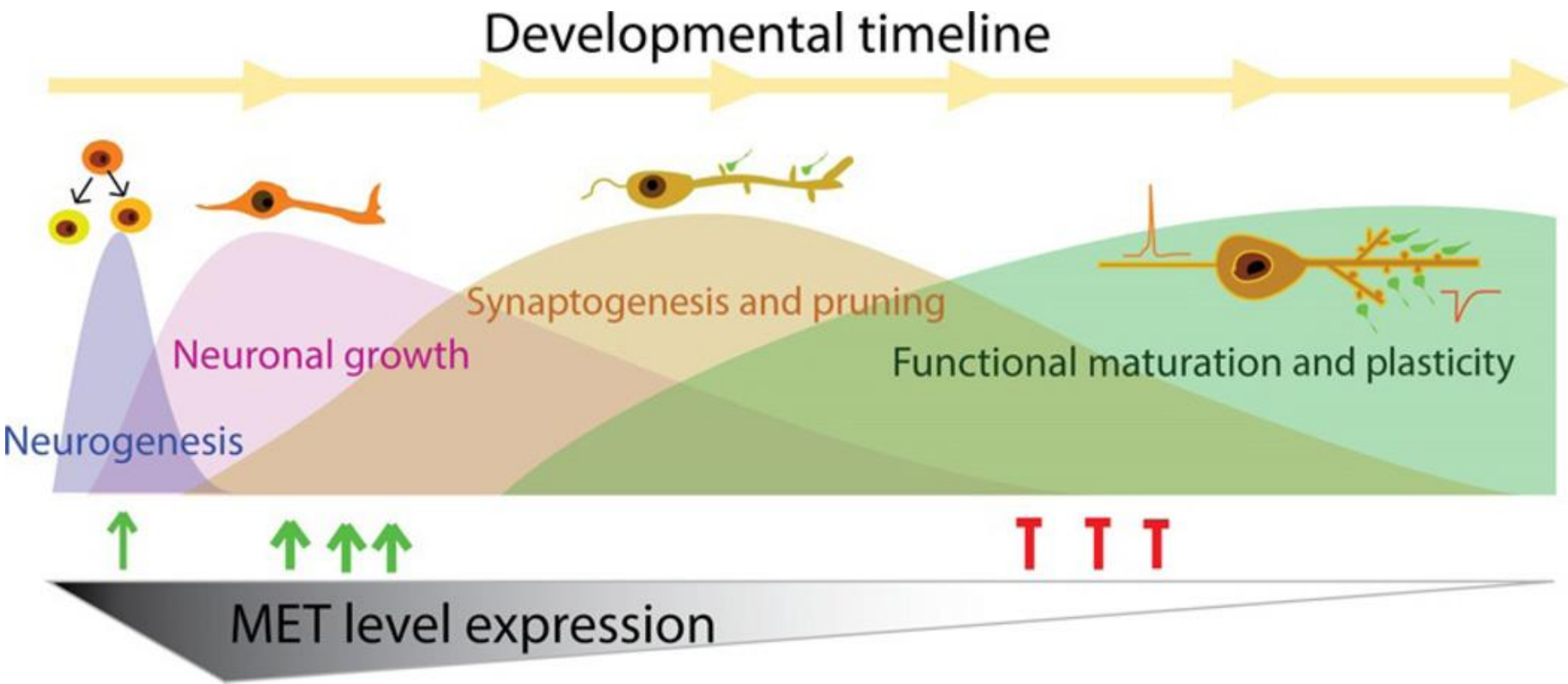
| Reference | Patients with ASD, n | De novo mutations, n (%) | Mutations/ deletions | Clinical phenotype |
|---|---|---|---|---|
| Lam *et al*[48] | 21 (all F) | 1/21 (4.8) | IVS2+2delTAAG | Autism and MR. No regression, epilepsy or microcephaly |
| Vourc'h *et al*[51] | 59 (42M,17F) | — | — | — |
| Beyer *et al*[52] | 202 (154M,48F) | — | — | — |
| Carney *et al*[49] | 69 (all F) | 2/69 (2.9) | 1157del41, R294X | Autism, MR and history of regression. No stereotypies, epilepsy or microcephaly |
| Zappella *et al*[50] | 19 (all F) | 2/19 (4.7) | R133C, R453X | Preserved speech variant of Rett syndrome |
| Shibayama *et al*[53] | 24‡ | 0/24 (0) | c1638 G→C,* c 6809 T→C,* P376R† | — |
| Lobo-Menendez *et al*[54] | 99 (58M,41F) | – | – | — |
| Li *et al*[55] | 65 (49M,16F) | — | — | — |
| Xi *et al*[56] | 31 (all M) | 0/31 (0) | 1 3′UTR variant* | — |
| Harvey *et al*[57] | 401 (266M,135F) | — | — | — |
| Coutinho *et al*[58] | 172 (141M,31F) | 0/172 (0) | 12 3′UTR variants.† 2 intronic variants,† G206A* | — |
| Total | 397F, 741M‡ | 5/397 females (1.3), 0/741 males | — | — |

ASD, autistic spectrum disorders; MR, mental retardation; UTR, untranslated region.

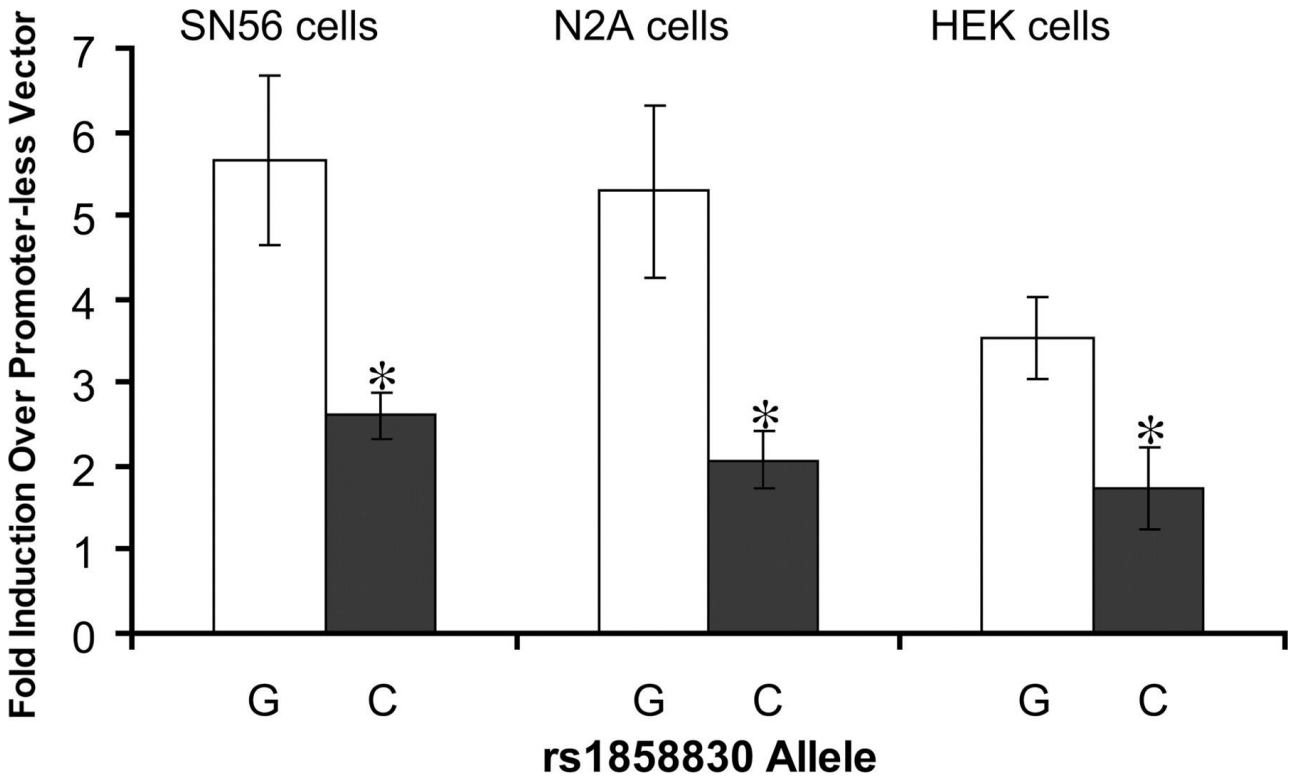
- Mutations in MECP2 associated with RETT syndrome and autism
- RETT – severe neurological genetic disease
- Mutations can dysregulate neuronal function and development – link with autism

# MET AND AUTISM

- MET is a tyrosine kinase involved in cell signaling
- Neurogensis and neuronal maturation



# MET AND AUTISM

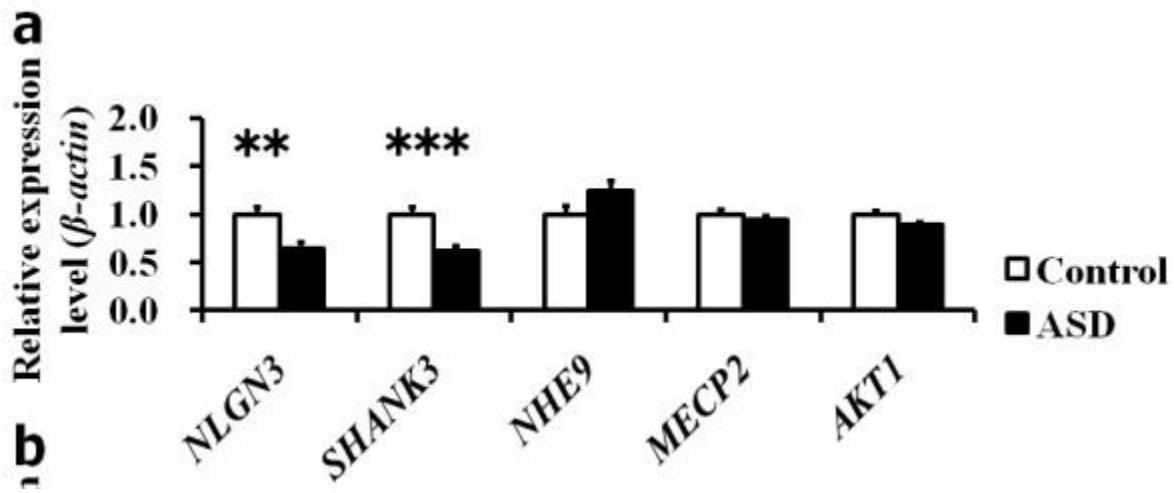



- C allele associated with reduced MET levels
- Associated with increased fetal autoantibodies
- Potential immunological link ?
- Neuronal link?

# NLGN3/4 AND AUTISM

- NLGN3 and 4 encodes for neuronal surface proteins
- Plays a role in synapse function and synaptic signal transmission, and may mediate its effects by clustering other synaptic proteins.
- May promote the initial formation of synapses, but is not essential for this. May also play a role in glia-glia or glia-neuron interactions in the developing peripheral nervous system.

# NLGN3/4 AND AUTISM



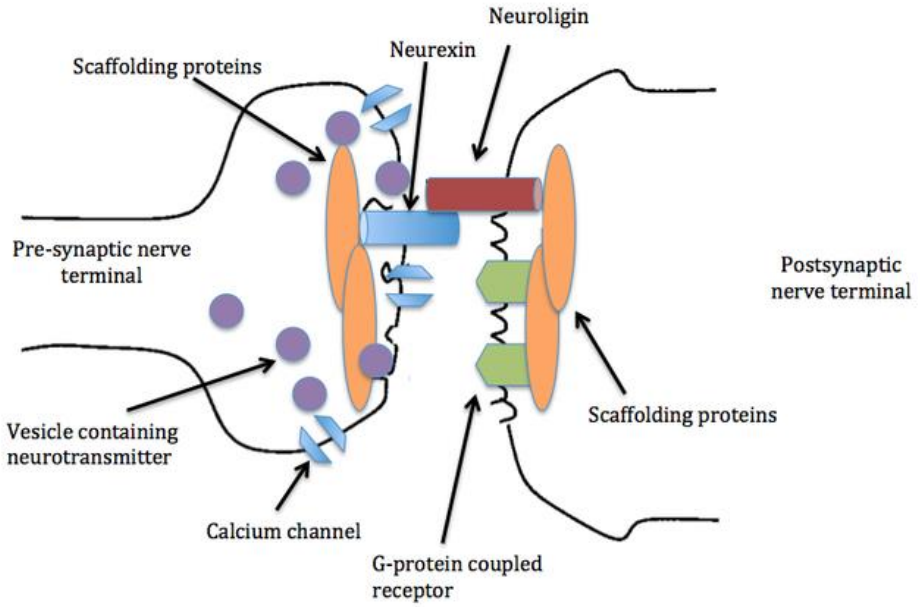

- Reduced NLGN3/4 levels associated with autism (impact on neuronal function and development )

- C to T transition in NLGN3 inherited from the mother and changing a highly conserved arginine residue into cysteine (R451C) within the esterase domain

- A frameshift mutation (1186insT) was identified in NLGN4 . This mutation creates a stop codon at position 396, leading to premature termination of the protein before the transmembrane domain.

# NRXN1 AND AUTISM

- NRXN1 encodes for Neurexin 1 – alpha
- Presynaptic protein that helps to connect neurons at the synapse



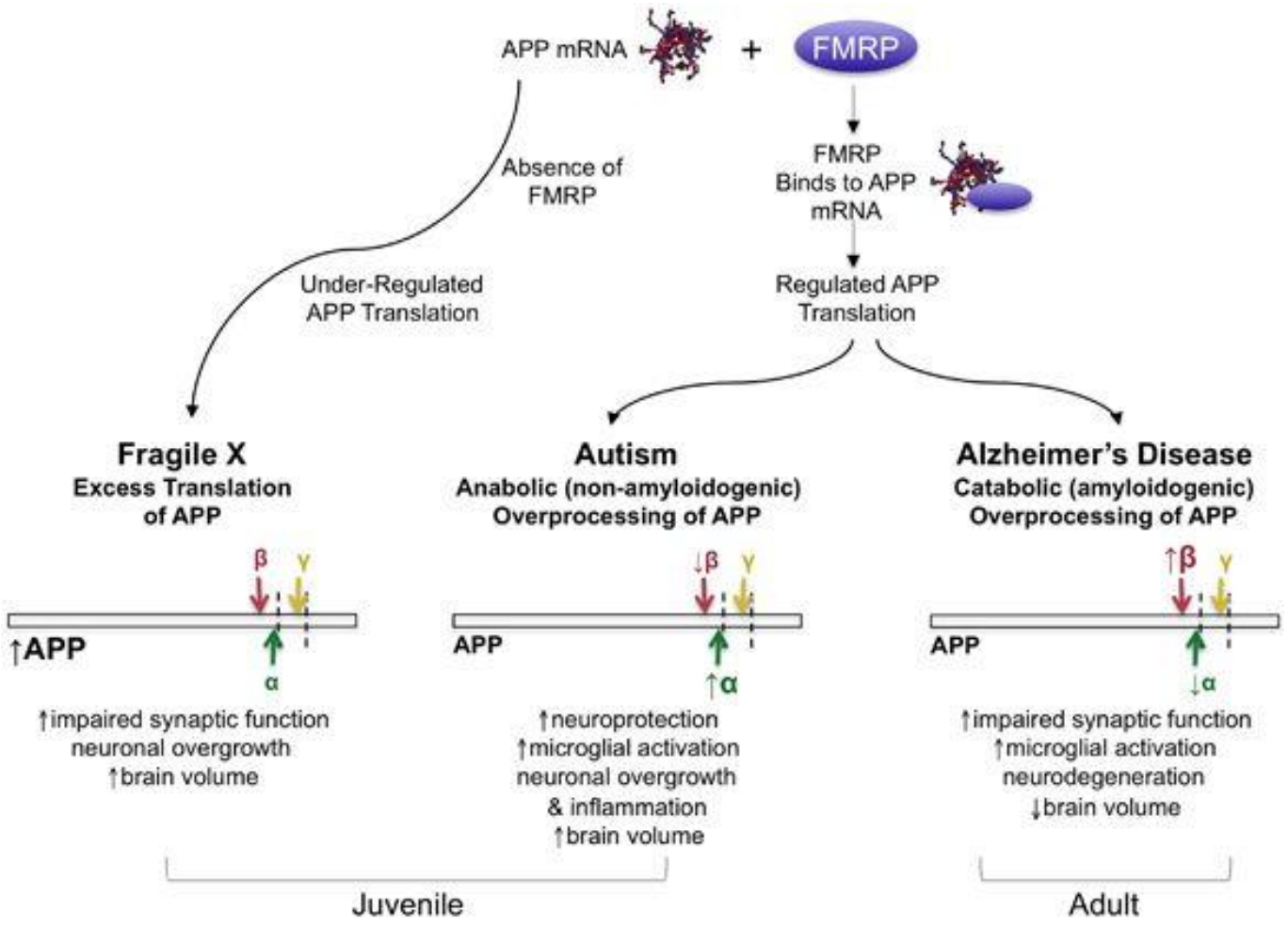
# NRXN-1 AND AUTISM

| *Location* | *α-NRXN1: NM_001135659.1 (exon 1–17, 19–24) (NP_001129131.1/ 1547 AA)* | *β-NRXN1: NM_138735.2 (exon 18–20, 22–24) (NP_620072.1/442 AA)* | *Variant types* | *Patients (n = 115)/ (no. found in controls)* |
|---|---|---|---|---|
| Exon 1 | c.511C > T (p.L171L) | NA | rs1045874 | 35/ND |
| Intron 3 | c.889 + 117T > C[a] | NA | Novel intronic | 1/ND |
| Intron 5 | c.930-56C > T[a] | NA | Novel intronic | 1/ND |
| Exon 6 | c.999C > T (p.P333P) | NA | rs2303298 | 7/ND |
| Intron 8 | c.1440 + 85C > T | NA | rs186874890 | 1/ND |
| Intron 11 | c.2467 + 55_2467 + 56insT | NA | rs5831131 | 30/ND |
| Intron 11 | c.2467 + 60G > A[a] | NA | Novel intronic | 1/ND |
| Exon 14 | c.2713T > A[a] (p.F905I) | NA | Novel missense | 1/(0/310) |
| Intron 17 | c.3484 + 20T > C | NA | rs3213756 | 41/ND |

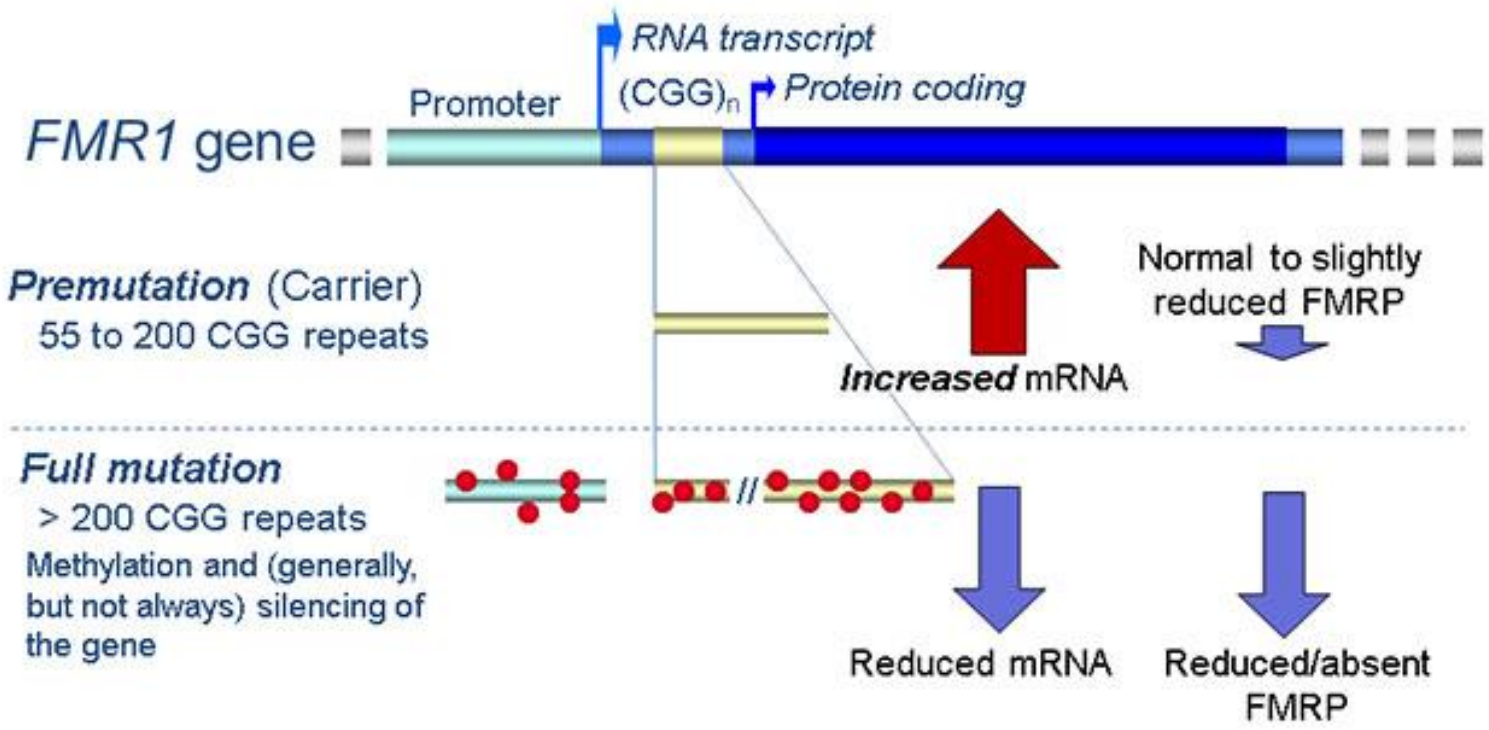
- NRXN1 mutations impact synaptic signaling
- Link with autism

# FMR-1 AND AUTISM

**FMR1** (fragile X mental retardation is a human gene that codes for a protein called fragile X mental retardation protein, or FMRP. This protein, most commonly found in the brain, is essential for normal cognitive development and female reproductive function.



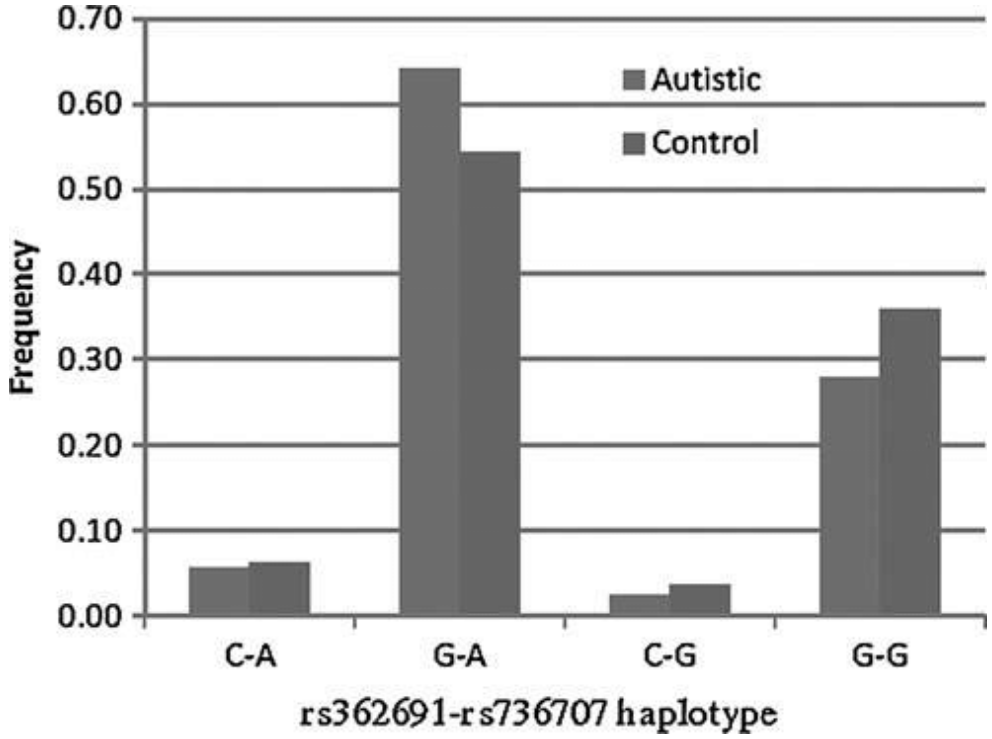
# FMR1 AND AUTISM



# RELN AND AUTISM

- The RELN gene provides instructions for making a protein called reelin.
- This protein is produced in the brain both before and after birth. Reelin is released by certain brain cells; then it attaches (binds) to specific receptor proteins. In the developing brain, this binding turns on (activates) a signaling pathway that triggers nerve cells (neurons) to migrate to their proper locations.
- Reelin also controls release of neurotransmitters

# RELN AND AUTISM




- RELN mutations impact neuronal signaling and neuronal developing – associated with autism

# IF SOMEONE IS AT RISK OF AUTISM?

- Defects in folate genetics
- Reduce exposure to toxic chemicals and pollutants
- Increase methylfolate intake, vitamin B12 intake
- Intake of dark leafy vegetables
- Keto diet as a treatment for autism

# Schizophrenia is a PSYCHOTIC DISORDER

A severe mental disorder in which thinking and emotion are so impaired that the individual is seriously out of contact with reality.

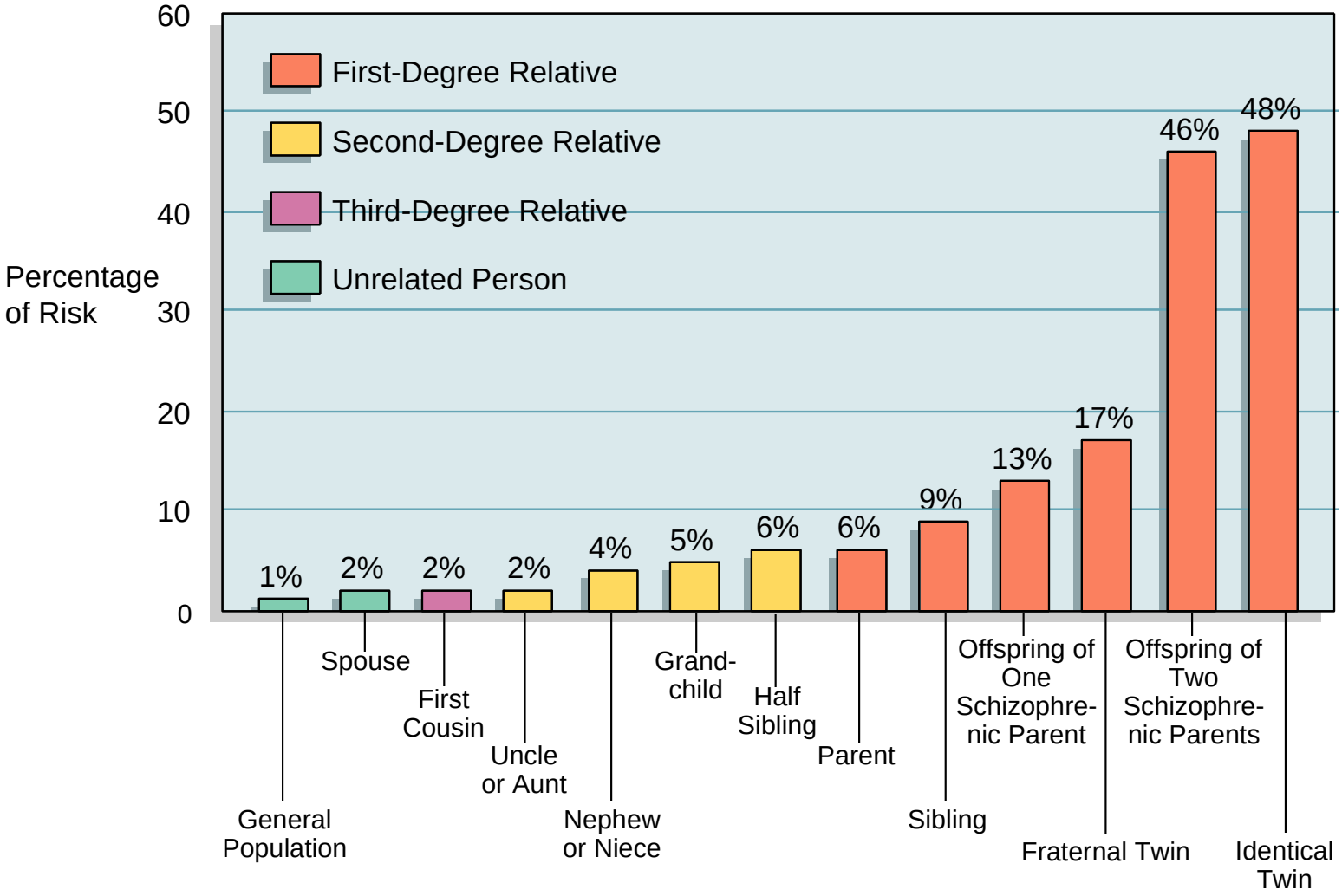
It is not multiple personality disorder

# Positive Symptoms

- Distortions or excesses of normal functioning
  - delusions,
  - hallucinations,
  - disorganized speech,
  - thought disturbances,
  - motor disturbances
- Positive symptoms are generally more responsive to treatment than negative symptoms

# Delusions

- False beliefs that are firmly and consistently held despite disconfirming evidence or logic
- Individuals with mania or delusional depression may also experience delusions.
- However, the delusions of patients with schizophrenia are often more bizarre (highly implausible).


60
50
40
30
20
10
0
Percentage of Risk
First-Degree Relative
Second-Degree Relative
Third-Degree Relative
Unrelated Person
1%
2%
2%
2%
4%
5%
6%
6%
9%
13%
17%
46%
48%
General Population
Spouse
First Cousin
Uncle or Aunt
Nephew or Niece
Grand-child
Half Sibling
Parent
Sibling
Offspring of One Schizophre-nic Parent
Fraternal Twin
Offspring of Two Schizophre-nic Parents
Identical Twin
Relationship to Schizophrenic Person

# Biological Finding

- ***The Dopamine Hypothesis***
  - Disturbed functioning in dopamine system (i.e., excess dopamine activity at certain synaptic sites)
- Supportive evidence:
  - Phenothiazines reduce dopamine activity and psychotic symptoms are reduced;
  - L-Dopa and amphetamines increase dopamine activity and can produce psychotic symptoms

POLYGENIC DISEASE

# SCHIZOPHRENIA GENETICS AND HLA

- HLA alleles have been implicated in both the risk of developing schizophrenia along with having a protective effect
- Infectious diseases during neurodevelopment are thought to implicate the risk of developing schizophrenia
- HLA are central mediators of immunity
- Autoimmune response causing damage to anatomical structures during neuronal development

# SCHIZOPHRENIA GENETICS AND HLA

**Table 2.** Association of HLA alleles DQA1*0501, DQB1*02 and DQA1*0301, DQB1*0302 and the DQ2 and DQ8 heterodimers in patients with schizophrenia and controls

| HLA | Patients N=104 | Controls N=60 | Odds Ratios (95% CI) | Fisher exact P value |
|---|---|---|---|---|
| DQA1*0501 | 42 (40.38%) | 31 (51.66%) | 0.634 (0.334-1.202) | 0.108 |
| DQB1*02 | 67 (64.42%) | 26 (43.33%) | 2.368 (1.237-4.534) | 0.007*, ** |
| DQA1*0301 | 37 (35.57%) | 10 (16.66%) | 2.803 (1.273-6.172) | 0.006*, ** |
| DQB1*0302 | 33 (31.73%) | 10 (16.66%) | 2.357 (1.064-5.221) | 0.0230* |
| DQ2+ | 19 (18.26%) | 13 (21.66%) | 0.808 (0.367-1.781) | 0.369 |
| DQ8+ | 13 (12.5%) | 10 (16.66%) | 0.714 (0.292-1.746) | 0.302 |

* P value <0.05

** P value corrected after bonferroni P< 0.017 (alpha=1-0.95 $^{1/N}$)

# SCHIZOPHRENIA GENETICS AND CACN1C

- Belongs to a family of genes that provide instructions for making calcium channels. These channels, which transport positively charged calcium atoms (calcium ions) into cells, play a key role in a cell's ability to generate and transmit electrical signals.
- Voltage-gated calcium channels couple depolarization of the cell-surface membrane to entry of calcium, which triggers secretion, contraction, neurotransmission, gene expression, and other physiological responses.

- The calcium channel produced from the CACNA1C gene is known as CaV1.2. These channels are found in many types of cells, although they appear to be particularly important for the normal function of heart and brain cells.

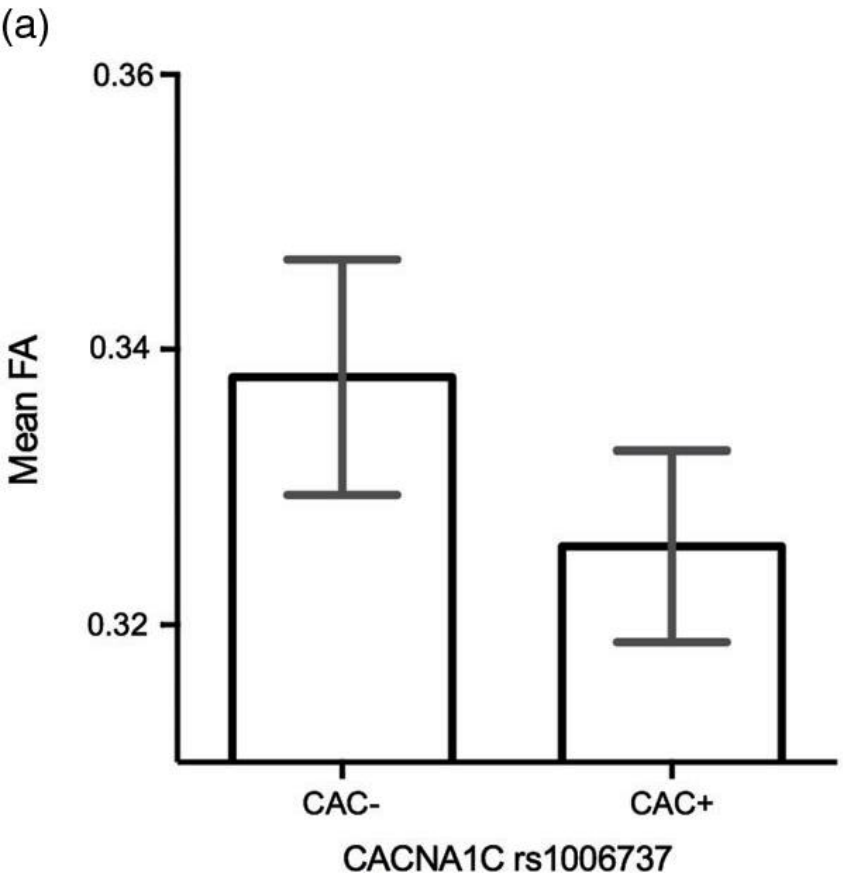
# SCHIZOPHRENIA GENETICS AND CACN1C

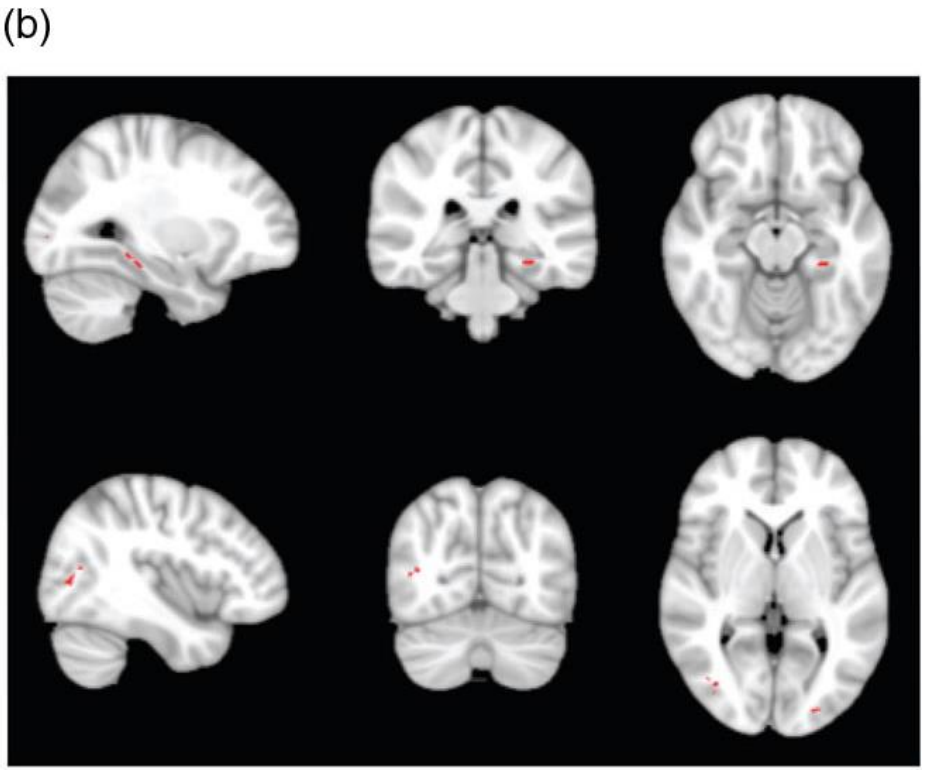
| *Chromosome 12p13* (CACNA1C) | | | | | | | *For comparison* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Schizophrenia (combined Denmark I, II and III)* | | | | | | *Bipolar disorder (combined WTCCC/STEP-UCL/ED-DUB-STEP2)* | | | |
| | *Genotype counts (AA/AG/GG)* | | *MAF* | | | | *MAF* | | | |
| *SNP, minor allele* | *SZ* (n = *976*) | *CON* (n = *1489*) | *SZ* | *CON* | P-*value* | *OR* | *BPD* (n = *4387*) | *CON* (n = *6209*) | P-*value* | *OR* |
| rs1006737, A | 130/444/402 | 158/675/656 | 0.361 | 0.333 | 0.015 | 1.160 | 0.356 | 0.324 | $7.0 \times 10^{-8}$ | 1.181 |

Abbreviations: BPD, bipolar disorder; CON, control individuals; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; SZ, schizophrenia; WTCCC, Wellcome Trust Case-Control Consortium.

# SCHIZOPHRENIA GENETICS AND CACN1C

A allele carriers of CACNA1C (CAC+) have lowered fractional anisotropy (index of myelination)



# SCHIZOPHRENIA GENETICS AND CACNB2

•At synapses, these channels form large signaling complexes in the presynaptic nerve terminal, which are responsible for the calcium entry that triggers neurotransmitter release and short-term presynaptic plasticity.

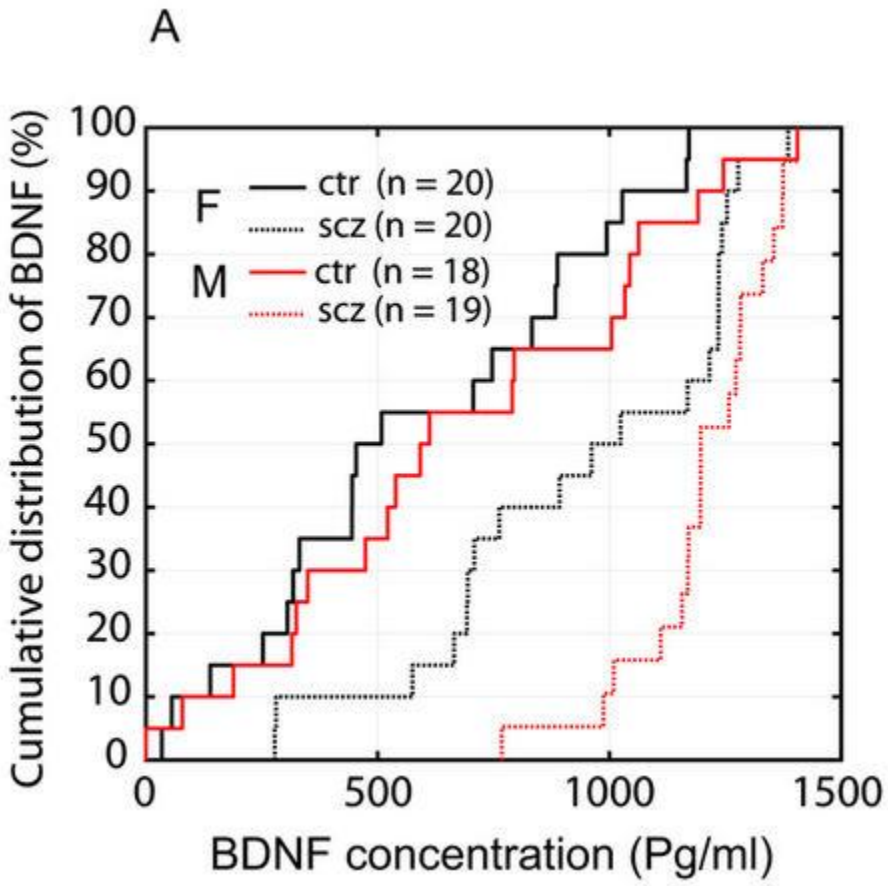
CACNB2 (intronic SNP rs17691888) which encodes the β2 subunit of L-type calcium channels (Cav β2).

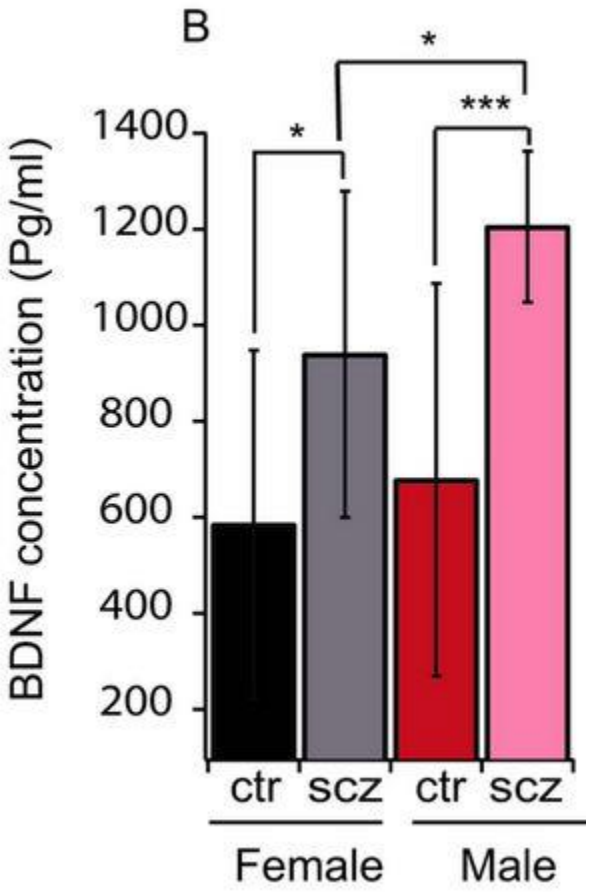
- G allele of CACNB2 associated with Schizophrenia
- Impact on voltage gated calcium channel activity which impacts neurotransmitter physiology and homeostasis

# SCHIZOPHRENIA GENETICS AND BDNF

- The brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophin in the brain, and it has been associated with several psychiatric disorders.
- An inadequate neurotrophic support during brain development could lead to a structural disorganization of networks in which neural connections are established in a sub-optimal manner.
- Therefore, alterations in BDNF may contribute to altered brain development, failures in neuroplasticity, and synaptic disconnectivity,
- Explain at least in part some of the morphological, neurochemical, and cytoarchitectonic abnormalities found in the brains of patients with schizophrenia

# Increased BDNF levels associated with Schizophrenia



# SCHIZOPHRENIA GENETICS AND BDNF

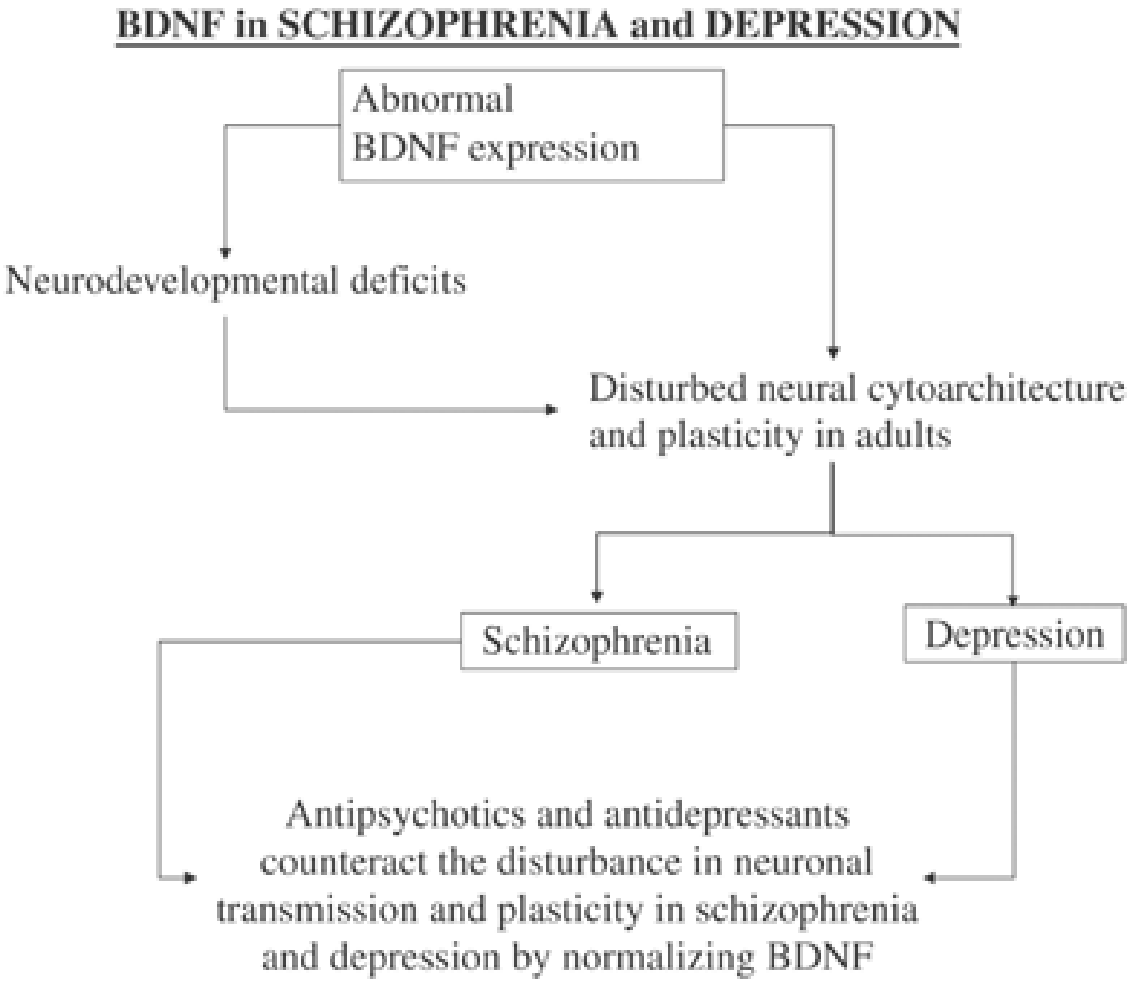
- Val66Met polymorphism
- Rs6265 – A allele – associated with Schizophrenia
- A allele also associated with increased suicide risk among schizophrenics

History of suicide attempts by genotype and allele frequencies in schizophrenia patients when dividing genotypes based on the existence of the specific allele

| | Genotype | | $\chi^2$ | p * |
|---|---|---|---|---|
| 196G/A | Patients without A allele | Patients with A allele | 4.037 | 0.045 |
| Suicide attempt | 3 | 22 | | |
| None | 42 | 90 | | |
| 11757G/C | Patients without C allele | Patients with C allele | 0.024 | 0.877 |
| Suicide attempt | 10 | 15 | | |
| None | 55 | 77 | | |

*genotypes which included the A allele, G/A and A/A, showed a statistically higher history of previous suicide attempts

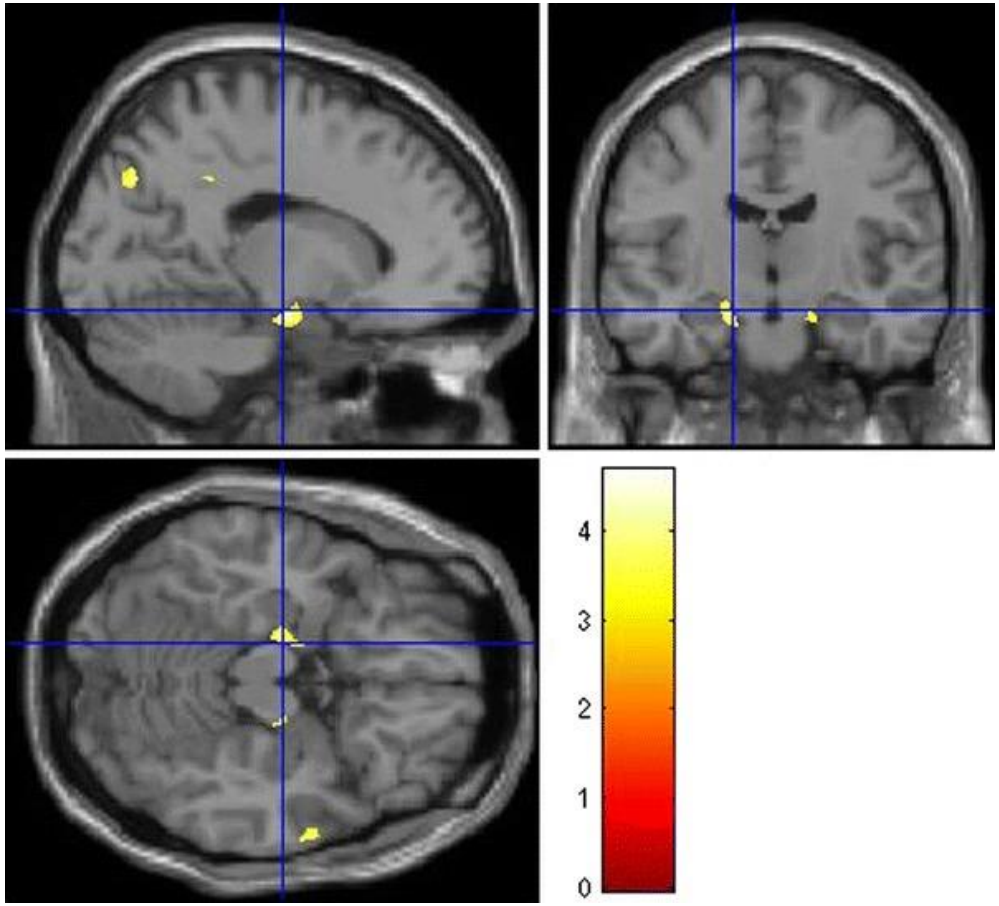
# BNDF and Schizophrenia



# SCHIZOPHRENIA GENETICS AND DTNB1

- Encodes for dysbindin
- dysbindin is found in neural tissue of the brain, particularly in axon bundles and especially in certain axon terminals, notably mossy fiber synaptic terminals in the cerebellum and hippocampus
- DTNBP1 interacts with the dystrophin glycoprotein complex at postsynaptic sites and might thereby alter neuronal synaptic mechanisms.
- Mutations in dysbindin associated with risk of Schizophrenia
- DTNB1 levels reduced in Schizophrenia
- Rs2619522 – G allele risk allele
- Rs1018381 – T allele risk allele

# SCHIZOPHRENIA GENETICS AND DTNB1

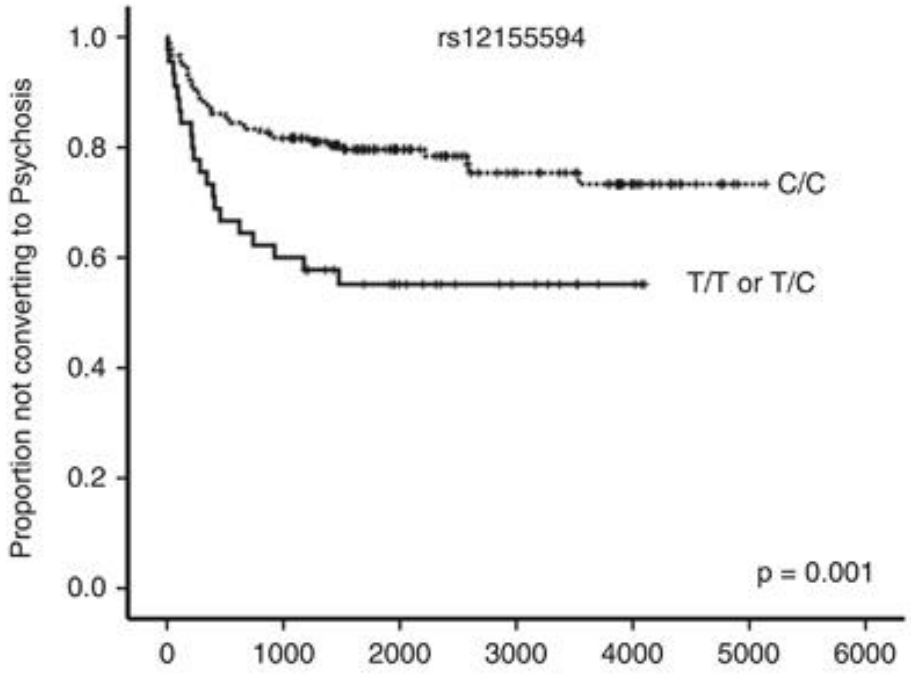


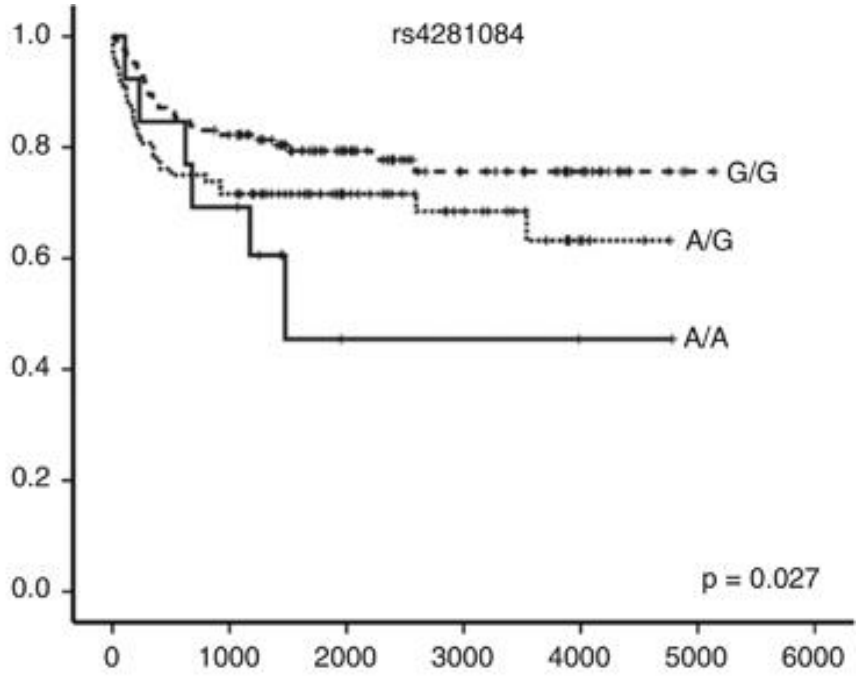

- Grey matter refers to unmyelinated neurons and other cells of the central nervous system. It is present in the brain, brainstem and cerebellum, and present throughout the spinal cord.
- Risk allele carriers – increased grey matter
- Progressive dysregulation ?

# SCHIZOPHRENIA GENETICS AND NRG1

- • Encodes for Neuregulin 1 – involved in development of neurons
- Neuregulin 1 is thought to play a role in synaptic plasticity. It has been shown that a loss of Neuregulin 1 within cortical projection neurons results in increased inhibitory connections and reduced synaptic plasticity (important for learning and memory)
- Careful regulation of the amount of Neuregulin 1 must be maintained in order to preserve an intricate balance between excitatory and inhibitory connections within the central nervous system
- Dysregulation associated with Schizophrenia
- rs4281084 and rs12155594

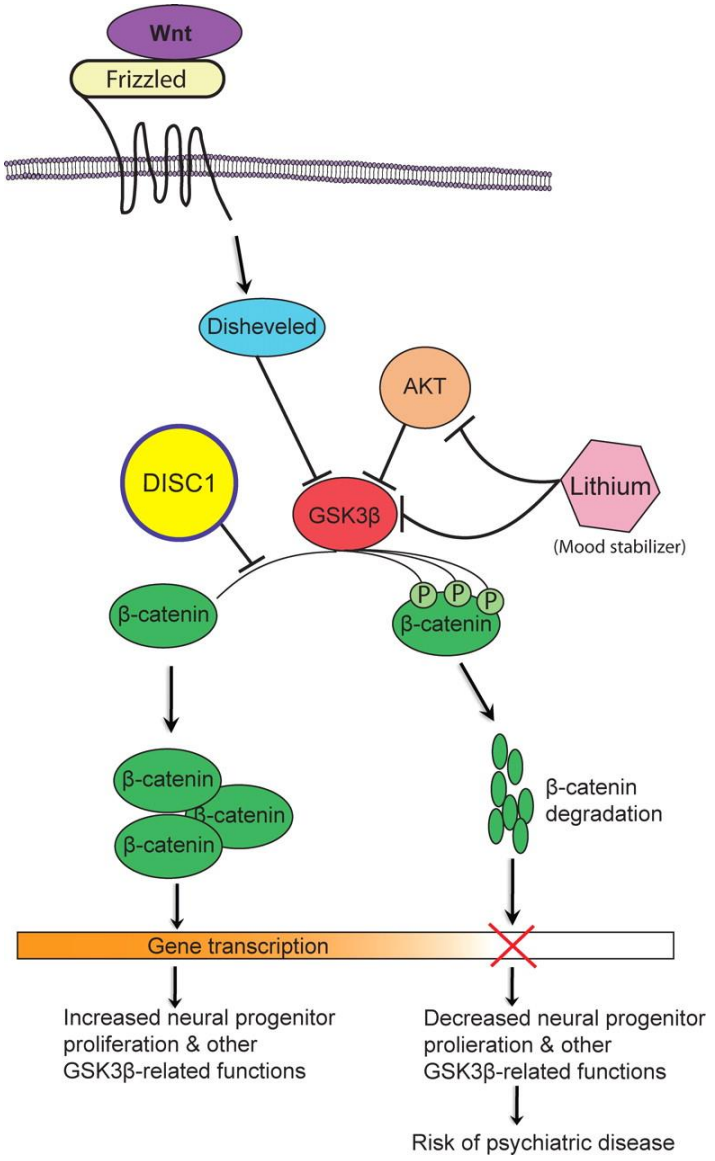
# NRG1 SNPs and Psychosis



# SCHIZOPHRENIA GENETICS AND DISC1

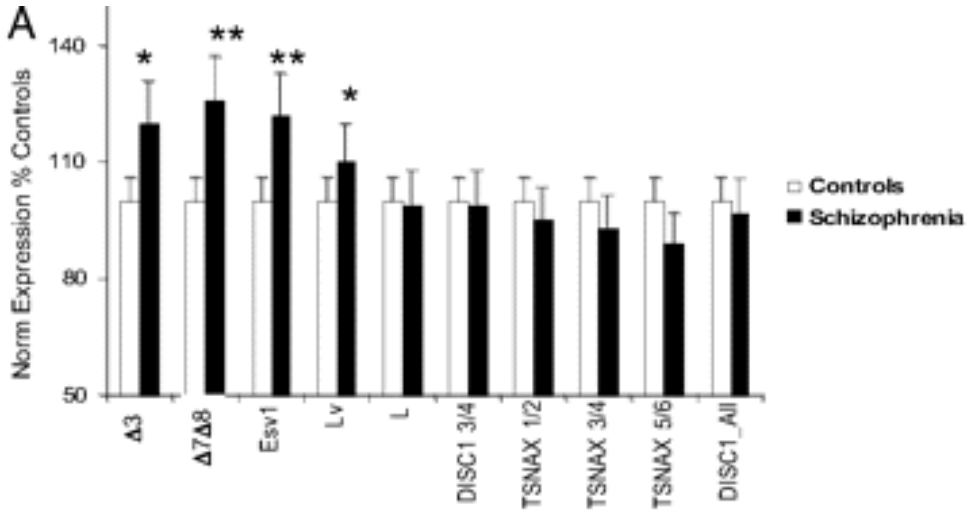
- • This gene encodes a scaffold protein with multiple coiled coil motifs which is located in the nucleus, cytoplasm and mitochondria.
- The protein is involved in neurite outgrowth and cortical development through its interaction with other proteins.
- Mutations associated with Schizophrenia

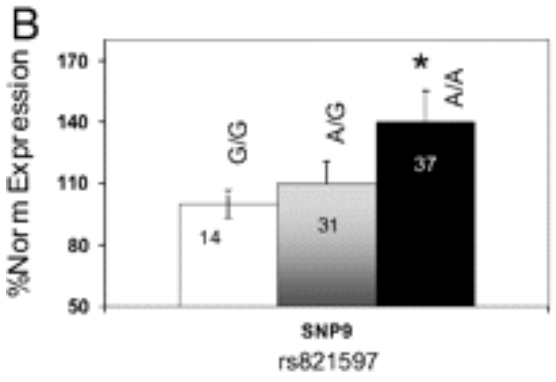


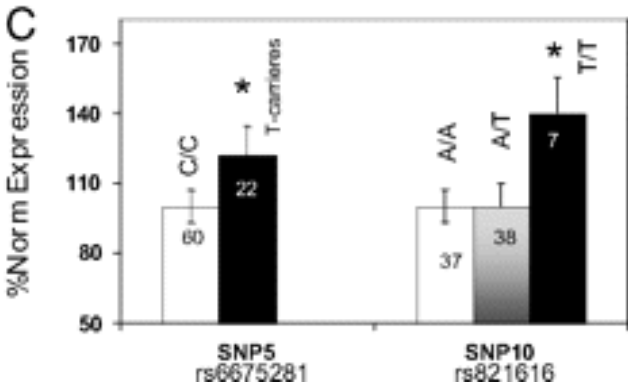
# SCHIZOPHRENIA GENETICS AND DISC1

- Mutations resulting in truncated protein associated with schizophrenia

- Higher expression of defective transcripts

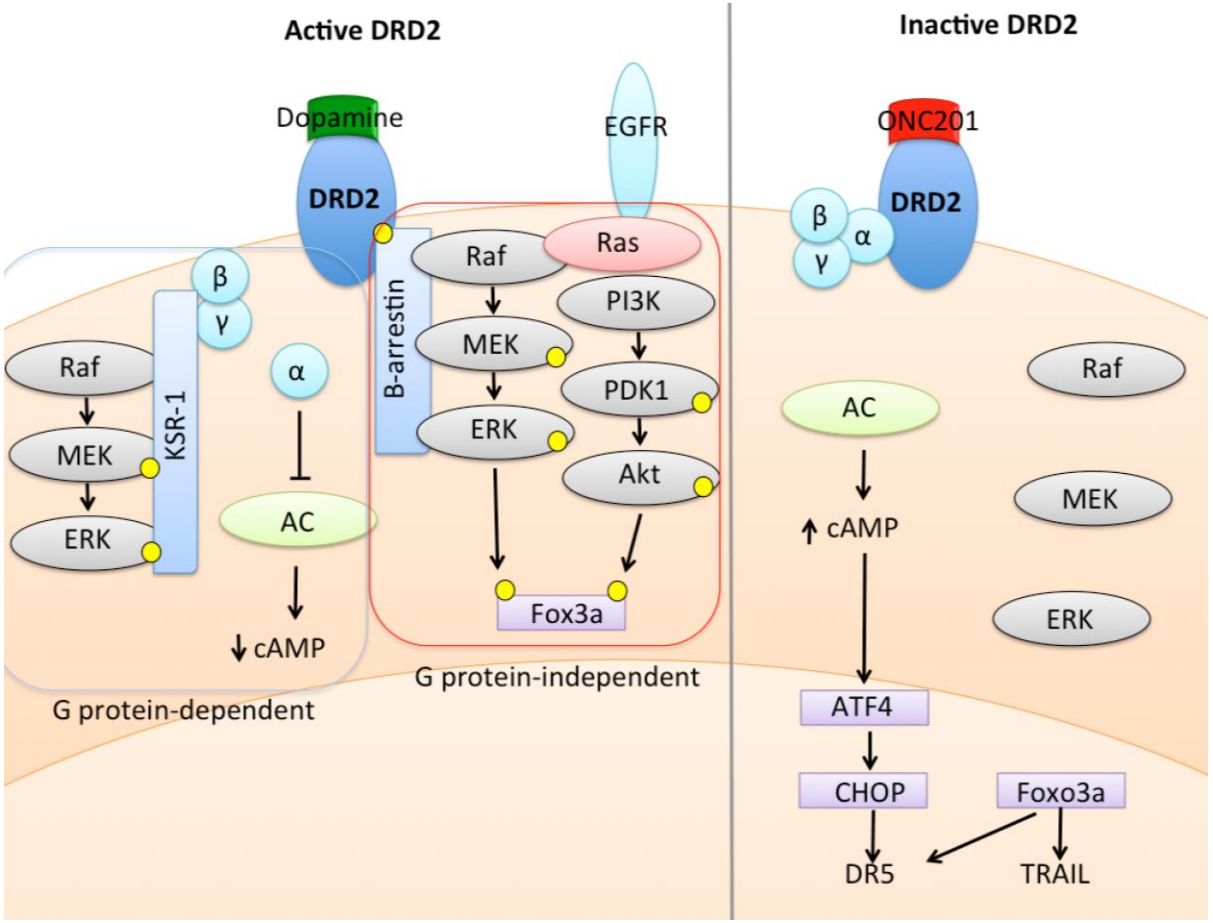


# SCHIZOPHRENIA GENETICS AND DRD2

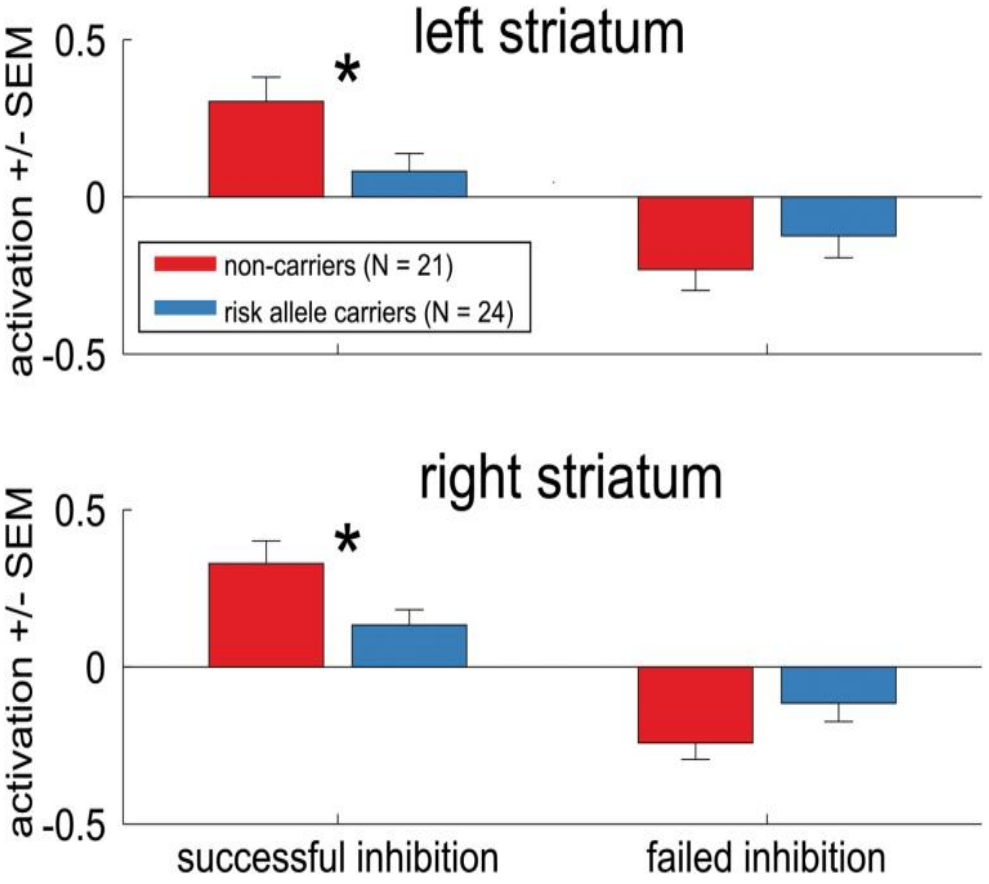
- DRD2 – Dopamine Receptor D2
- Important for dopamine induced signaling
- Mutations impact dopamine signaling



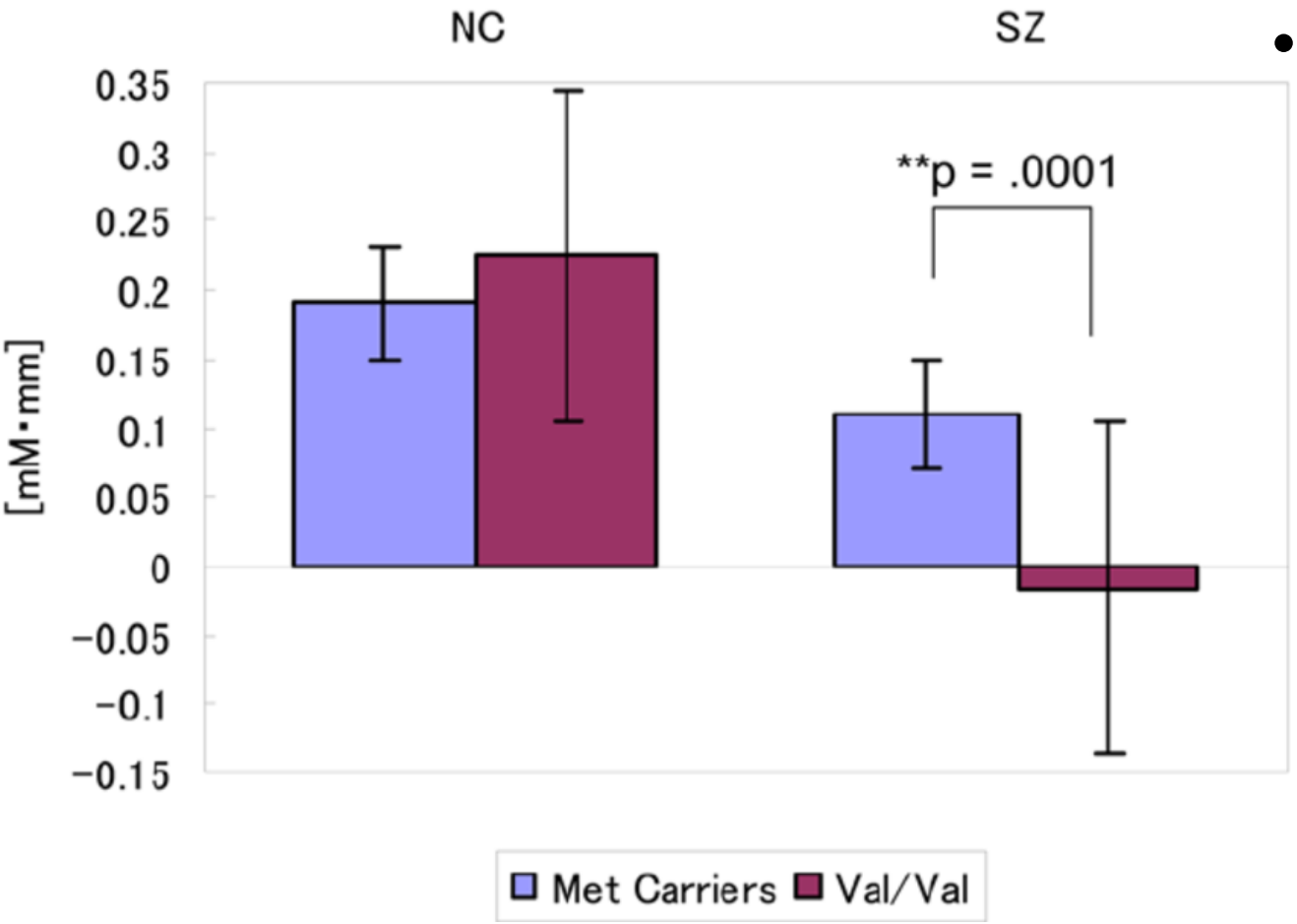
# SCHIZOPHRENIA GENETICS AND DRD2

- DRD2 risk allele rs2514218

Risk allele carriers showed a diminished striatal (basal ganglia) response to increasing proactive inhibitory control demands, whereas overall level of striatal activation (voluntary response) in carriers was elevated compared to noncarriers.

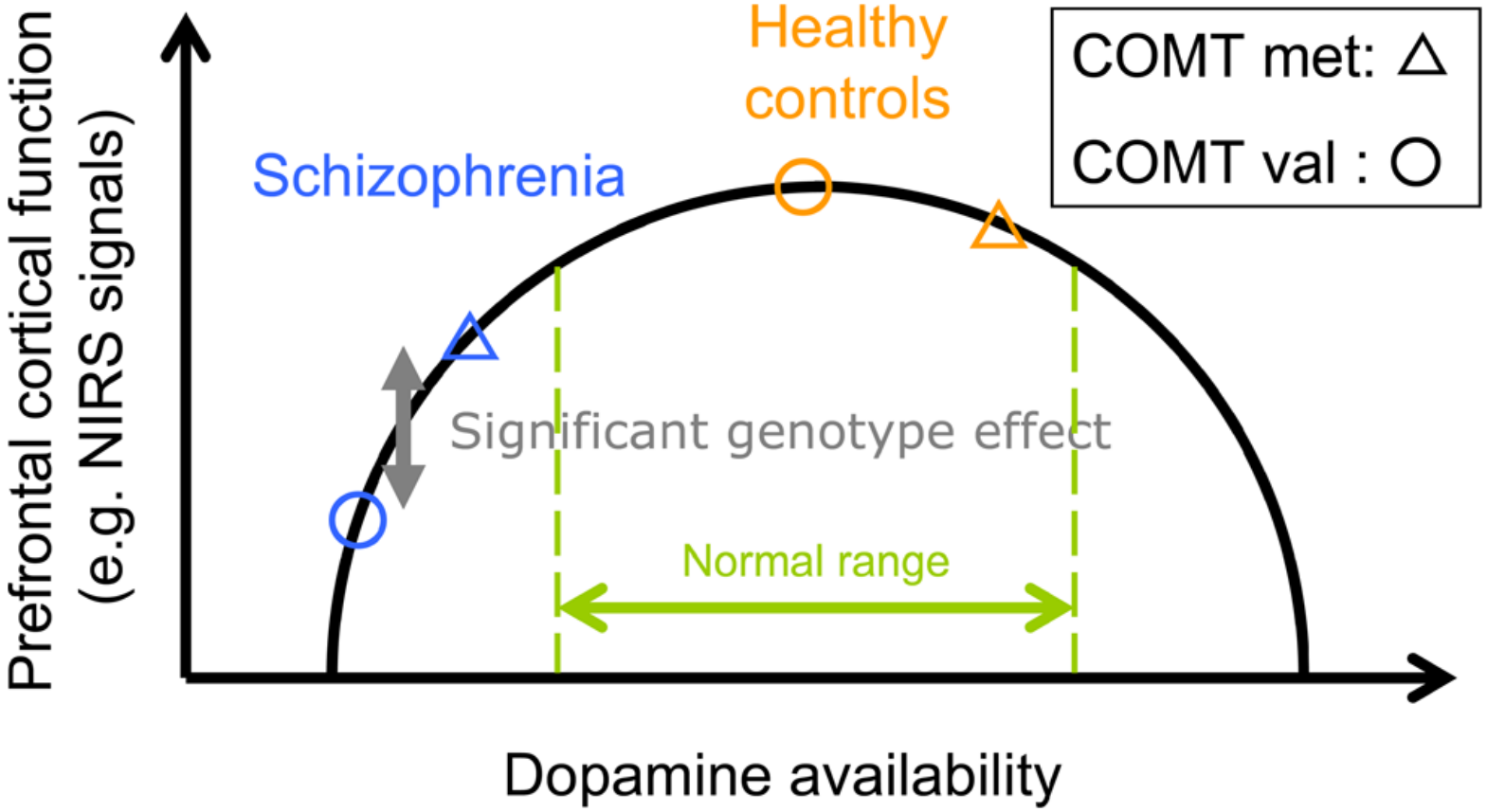


# COMT AND SCHIZOPHRENIA




- Prefrontal hemodynamic activation – significantly reduced in Val carriers – Fast COMT metabolizers

# COMT AND SCHIZOPHRENIA



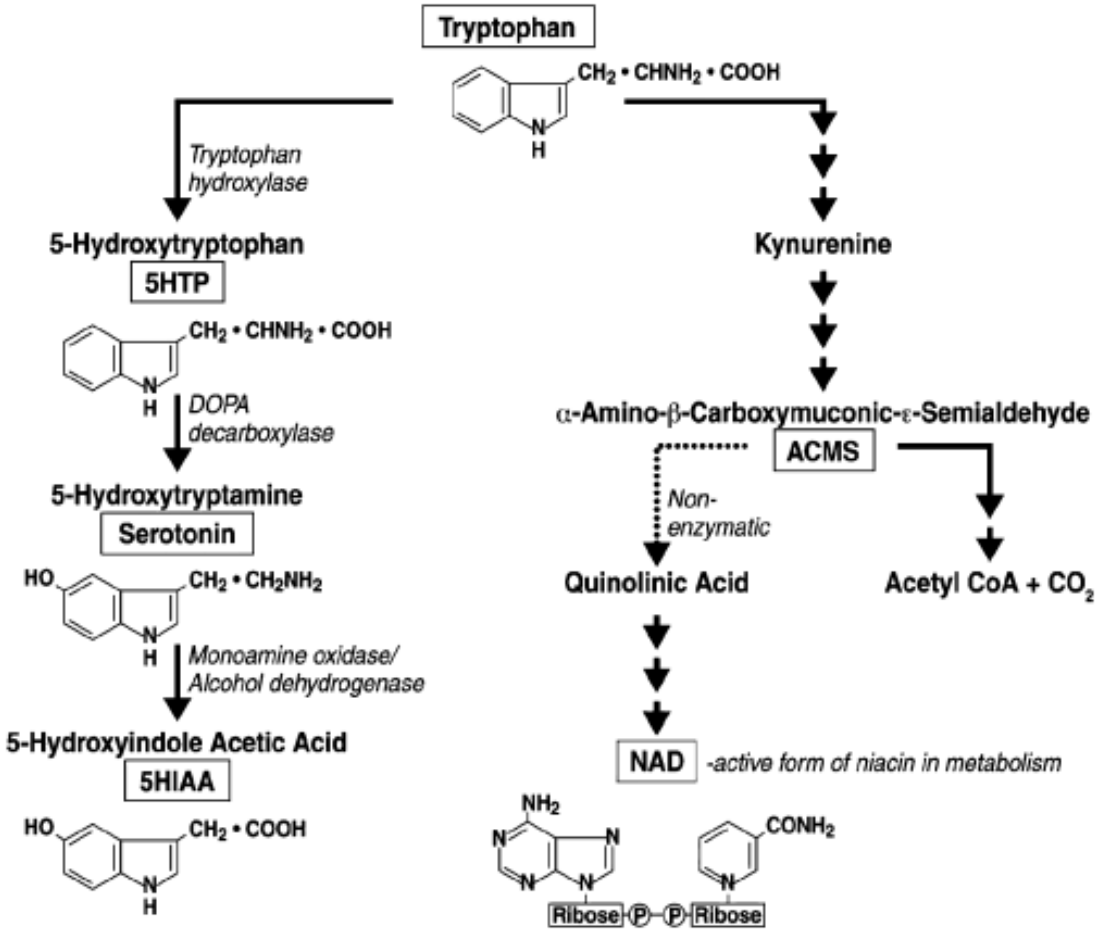
# WHAT TO DO IF AT INCREASED RISK?

- Don't use street drugs, and moderate any use of alcohol
- Make an ongoing effort to develop your social skills as much as you can
- Avoid social isolation
- Make an ongoing effort to maintain friendships with adults
- Make an extra effort to learn positive perspectives on the world
- Make extra effort to learn how to deal with stress and anxiety
- Seek Help from Qualified Psychologists and Psychiatrists if you have problems coping

# Serotonin

- Serotonin is a chemical / neurotransmitter that has a wide variety of functions in the human body.
- It is sometimes called the happy chemical, because it contributes to wellbeing and happiness.
- The scientific name for serotonin is 5-hydroxytryptamine, or 5-HT. It is mainly found in the brain, bowels, and blood platelets.

# SEROTONIN PATHWAY



# Endogenous Function

- Central neurotransmitter
- Precursor of melatonin
- GI tract: uncertain;  motility?
- In carcinoid tumors (neuroendocrine tumors): large amounts released leading to diarrhea, bronchoconstriction and edema
- Platelets: 5-HT2 receptors → aggregation and vasoconstriction

# Serotonin Pharmacological Effects

- Respiratory system: bronchoconstriction if asthmatic; stimulation of aortic and carotid chemoreceptors → ↑ respiratory rate and minute vol. (air exhaled or inhaled per minute)
- GI tract: small intestine very sensitive to serotonin → intense rhythmic contractions due to direct and indirect (ganglia in wall) effects.

  Also stimulates vomiting (5-HT3 receptors on vagal afferents and centrally).

# Serotonin Pharmacological Effects -2

- Cardiovascular system: Multiple direct and indirect effects:

1. Direct vasoconstriction (large arteries) and indirect vasodilation (NO and $PGI_2$ – mediated)
2. Heart: direct inotropic (impact on muscles) and chronotropic effects (electric conduction)
3. Reflex mechanisms due to change in BP
4. Stimulation of sensory nerve endings in baroreceptors (sense change in pressure) and in vagal afferents in coronary circulation (Bezold Jarrisch reflex) → bradycardia (slow heart rate) and hypotension

# Serotonin in the Central Nervous System

- Pain perception
- Sleep/Wakefulness
- Various behaviors normal/abnormal: depression, schizophrenia, obsessive compulsive behavior, etc.
- Neuroendocrine regulation – controls hypothalamic cells involved in release of several anterior pituitary hormones.

# Depression

- Depression is among the top five leading causes of disability and disease burden throughout the world
- Stressful events involve threat, loss, humiliation, or defeat
  - Influence the onset and course of depression

# Predisposition

- Stress theories of depression predict that an individual's sensitivity to stressful events depends on their genetic makeup
- Gene x environment (GxE) interaction
  - Do specific genes exacerbate or buffer the effect of stressful life events on depression?
  - Studies have shown polymorphisms in Serotonin pathway along with environment stimuli as predisposition to depression

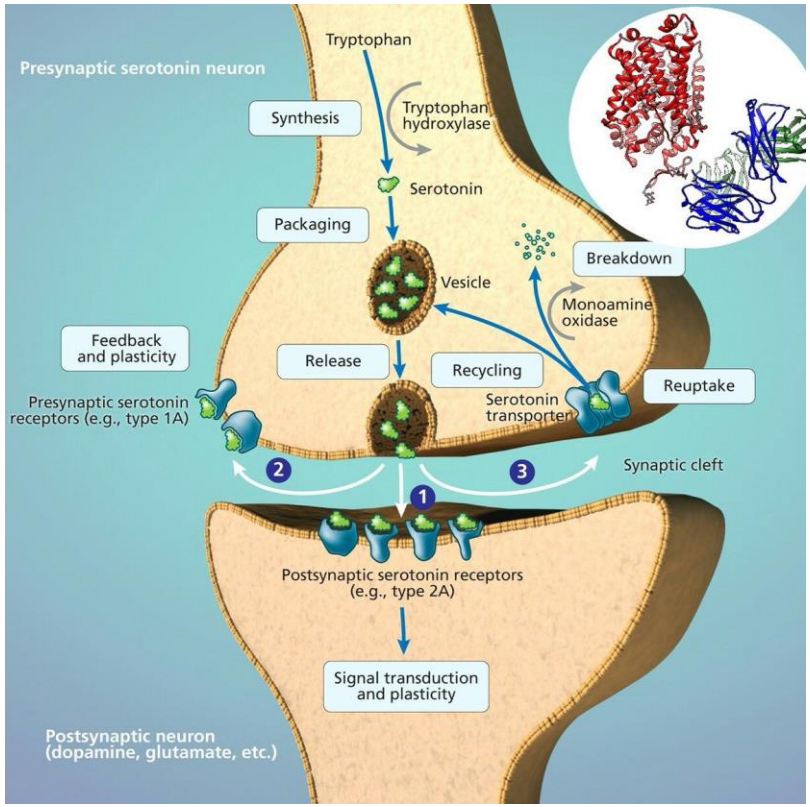
# Serotonin (5-HT) and Fear

5-HT strongly implicated in emotional behavior:

- 5-HT synapses targeted by mood-altering drugs
- SSRIs (serotonin reuptake inhibitor) effective against panic, anxiety & depression
- $5\text{-}HT_{1A}$ partial agonists are effective anxiolytics (drugs to reduce anxiety)
- $5\text{-}HT_{1A}$ knockout mice exhibit increased fear/anxiety
- 5-HTT knockout mice exhibit increased fear/anxiety

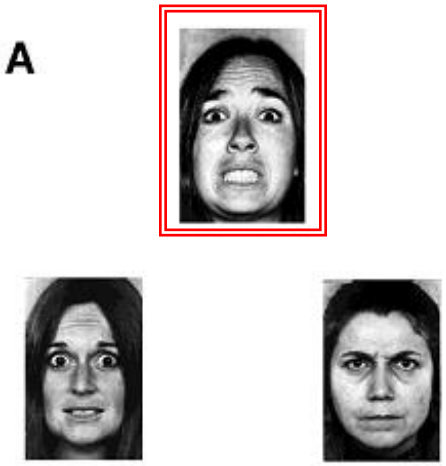
# 5-HTT Gene Polymorphism

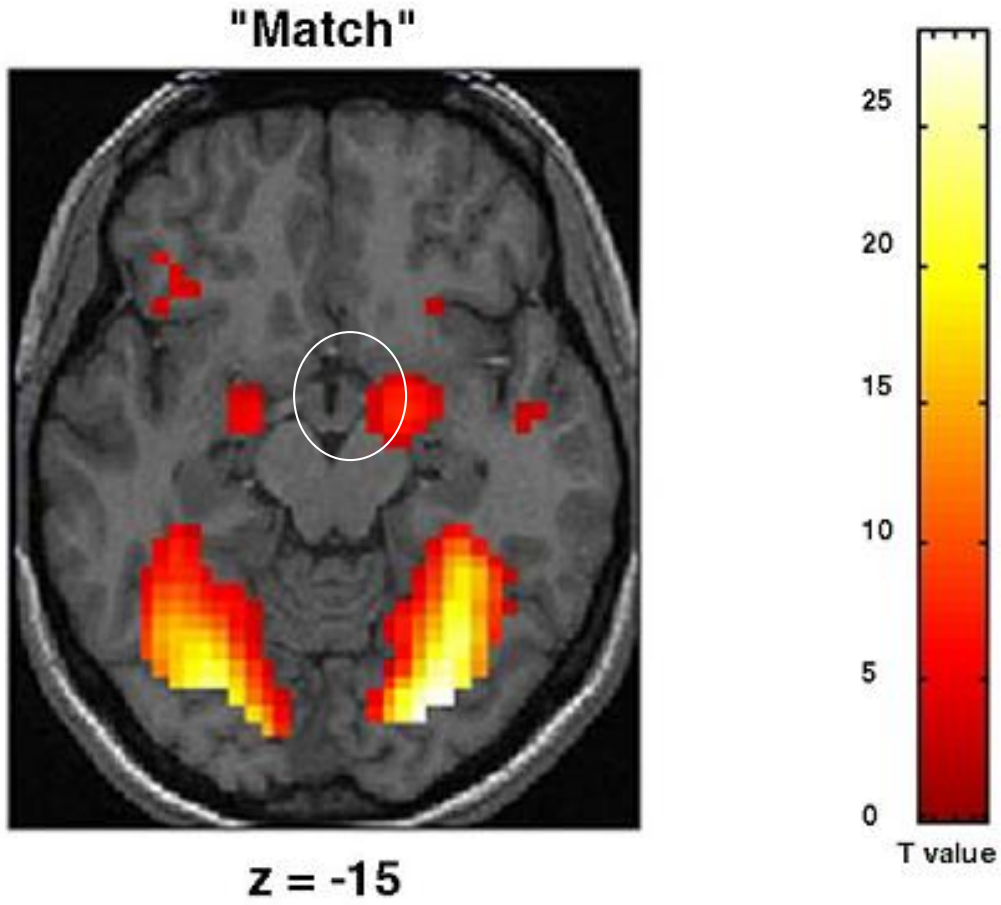
- The serotonin system is a logical source of candidate genes for depression (SSRI)
- Serotonin transporter gene (SLC6A4) has a functional polymorphism in the 5’ promoter region
- The short “s” allele is associated with lower transcriptional efficiency of the promoter compared with the long “l” allele



# Perceptual processing of fearful faces engages the amygdala

- Amygdala works in serotonin dependent fashion

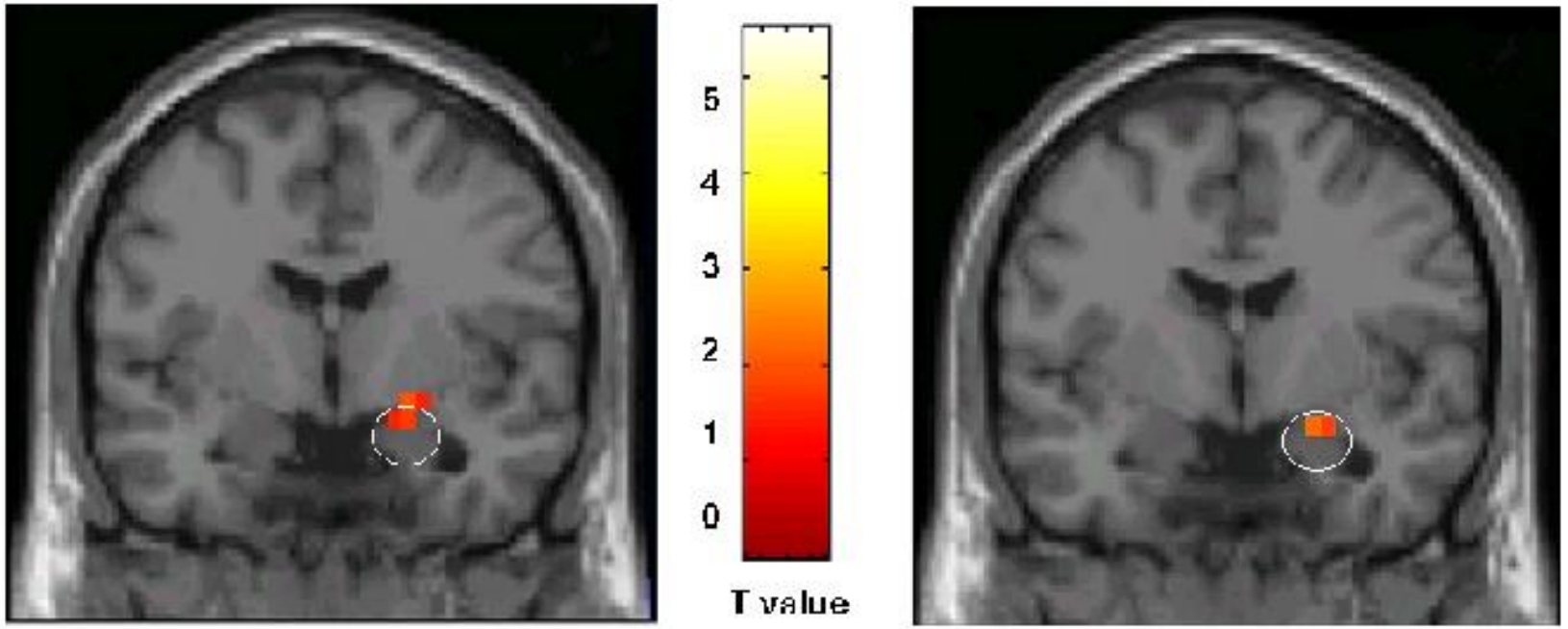


# 5-HTTLPR ALLELES

- 5-HTTLPR (serotonin-transporter-linked polymorphic region) is a degenerate repeat polymorphic region in SLC6A4, the gene that codes for the serotonin transporter.
- The 5-HTTLPR is a repeat polymorphism with long (L) and short (S) alleles. The S allele is associated with lower 5-HTT expression and function, as well as anxiety and negative mood in healthy individuals.

# 5’-HTTLPR genotype and fMRI during perceptual processing of fearful faces




first cohort
n=14

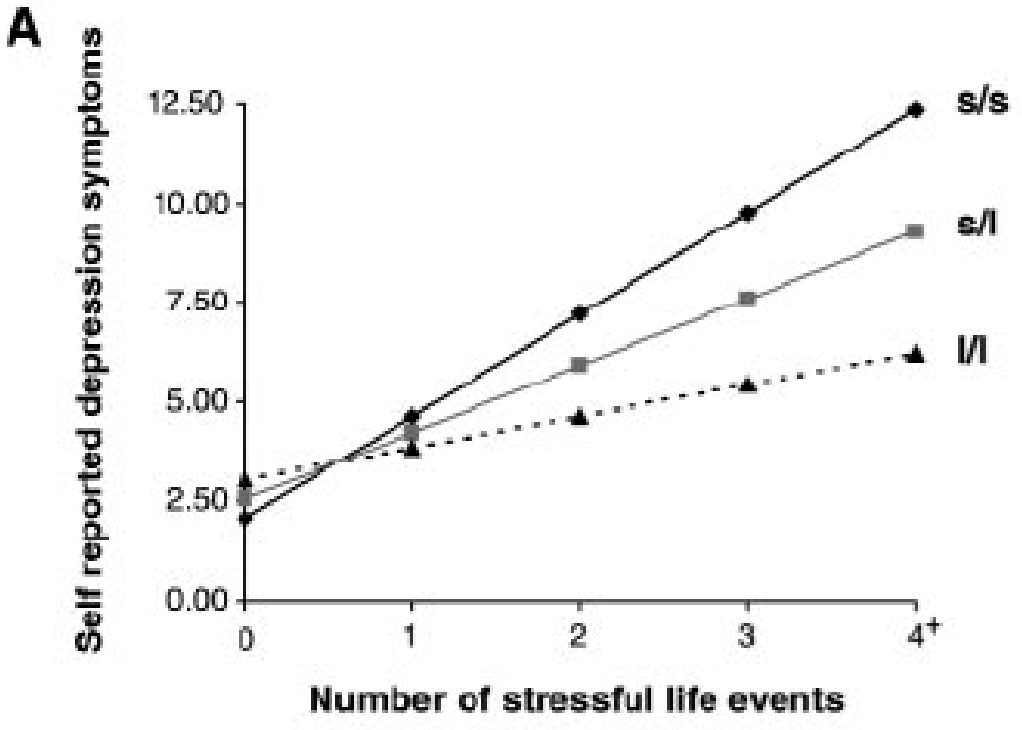
p<.05 corrected

second cohort
n=14

*s* allele carriers show a greater amygdala response than *ll* homozygous individuals

# SHORT ALLELE AND DEPRESSION

The interaction between genotype and life events showed that the effect of life events on self reports of depression symptoms at age 26 was significantly stronger (P=0.02) among individuals carrying an “s” allele than among l/l homozygotes

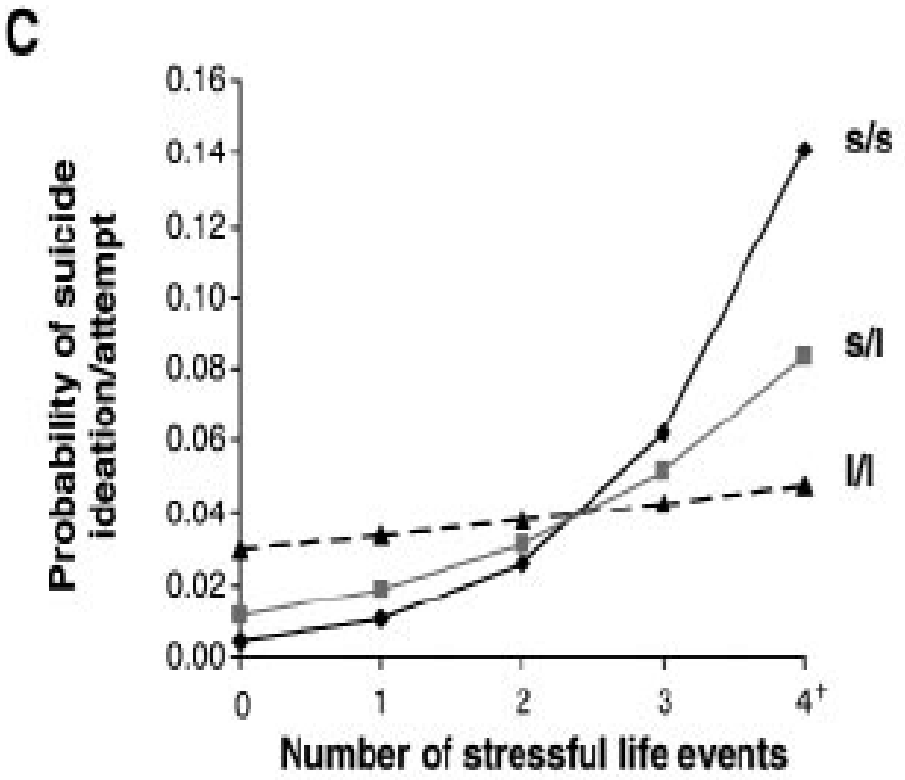


# SHORT ALLELE AND DEPRESSION

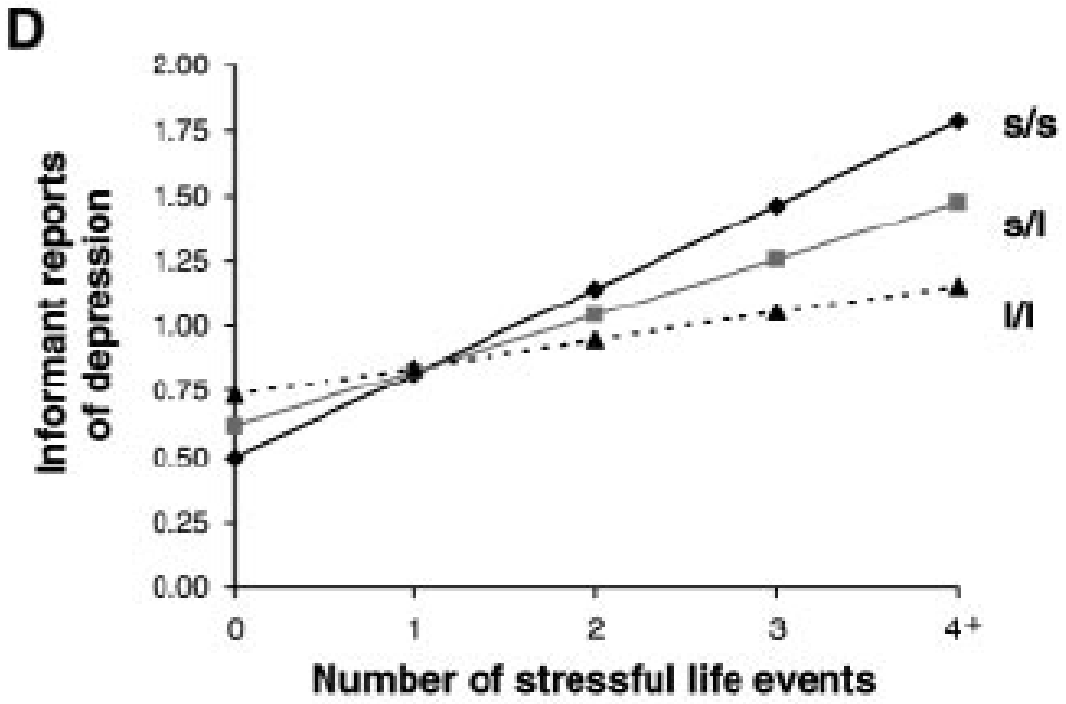

Stressful life events predicted a diagnosis of major depression among carriers of an s allele, but not among l/l homozygotes (P=0.056)

# SHORT ALLELE AND DEPRESSION ASSOCIATED SUICIDE

Stressful life events predicted suicide ideation or attempt among individuals carrying an s allele but not among l/l homozygotes (P=0.05)



# DEPRESSION RISK AND STRESSFUL EVENTS

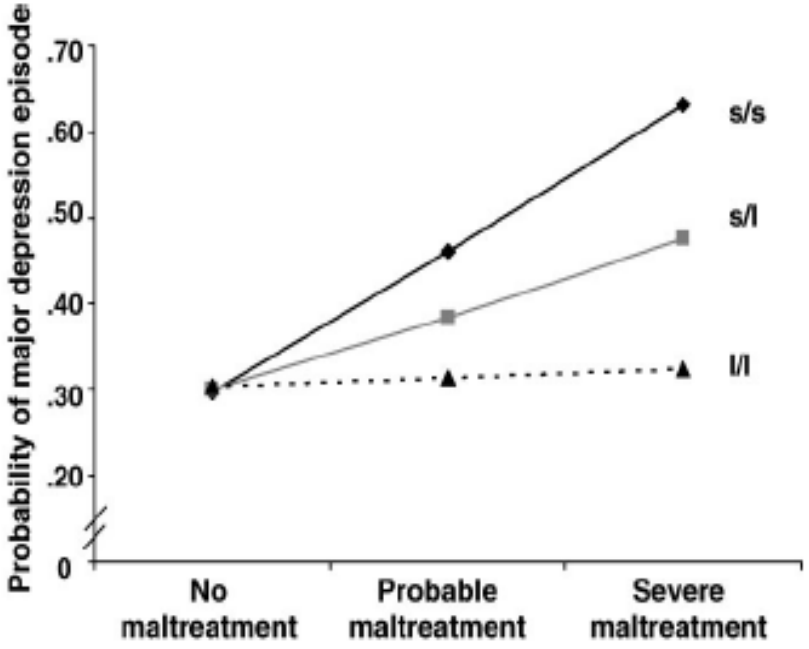



GxE interaction shows that the effect of life events on informant reports of depression was stronger among individuals carrying an s allele than among l/l homozygotes

# ENVIRONMENT EFFECTS

- If 5-HTT genotype moderates the depressogenic influence of stressful life events, it should moderate the effect of life events that occurred not just in adulthood, but also in earlier developmental stages

# SHORT ALLELE AND CHILDHOOD MALTREATMENT

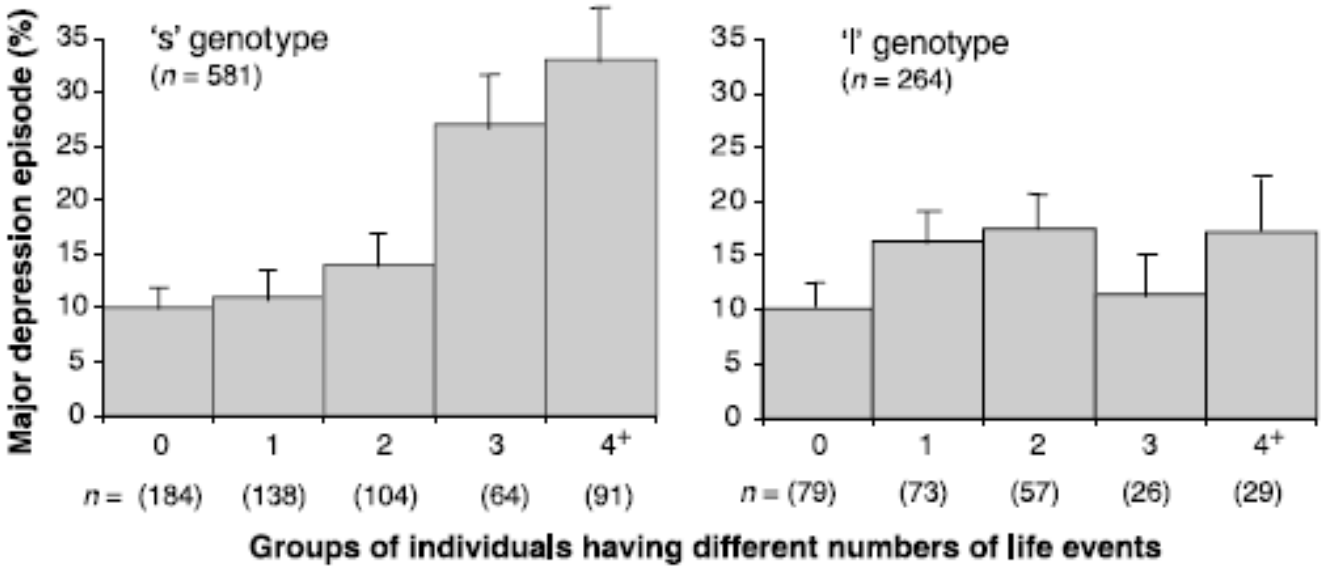



The interaction between GxE shows that childhood maltreatment (that occurred during the first decade of life) predicted adult depression only among individuals with an s allele and not among l/l homozygotes (P=0.05)

# MAOA involvement in depression

- Studies have previously shown that monoamine oxidase A moderates children's sensitivity to maltreatment
- MAOA has a high affinity for 5-HTT, but does not have a protective effect on l/l because the moderation of life stress on depression was observed regardless of MAOA gene status

# SHORT ALLELE AND DEPRESSION – RELATION TO STRESSFUL EVENTS

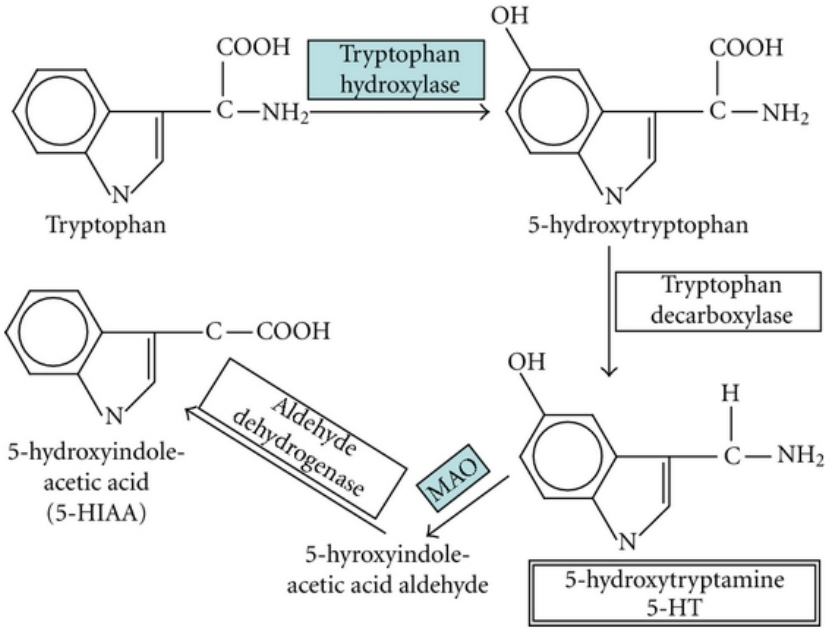



The percentage of individuals meeting diagnostic criteria for depression at age 26, as a function of 5-HTT genotype and the number of stressful events between ages 21-26

Among cohort members suffering 4 or more stressful life events, 33% of individuals with an s allele became depressed, whereas only 17% of l/l homozygotes developed depression

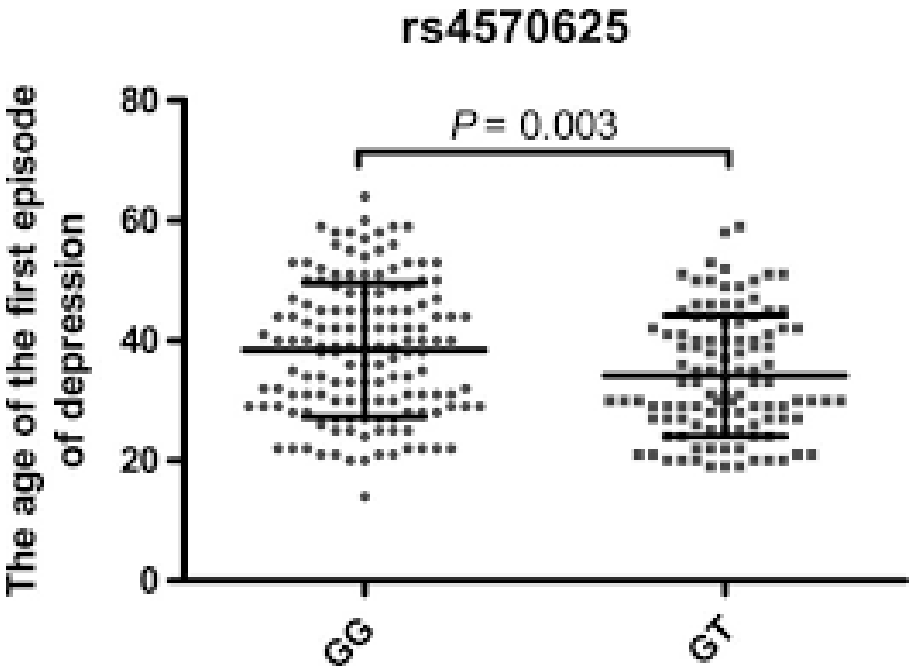
# Tryptophan hydroxylase and depression

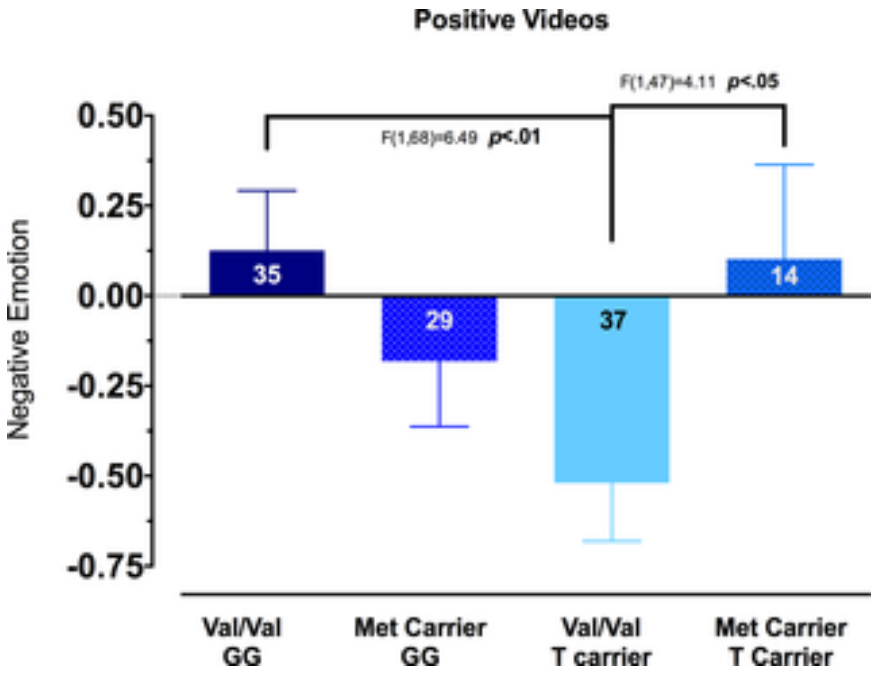
- Tryptophan hydroxylase is an enzyme that catalyzes the reaction that produces serotonin from the amino acid tryptophan. Iron is a co-factor, and BH4 (Tetrahydrobiopterin) is also used in the reaction.
- There are two genes that code for tryptophan hydroxylase:
- TPH1 is found mainly in the gut, skin, and pineal gland
- TPH2 works in the central nervous system.



# TPH and Depression

Variation in the tryptophan hydroxylase-2 gene (TPH2) coding for the rate-limiting enzyme of serotonin (5-HT) synthesis in the brain modulates responses of limbic circuits to emotional stimuli and has been linked to a spectrum of clinical populations characterized by emotional dysregulation.



# TPH and OCD

**Obsessive–compulsive disorder** (**OCD**) is a mental disorder in which a person feels the need to perform certain routines repeatedly (called "compulsions"), or has certain thoughts repeatedly (called "obsessions").

| | Genotypes | | | Alleles | |
|---|---|---|---|---|---|
| Marker | G/G | G/T | T/T | G | T |
| rs4570625 | 53.5% | 42.3% | 4.2% | 74.6% | 25.4% |
| | C/C | C/T | T/T | C | T |
| rs4565946 | 33.8% | 50.7% | 15.5% | 59.2% | 40.8% |

# SEROTONIN RECEPTORS

| Receptor Subtypes | Signaling Mechanism | Distribution | Effects |
|---|---|---|---|
| 5-$HT_{1A}$ | Gi, ↓ cAMP | Raphe nuclei, hippocampus | Regulates sleep, feeding and anxiety |
| 5-$HT_{1B}$ | Gi, ↓ cAMP | Substantia nigra, globus pallidus, basal ganglia | Neuronal inhibition, behavioral changes |
| 5-$HT_{1D}$ | Gi, ↓ cAMP | Brain | Vasoconstriction |
| 5-$HT_{1E}$ | Gi, ↓ cAMP | Cortex, hippocampus | Memory |
| 5-$HT_{1F}$ | Gi, ↓ cAMP | Globus pallidus, putamen | Anxiety, vasoconstriction |
| 5-$HT_{2A}$ | Gq, ↑ $IP_3$ | Platelets, cerebral cortex | Cellular excitaton, muscle contraction |
| 5-$HT_{2B}$ | Gq, ↑ $IP_3$ | Stomach | Appetite |
| 5-$HT_{2C}$ | Gq, ↑ $IP_3$ | Hippocampus, substantia nigra | Anxiety |
| 5-$HT_3$ | $Na^+$-$K^+$ ion channel | Area postrema, enteric nerves | Vomiting |
| 5-$HT_4$ | Gs, ↑ cAMP | Cortex, smooth muscle | Gut motility |
| 5-$HT_{5A,B}$ | Gi, ↓ cAMP | Brain | Locomotion, sleep |
| 5-$HT_6$ | Gs, ↑ cAMP | Brain | Cognition, learning |

- Mutations in HTR1A, HTR1B and HTR2A associated with behavioral changes

# HTR1A GENE AND IMPULSIVENESS

- Serotonin-1A (5-HT(1A)) receptors are known to play a role in impulsivity-related behavior.
- The C(-1019)G functional polymorphism (rs6295) has been suggested to regulate the 5-HT(1A) receptor gene (HTR(1A))
- Impacts expression in presynaptic neurons
- Increased receptor concentration and reduced neuronal firing could be associated with the G allele.
- This polymorphism is also associated with aggression, suicide, and several psychiatric disorders
- RS6295 - Subjects carrying GG genotype showed significantly higher impulsiveness scores

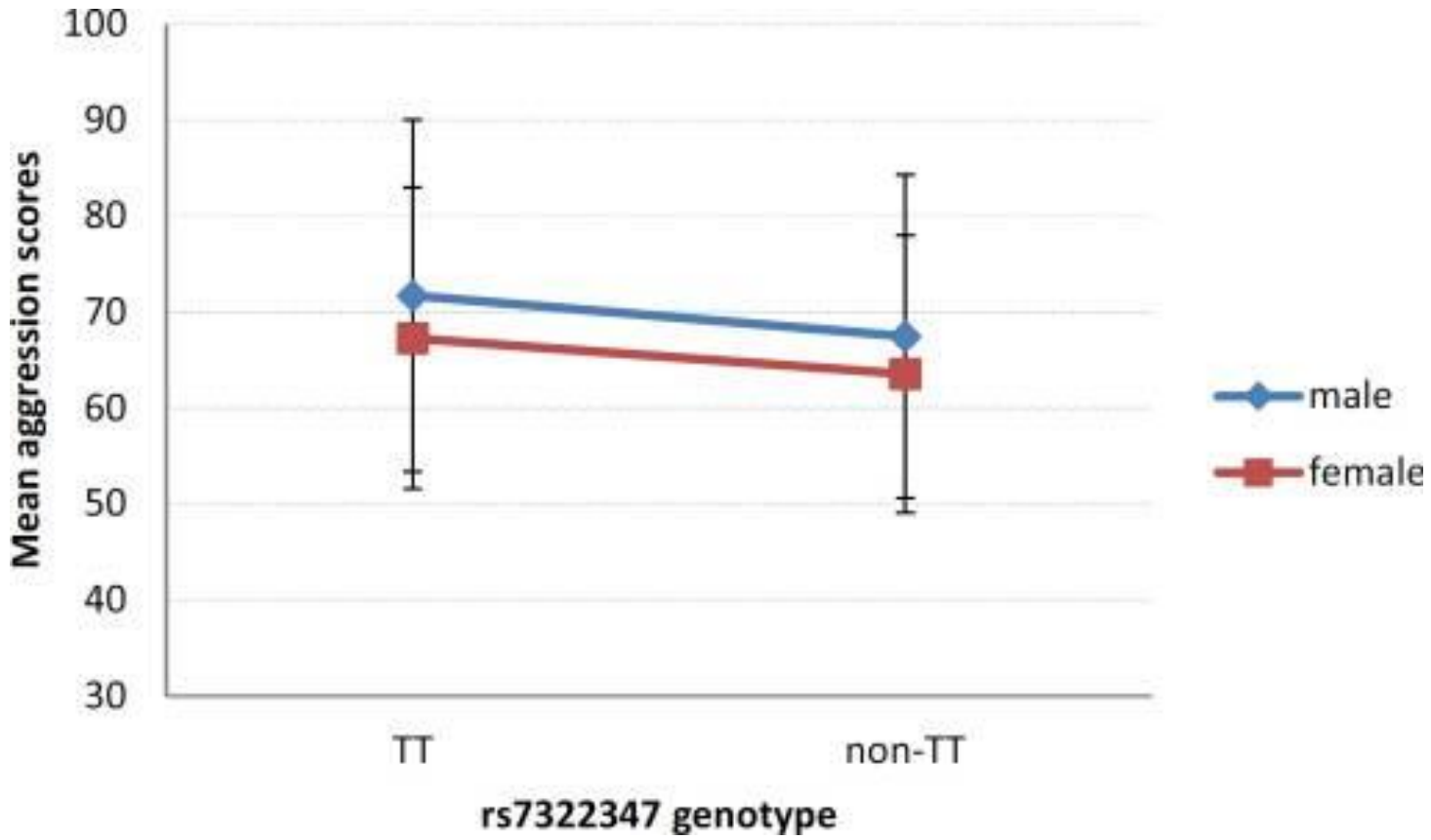
# HTR1B GENE AND DEPRESSION

- The protein encoded by this intronless gene is a G-protein coupled receptor for serotonin (5-hydroxytryptamine).
- Ligand binding activates second messengers that inhibit the activity of adenylate cyclase and manage the release of serotonin, dopamine, and acetylcholine in the brain.
- The encoded protein may be involved in several neuropsychiatric disorders and therefore is often a target of antidepressant and other psychotherapeutic drugs.
- The rs6296-C allele lowered the level of HTR1B mRNA, causing individuals with depression to display more hostility and aggressive behavior, which may lead to suicidal ideation.

# HTR2A GENE AND DEPRESSION

- Predominantly excitatory receptor for serotonin
- Rs6314 – A allele – reduced receptor levels
- Reduced ability to activate receptors or cause downstream signals. This means it causes a blunted signal after activation
- Also associated with Post Traumatic Stress Disorder
- Better response to antidepressants/SSRIs (paxil)

# HTR2A AND AGGRESSION



# Serotonin and Depression

- Genotype with environmental interaction extends to the natural development of depression in a representative sample of humans

- Individuals with 1 or 2 copies of an short allele polymorphism in the promoter region of 5-HTT exhibited more depressive symptoms, diagnosable depression, and suicidality in relation to stressful life events than individuals homozygous for the long allele
- s allele carriers have reduced amygdala volume and exaggerated amygdala excitation during perceptual processing of fearful stimuli, a likely mechanism of their relatively excessive fearfulness (and anxiety and neuroticism)  and susceptibility for depression

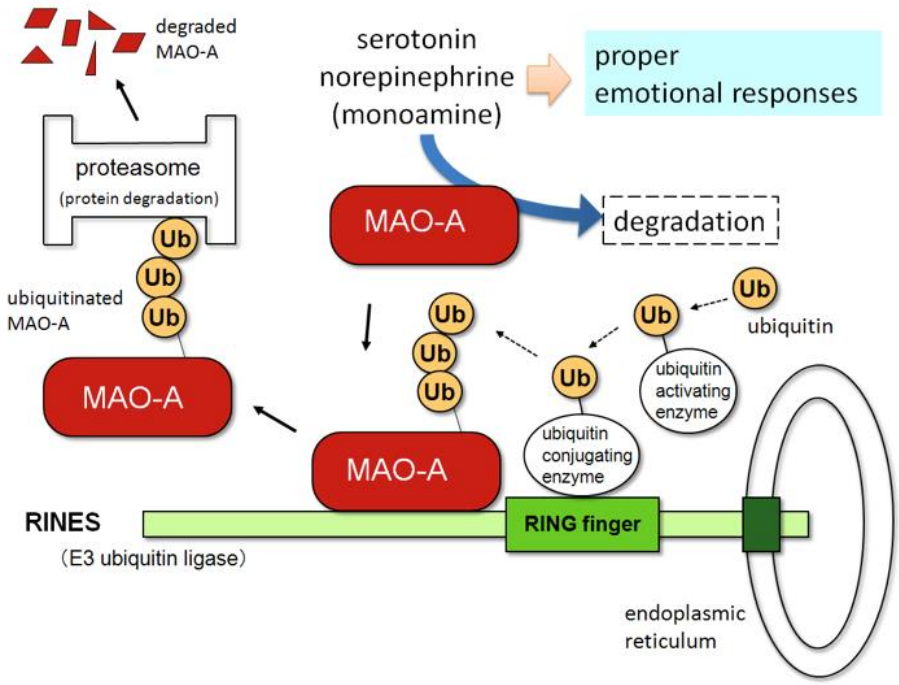
- Mutations in TPH and serotonin receptors further impact predisposition to depression and aggression.

# What to do if someone is at an increased risk?

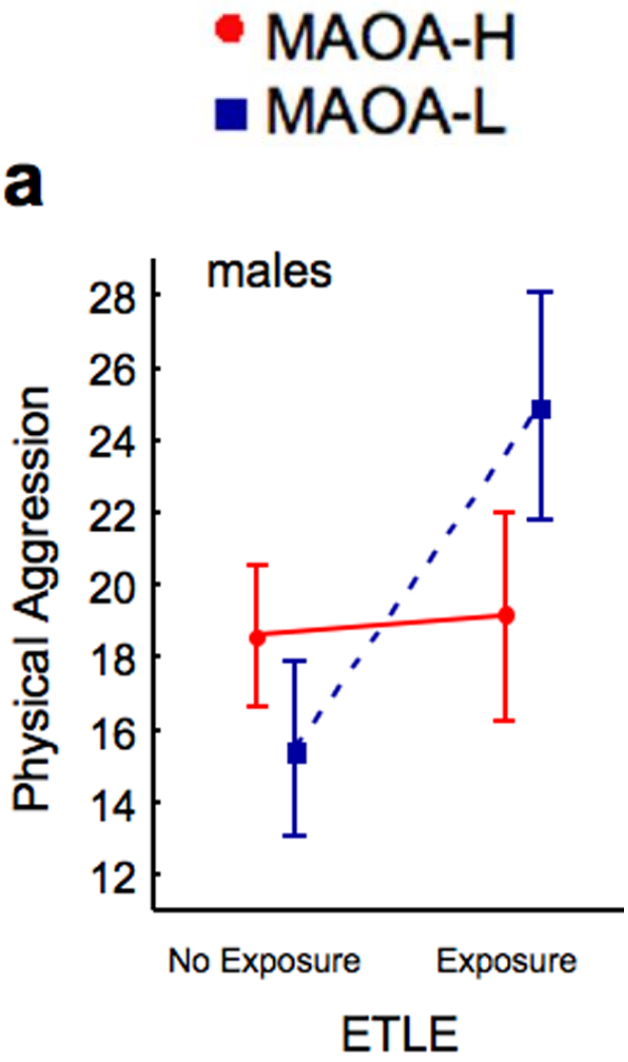
- Genetic predisposition to depression associates with reduced serotonin – impact on genes which are involved in serotonin production or serotonin receptors
- Increasing intake of foods rich in tryptophan (precursor to serotonin)
- Tryptophan is not made in our body – only dietary sources
- Majority serotonin made in gut
- Tryptophan intake with carbohydrates in necessary to help increase serotonin levels

# MAOA

- MAOA encodes for Monoamine Oxidase
- Associated with aggressiveness
- Involved in breakdown of neurotransmitters in synapses
- Genetic polymorphisms impact the levels of MAOA
- Low MAOA activity associated with aggressive behavior



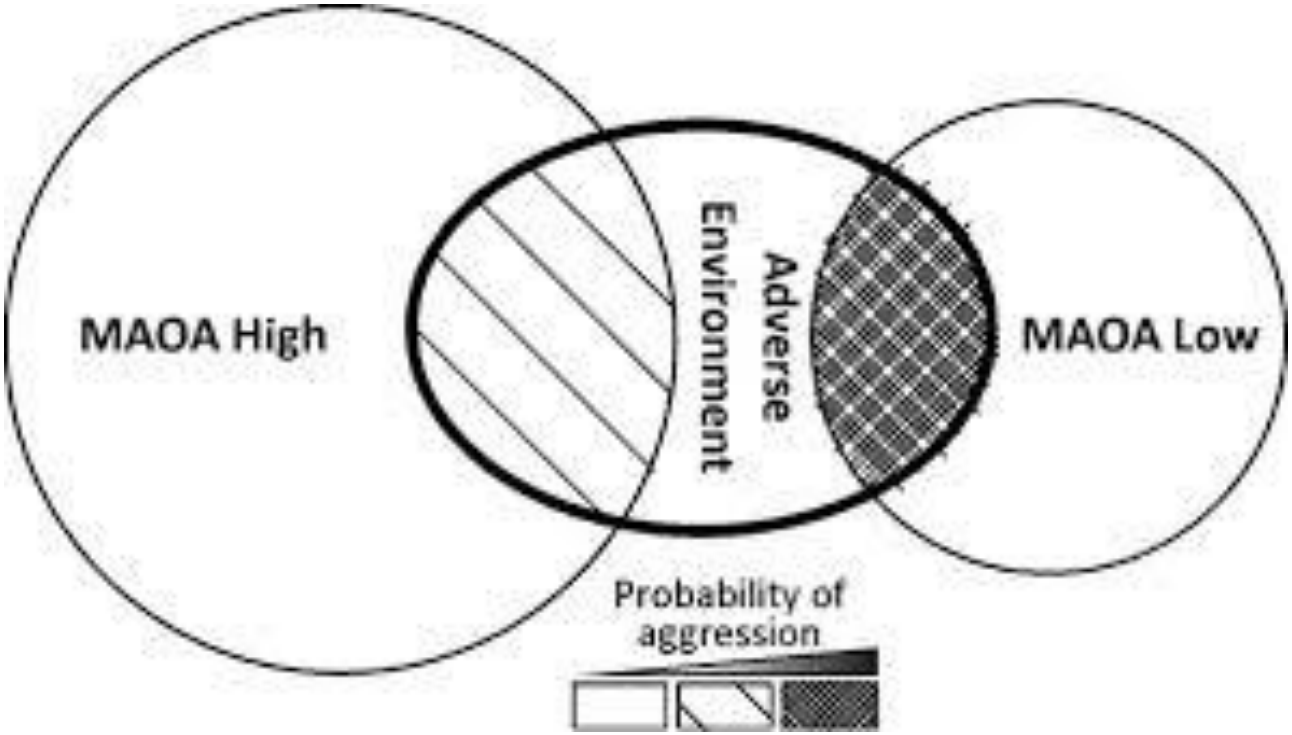
# MAOA AND AGGRESSION



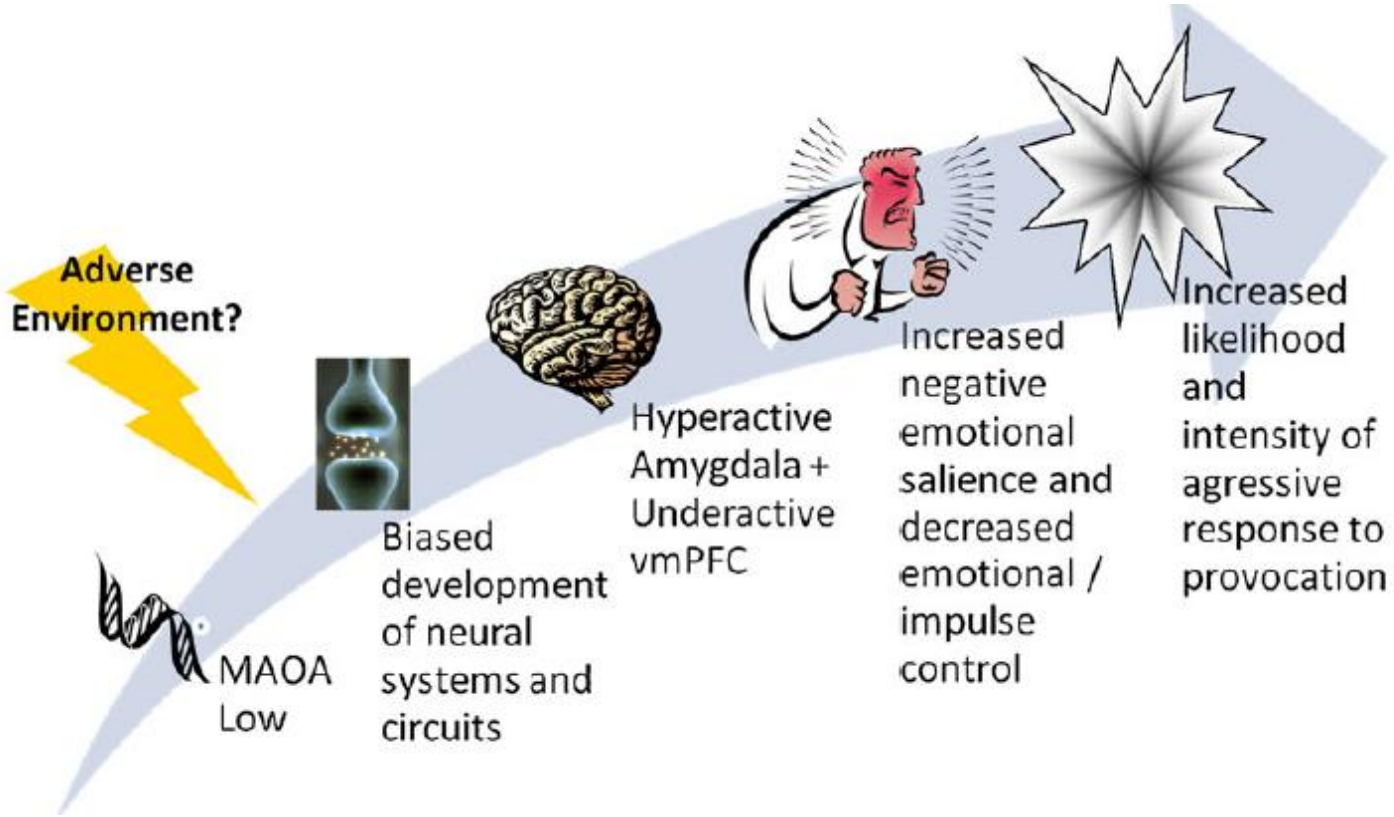

- Early traumatic life events significantly increases the risk of aggressiveness in individuals with low MAOA activity
- This gene has been referred to as the “criminal gene”
- Has also been used in court to reduce sentence

# MAOA AND AGGRESSION

- Rs6323 – G allele associated with increased activity
- Rs6323 – T allele associated with reduced activity
- At the same time someone can have multiple repeats of the gene



# MAOA AND AGRESSION



# WHAT TO DO IF SOMEONE IS AT RISK?

- Prevent adverse environment
- Low estrogen – ie boost estrogen breakdown – increase MAOA activity
- Prevent excessive exercise
- Avoid serotonin enhancing diets and drugs
- SSRIs, MAOIs (including reversible MAOIs like moclobemide), triptans for migraines, St. John's Wort, 5-HTP, the antibiotic linezolid (Zyvox) methylene blue and atypical opioids such as tramadol.

# Attention-Deficit/Hyperactivity Disorder

- ADHD is a neurodevelopmental disorder of childhood that is characterized by developmentally inappropriate levels of:
- Hyperactivity
- Impulsivity
- Inattention

# ADHD: Prevalence

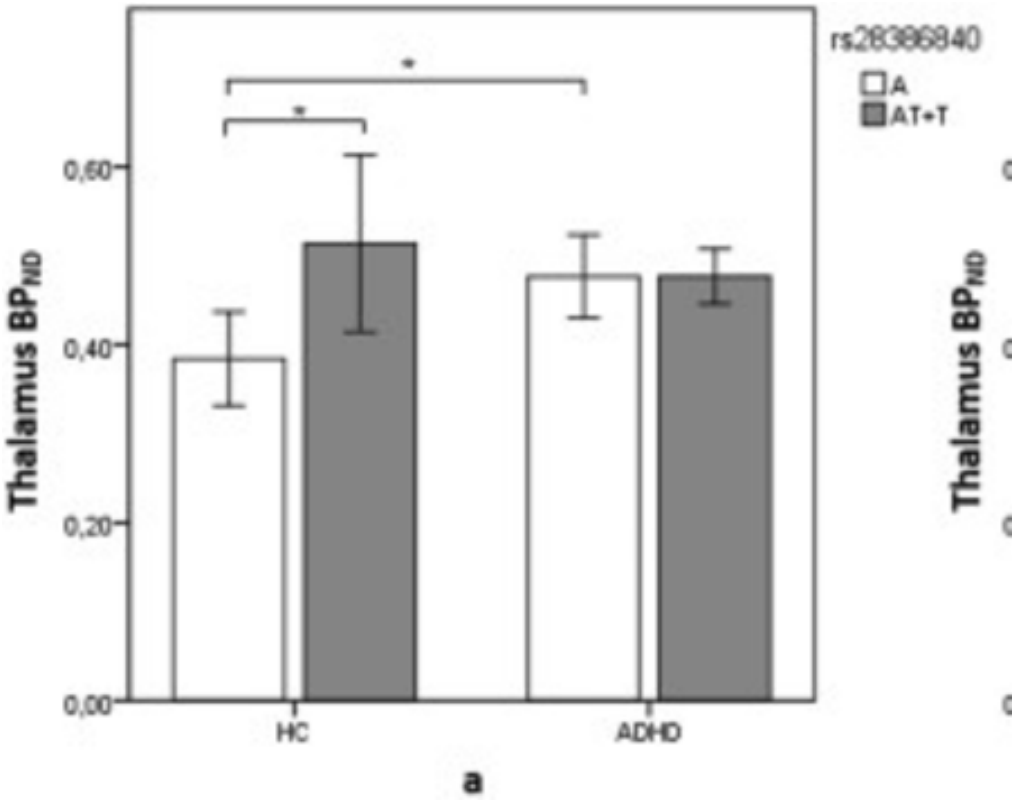
- 3-9% of the elementary school population
- more often in males than females, with the sex ratio being about 3:1 to 9:1
- most common disorders of childhood accounting for a large number of referrals to pediatricians, family physicians and child mental health professionals

# Norepinephrine and ADHD

- Norepinephrine (NE) (noradrenaline) is a key neurotransmitter in both the central and peripheral nervous systems and regulates many essential functions, including attention, memory, emotion, and autonomic function.
- The NE transporter (NET) is primarily responsible for reuptake of NE into presynaptic nerve terminals and is a regulator of NE homeostasis
- SLC6A2 - This gene encodes a member of the sodium:neurotransmitter symporter family. This member is a multi-pass membrane protein, which is responsible for reuptake of norepinephrine into presynaptic nerve terminals and is a regulator of norepinephrine homeostasis

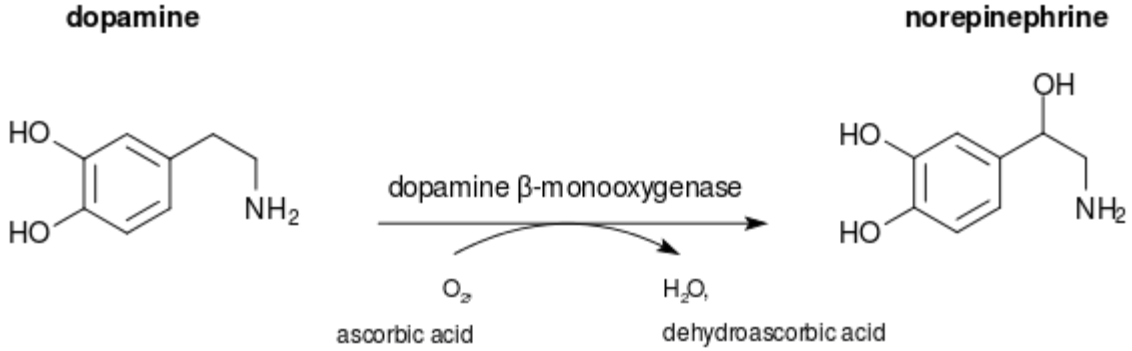
# Norepinephrine and ADHD

- -3081 A to T single nucleotide polymorphism (rs28386840) in the promoter region of the SLC6A2 and its association with ADHD



# Norepinephrine and ADHD

- DBH – Dopamine beta hydroxylase
- Synthesis of norepinephrine
- Reduced DBH levels associated with risk of ADHD



# DBH and ADHD

- Rs1611115
- Decreased levels of Dopamine-beta-Hydroxylase (T).
- Normal levels of Dopamine-beta-Hydroxylase (C).
- The Minor "T" allele is associated with:

- Higher dopamine and lower norepinephrine because of lower DBH (Dopamine-beta-hydroxylase), which converts dopamine to norepinephrine (T)
- More neuroticism (TT)
- More novelty seeking (TT)
- More impulsivity (TT)
- More aggression (TT)
- Increased risk of adult ADHD (TT) ]

# DRD4 AND ADHD

- The dopamine receptor D4 is a dopamine D2-like G protein-coupled receptor encoded by the DRD4 gene on chromosome 11 at 11p15.5
- activated receptor inhibits the enzyme adenylate cyclase, thereby reducing the intracellular concentration of the second messenger cyclic AMP
- The 7-repeat allele has been reported to encode a receptor with lower affinity for dopamine. In vitro studies indicate that the sensitivity of the 7R allele is half that of the 2R and 4R variants (SHORT variants)
- The 7R allele is associated with various psychiatric disorders including ADHD, dependences, pathological gambling, alcoholism, drug dependence and bulimia nervosa

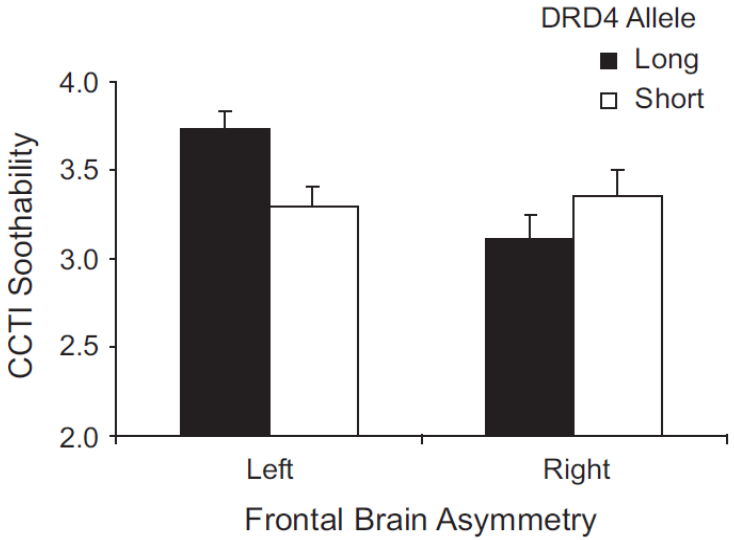
# DRD4 - ADHD

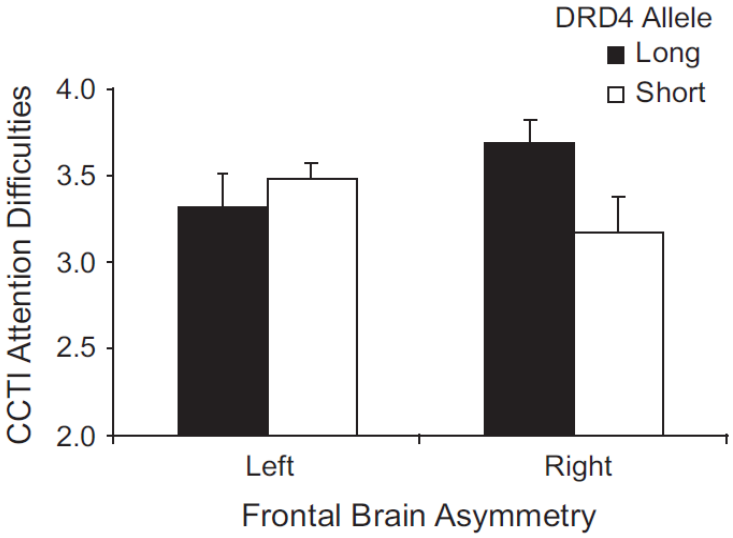
- DRD4 - Long Allele
  - Novelty/Sensation Seeking
  - Attention Problems/Aggression
  - Susceptibility to Parenting
- EEG Asymmetry
  - According to this model, an increase of the left prefrontal activity, either as a trait or as a state, is associated to approach-related emotions (e.g., positive), whereas an increase of the right prefrontal activity is associated to withdrawal-related emotions (e.g., negative).

"Long" versions of polymorphisms are the alleles with 6 to 10 repeats. 7R appears to react less strongly to dopamine molecules.

# DRD4 by Asymmetry

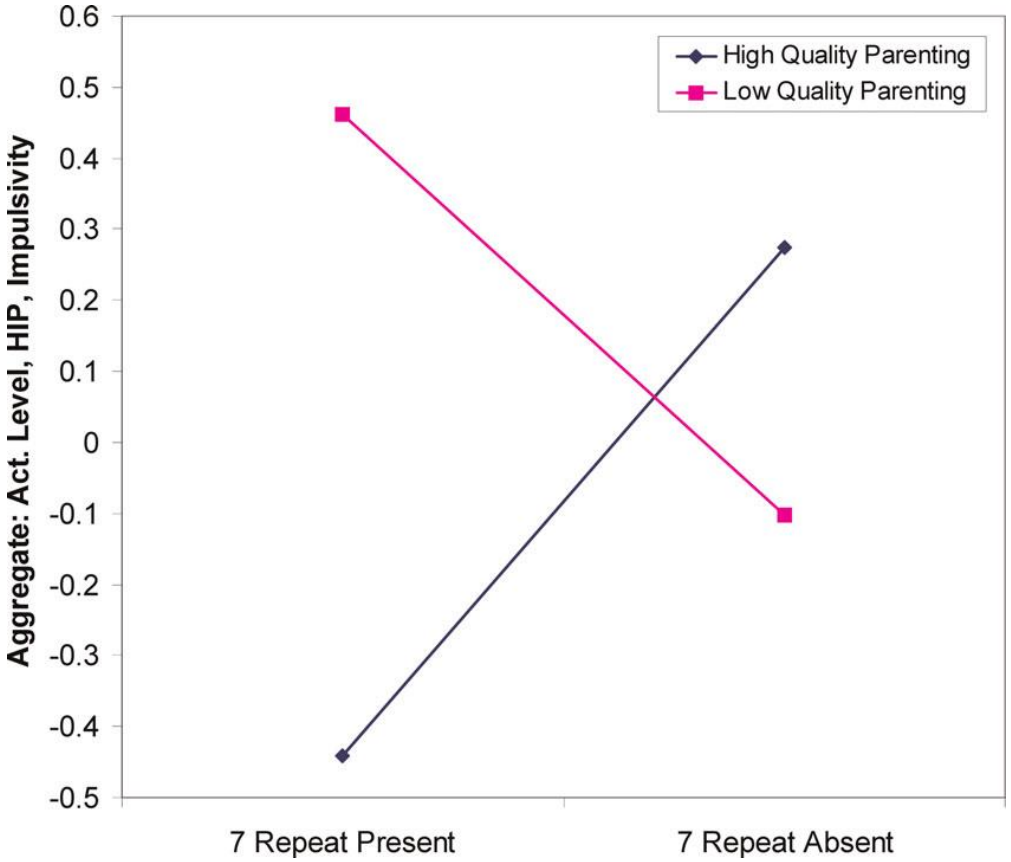
- Susceptibility to asymmetry in
  - Soothability
  - Attention Difficulties




Schmidt, Fox, Perez-Edgar & Hamer (2009)

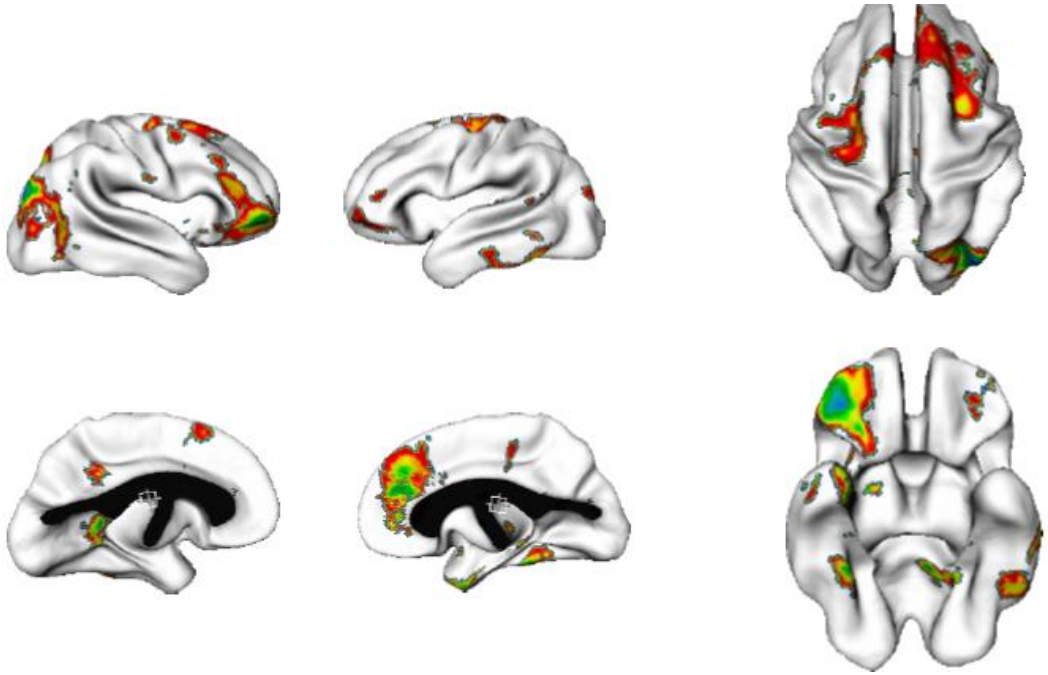
# Genes influence relation between parenting and temperament

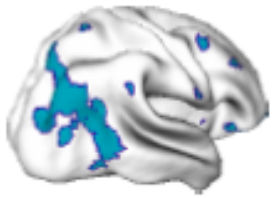


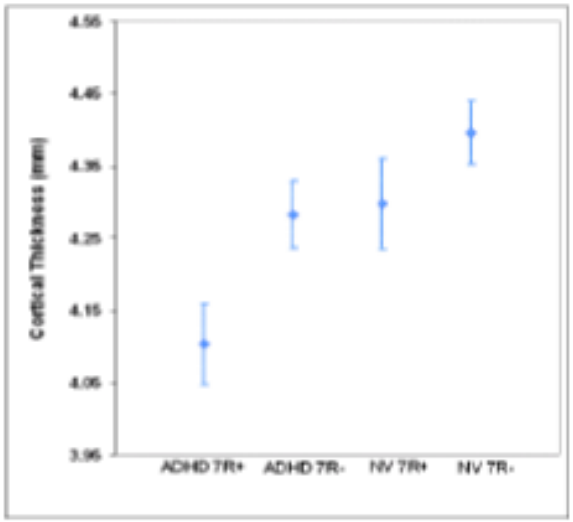

- 18-21 month olds
- **DRD4 48 (7-repeat allele) "long" allele** increased sensitivity to environmental factors such as parenting.
  - Lower quality parenting→ higher sensation seeking.
  - Higher quality parenting→ lower sensation seeking

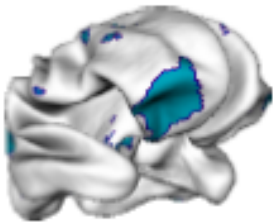
# Cortical Thickness & DRD4

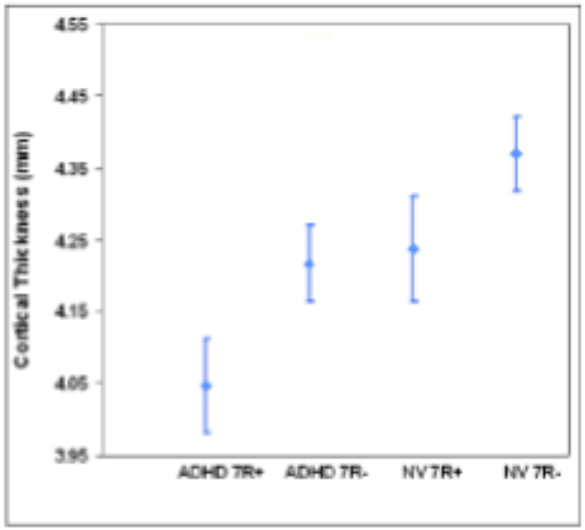
- 7R associated with reduced corticol thickness



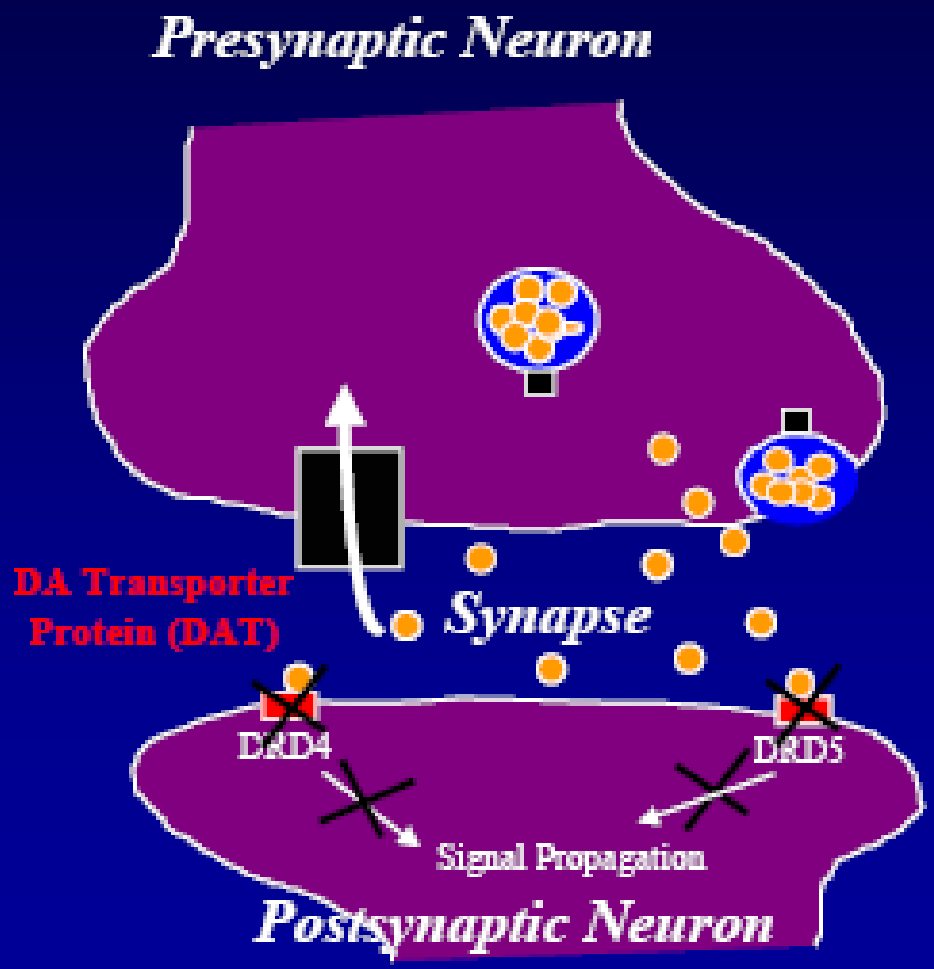






# Significance of DRD4 or DRD5 Dysfunction



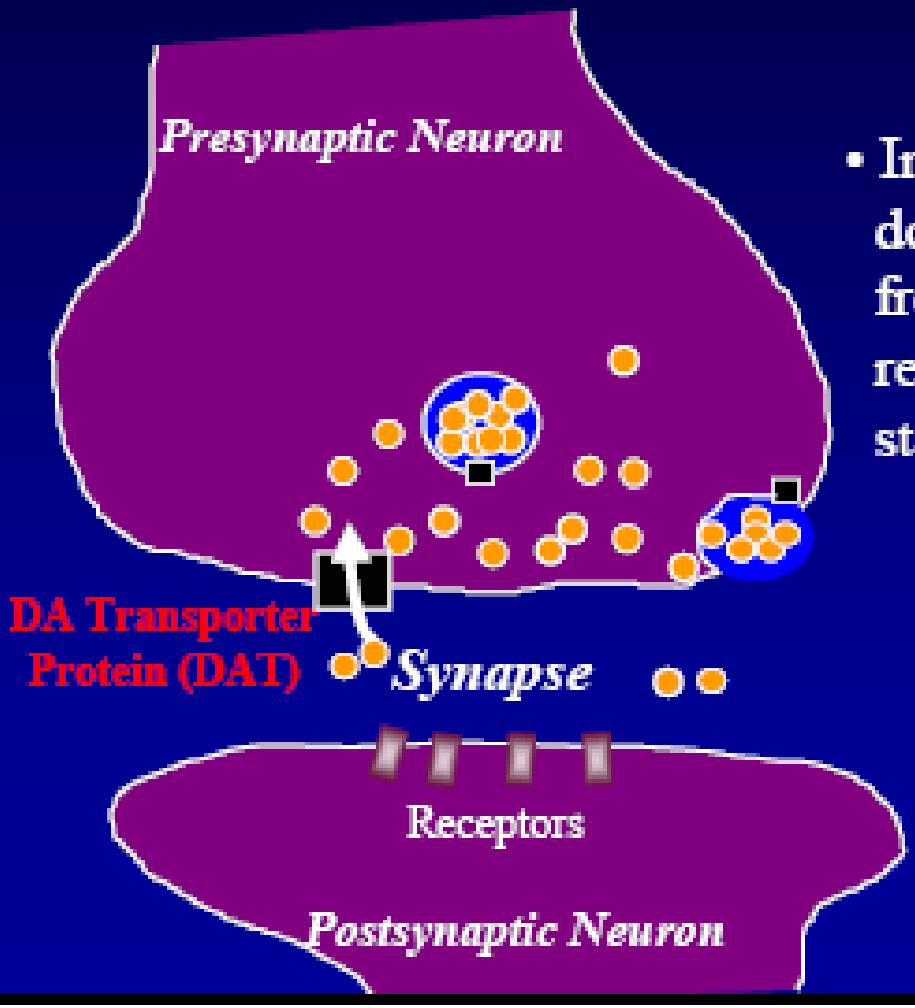

- Malfunction in DRD4 or DRD5 can mimic hypodopaminergic state (dopamine signals cannot effectively be transmitted to postsynaptic neuron)

Courtesy of S. Fanous, PhD.

# DOPAMINE TRANSPORTERS AND ADHD

- Dopamine transporter (DAT) is a principal target of the most widely used antihyperactivity medications (amphetamine and methylphenidate)
- DAT is a membrane-spanning protein that pumps the neurotransmitter dopamine out of the synaptic cleft back into cytosol.
- Increased DAT activity associated with ADHD as it leads to hypodopaminergic state

# Significance of DAT Dysfunction




- Increased reuptake of dopamine via DAT from the synapse can result in a hypodopaminergic state

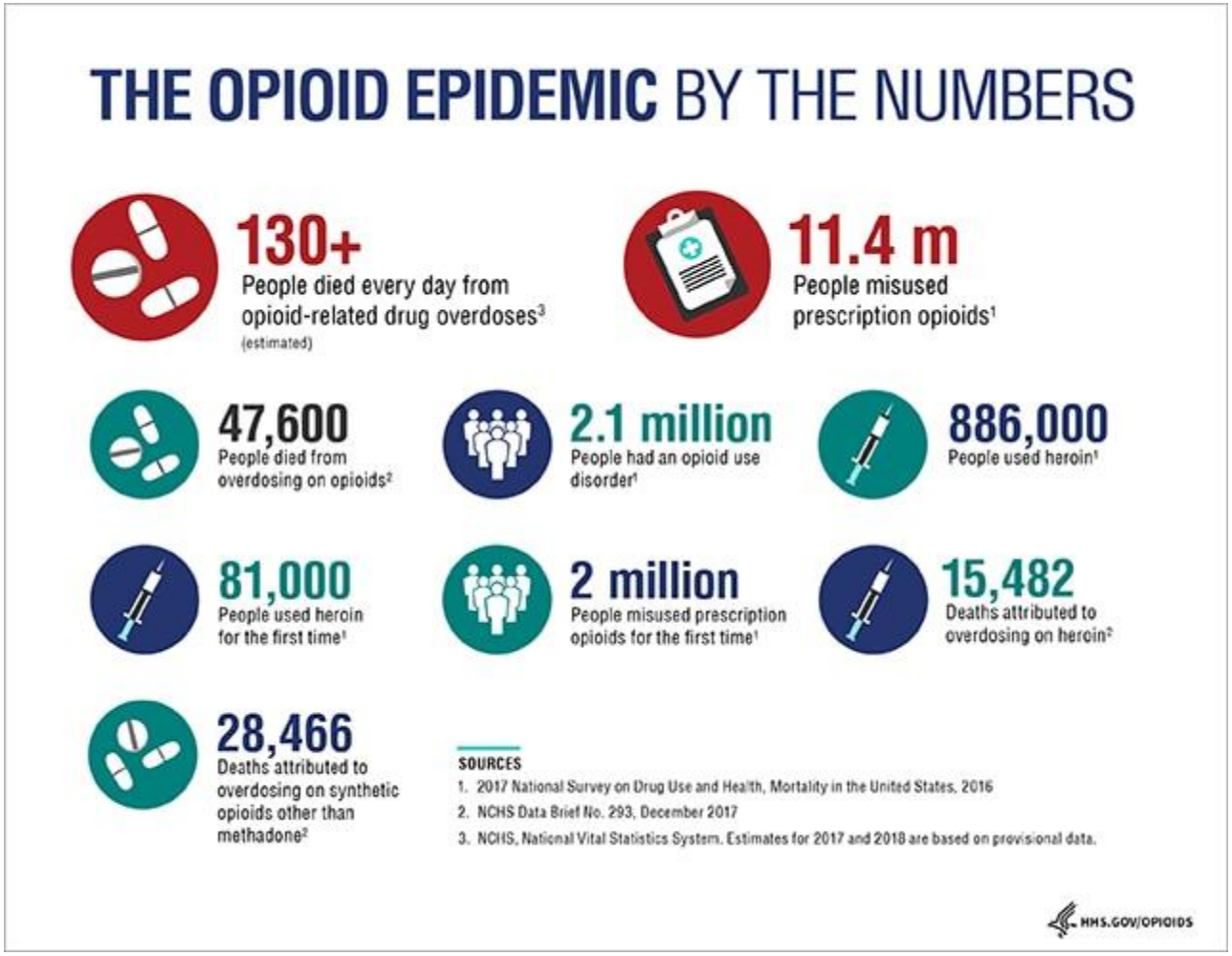
Courtesy of S. Faraone, PhD.

# DAT POLYMORPHISMS AND ADHD

- Rs27072, rs27048 – C allele associated with ADHD
- 3.5 x higher risk reported in carriers of the C allele
- Increased DAT activity
- Hypodopaminergic state

# WHAT TO DO IF SOMEONE IS AT HIGH RISK ?

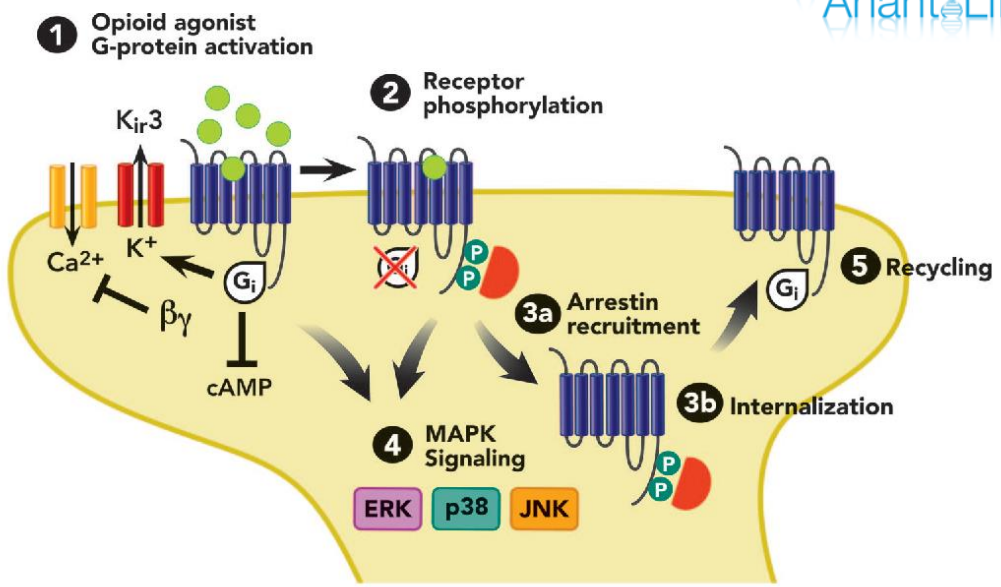
- Hypodopaminergic state
- Dysregulation of dopamine to norepinephrine
- Reduced norepinephrine
- Increase consumption of foods rich in tyrosine
- High protein foods
- Our body can produce tyrosine from phenylalanine


THE OPIOID EPIDEMIC BY THE NUMBERS
130+
People died every day from opioid-related drug overdoses[3]
(estimated)
11.4 m
People misused prescription opioids[1]
47,600
People died from overdosing on opioids[2]
2.1 million
People had an opioid use disorder[1]
886,000
People used heroin[1]
81,000
People used heroin for the first time[1]
2 million
People misused prescription opioids for the first time[1]
15,482
Deaths attributed to overdosing on heroin[2]
28,466
Deaths attributed to overdosing on synthetic opioids other than methadone[2]
SOURCES
1. 2017 National Survey on Drug Use and Health, Mortality in the United States, 2016
2. NCHS Data Brief No. 293, December 2017
3. NCHS, National Vital Statistics System. Estimates for 2017 and 2018 are based on provisional data.
HHS.GOV/OPIOIDS

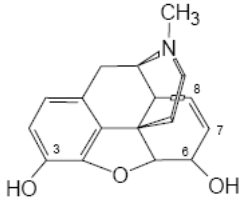
# Opioid Receptors

- Mu, Delta, Kappa
- All pure agonists act at Mu receptor
- Opioid receptors act on
  - CNS: cortex, thalamus, periaquaductal gray, spinal cord
  - Peripheral neurons
  - Inflammed tissue
  - Immune cells
  - Respiratory and GI tract

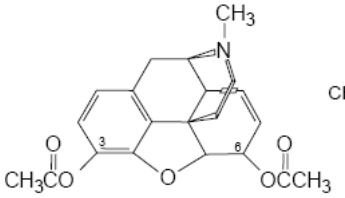
# OPIOID RECEPTORS



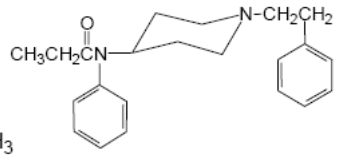

nary of opioid receptor signaling. Figure depicts opioid receptor signal transduction and trafficking. I



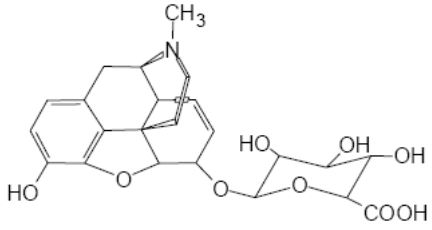

**Morphine**



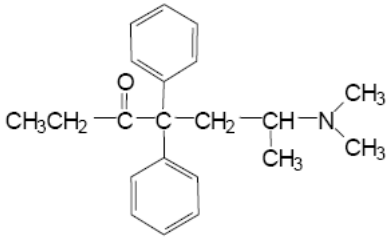

**Heroin**




**Fentanyl**




**Morphine 6β-Glucuronide**




**Methadone**

# Oxycodone

- Synthetic “cousin” to morphine
- Potency is 1.5-2X morphine
- Targets mu receptor and kappa receptors
- Not available in parenteral form in Canada

# Fentanyl

- Targets mu and delta receptors
- 80-100X potency of morphine
- Rapid onset and very short half-life – needs to be delivered as parenteral infusion or transdermal patch for constant analgesia
- No active metabolites
- Highly lipophilic – useful in renal dialysis

# MUTATIONS IN OPIOID RECEPTORS

| Gene | Receptor | Polymorphism – Your Genotype | Your Risk |
|---|---|---|---|
| OPRM1 | μ opioid receptor | Rs1799971 (GG) | At risk |
| OPRD1 | δ opioid receptor | rs2234918 (CC)<br>rs1042114 (GG) | At risk |
| OPRK1 | κ opioid receptor | rs1051660 (GG) | At risk |

- Mutations increase the threshold needed for receptor activation
- OPRM1 mutations also associated with alcohol addiction

# DOPAMINE RECEPTOR DRD2

This gene encodes the D2 subtype of the dopamine receptor. This G-protein coupled receptor inhibits adenylyl cyclase activity.

It is an inhibitory dopamine receptor which limits dopamine signaling

Mutations in DRD2 have been associated with addiction

rs1076560 , rs4648317 , rs1800497 , rs1801028

# DRD2 OPIOID ADDICTION

Table 2

Detailed results of genotypic association (2 d.f. test) of rs1076560 and opioid addiction in African Americans and European Americans.

| Population | Phenotype | CC | CA | AA | P-value |
|---|---|---|---|---|---|
| African American | Cases | 77.1% | 21.3% | 1.6% | 0.051 |
| | Controls | 82.8% | 16.7% | 0.5% | |
| European American | Cases | 68.5% | 28.7% | 2.8% | 0.046 (0.013 )* |
| | Controls | 74.3% | 23.4% | 2.3% | |

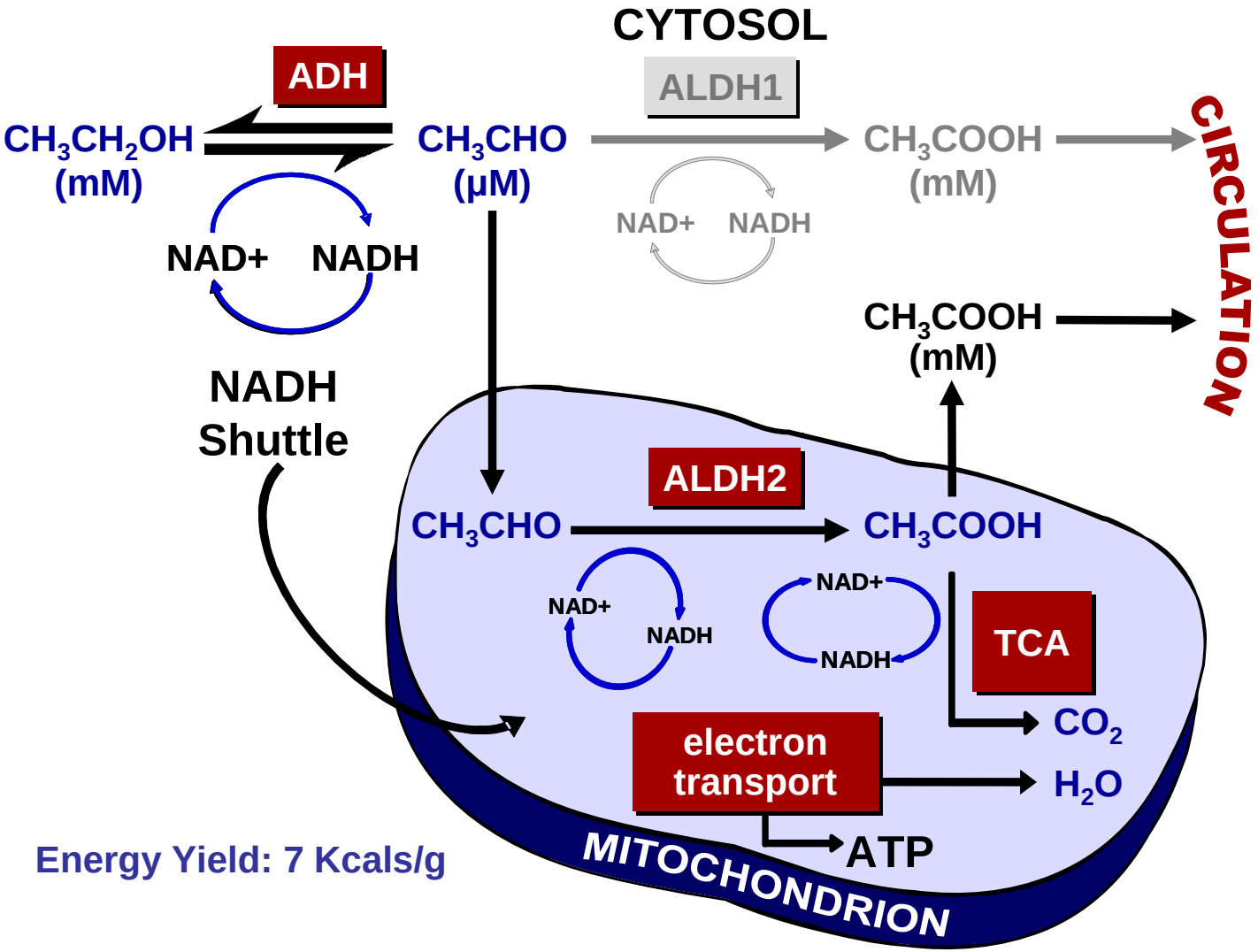
*Dominant test for association p-value. The Chi-square test was performed for European Americans and the Fisher's exact test for African Americans.

# WHAT TO DO IF AT INCREASED RISK?

- Increased opioid used increases risk of opioid addiction
- Explore alternatives for pain relief
- If opioids are required, maintain low dose and for a short duration

# Alcoholism: A Common Complex Disease

# Metabolism of Ethanol and Acetaldehyde in Hepatocyte



# ADH1B AND ALCOHOL ADDICTION

- Alcohol dehydrogenase 1B is an enzyme that in humans is encoded by the ADH1B gene.
- The encoded protein, known as ADH1B or beta-ADH, can form homodimers and heterodimers with ADH1A and ADH1C subunits, exhibits high activity for ethanol oxidation and plays a major role in ethanol catabolism (oxidizing ethanol into acetaldehyde).
- Mutations associated with reduced risk of alcohol addiction.

# ADH1B AND ALCOHOL ADDICTION

- rs1229984, that changes arginine to histidine at residue 47 of the mature protein
- G allele – reduced alcohol dependence
- A allele – increased alcohol dependence

# ALDH2 AND ALCOHOL FLUSH

- Encodes for alcohol dehydrogenase
- Aldehyde dehydrogenase is the second enzyme of the major oxidative pathway of alcohol metabolism.
- Key role in conversion of aldehyde to acetic acid
- Mitochondrial and cytosolic forms
- Mutation associated with reduced activity -> alcohol flush

# ALDH2 AND ALCOHOL FLUSH

- rs671(A) allele of the ALDH2 encodes a form of the aldehyde dehydrogenase 2 protein that is defective at metabolizing alcohol.
- This allele is known as the ALDH*2 form, and individuals possessing either one or two copies of it show alcohol-related sensitivity responses including facial flushing, and severe hangovers.
- Allele is extremely common among the Asian population

**Kreek et al. (Nature, Dec 2005) provides a reasonable list of candidate genes for substance use.**

**Table 1. Genes having one or more variants that have been reported to be associated with one or more addictions.**

| Gene | System | Protein | Chromosomal location | Drug |
|---|---|---|---|---|
| OPRM1 | Opioid | μ opioid receptor | 6q24-25 | Heroin/opiate; Alcohol |
| OPRK1 | Opioid | κ opioid receptor | 8q11.2 | Heroin/opiate |
| PDYN | Opioid | Preprodynorphin | 20pter-p12.2 | Cocaine/stimulants |
| TH | Dopaminergic | Tyrosine Hydroxylase | 11p15.5 | Alcohol |
| DRD2 | Dopaminergic | Dopamine receptor 2 | 11q23 | Alcohol |
| DRD3 | Dopaminergic | Dopamine receptor 3 | 3q13.3 | Alcohol |
| DRD4 | Dopaminergic | Dopamine receptor 4 | 11p15.5 | Heroin/opiate; Cocaine/stimulants; Alcohol |
| DBH | Dopaminergic | Dopamine β-hydroxylase | 9q34 | Cocaine/stimulants |
| DAT | Dopaminergic | Dopamine transporter | 5p15.3 | Alcohol |
| TPH1 | Serotonergic | Tryptophan hydroxylase 1 | 11p15.3-p14 | Alcohol |
| TPH2 | Serotonergic | Tryptophan hydroxylase 2 | 12q21.1 | Heroin/opiate; Alcohol |
| HTR1B | Serotonergic | Serotonin receptor 1B | 6q13 | Heroin/opiate; Alcohol |
| HTR2A | Serotonergic | Serotonin receptor 2A | 13q14-q21 | Alcohol |
| SERT | Serotonergic | Serotonin transporter | 17q11.1-q12 | Heroin/opiate; Alcohol |
| MAOA | CatecholaminergicSerotonergic | Monoamine oxidase A | Xp11.23 | Alcohol |
| COMT | Catecholaminergic | Catechol-*O*-methyl transferase | 22q11.2 | Heroin/opiate; Alcohol |
| GABRA1 | GABAergic | GABA receptor subunit α-1 | 5q34-q35 | Alcohol |
| GABRA6 | GABAergic | GABA receptor subunit α-6 | 5q31.1-q35 | Alcohol |
| GABRB1 | GABAergic | GABA receptor subunit β-1 | 4p13-p12 | Alcohol |
| CHRM2 | Cholinergic | Muscarinic acetylcholine | 7q35-q36 | Alcohol |
| CNR1 | Cannabinoid | Cannabinoid receptor 1 | 6q14-q15 | Cocaine/stimulants Alcohol |
| FAAH | Cannabinoid | Fatty acid amide hydrolase | 1p35-34 | Alcohol |
| NPY | Neuromodulatory | Neuropeptide Y | 7p15.1 | Alcohol |
| ADH1B | Ethanol Metabolism | Alcohol dehydrogenase 1B | 4q22 | Alcohol |
| ADH1C | Ethanol Metabolism | Alcohol dehydrogenase 1C | 4q22 | Alcohol |
| ALDH2 | Ethanol metabolism | Alcohol dehydrogenase 2 | 12q24.2 | Alcohol |
| CYP2D6 | Drug metabolism | Cytochrome CYP450 | 22q18.1 | Heroin/opiate |
| ANKK1 | Signal transduction | Ankyrin repeat and kinase domain-containing 1 | 11q23.2 | Alcohol |

# Brain Derived Neurotrophic Factor and neuronal plasticity/cognition

- increases cortical neuron survival
- sculpts glutamate innervation patterns
- increases synaptic efficacy of glutamate
- modulates LTP in hippocampus
- expression increased during spatial memory
- expression increased by antidepressant treatments
- genetic associations: Alzheimers Disease, Parkinson's Disease, bipolar disorder, schizophrenia, cognition

# BDNF: How do we get there from here ?

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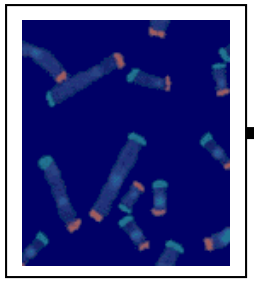
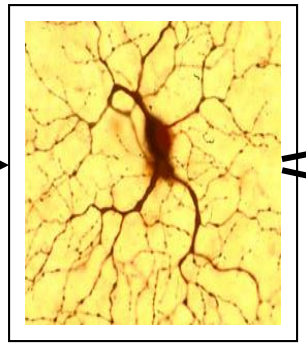
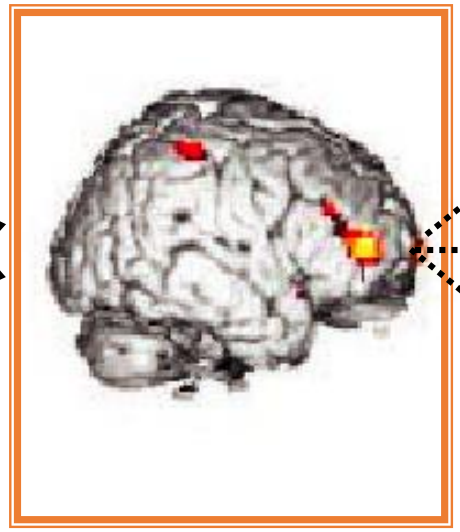


BDNF:
*val66met polymor-phism*



Cells:
*Intracellular trafficking and regulated secretion*

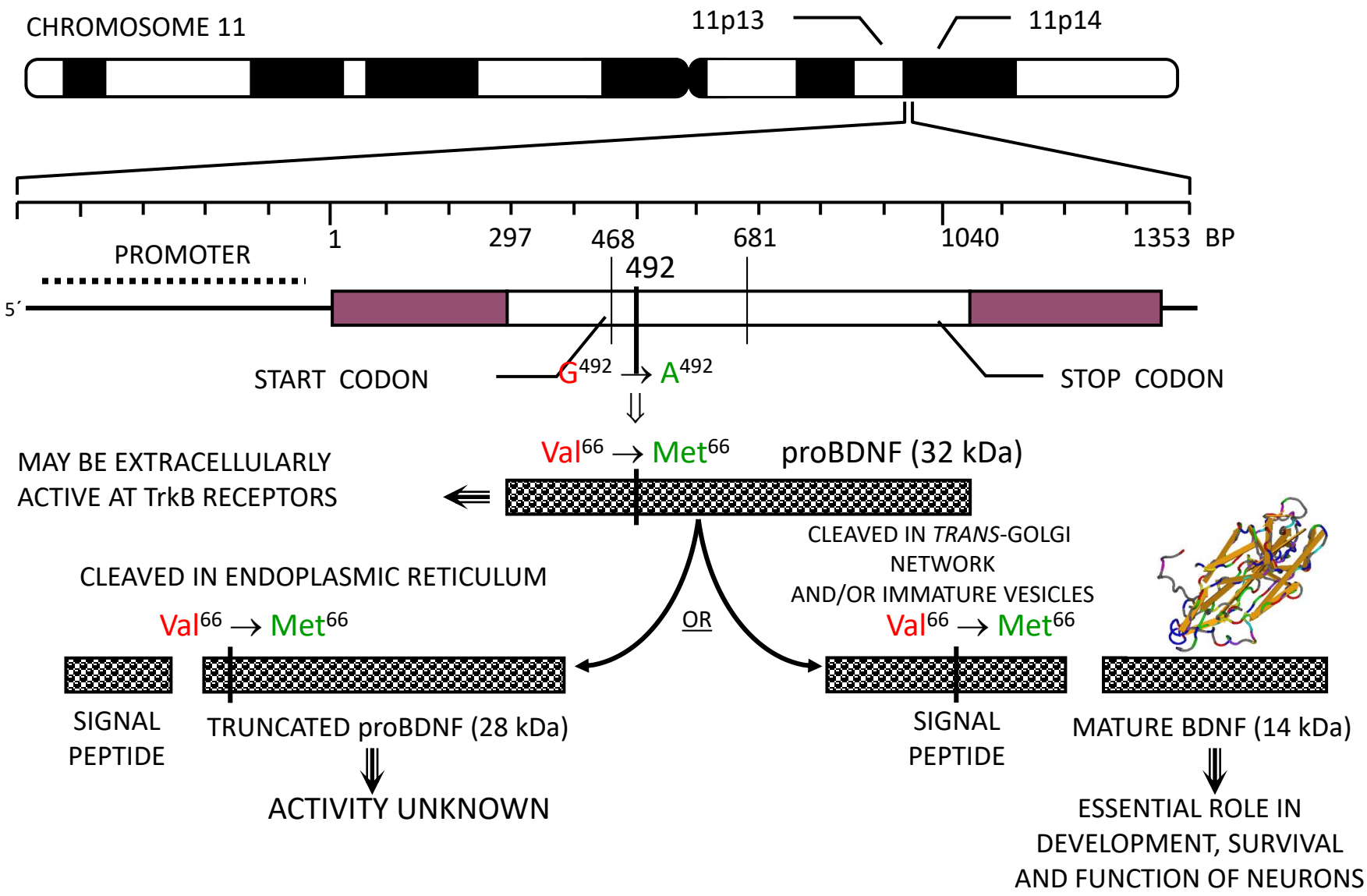


Systems
*hippocampal processing of memory*

bipolar disorder, schizophrenia Alzheimer's Disease, antidepressant effects

Behavior:
*complex functional interactions and emergent phenomena*

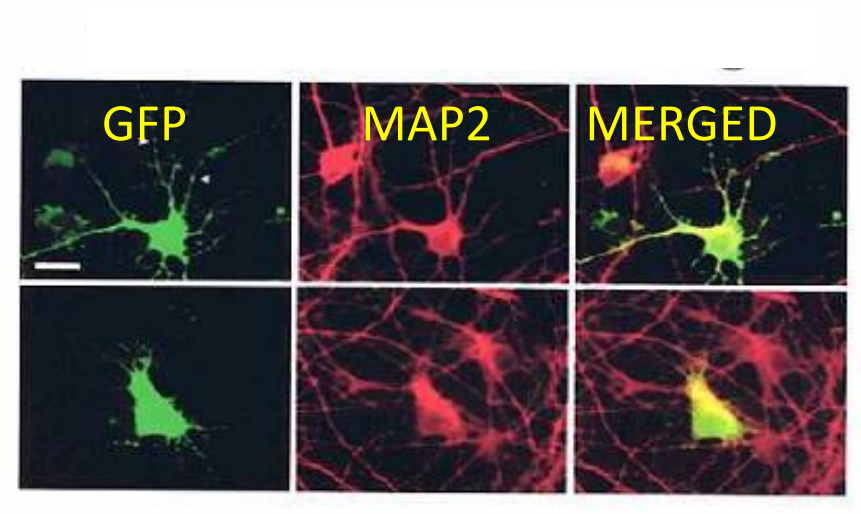
# The BDNF Gene



# Intracellular trafficking of BDNF alleles in cultured hippocampal neurons

Vector construct:

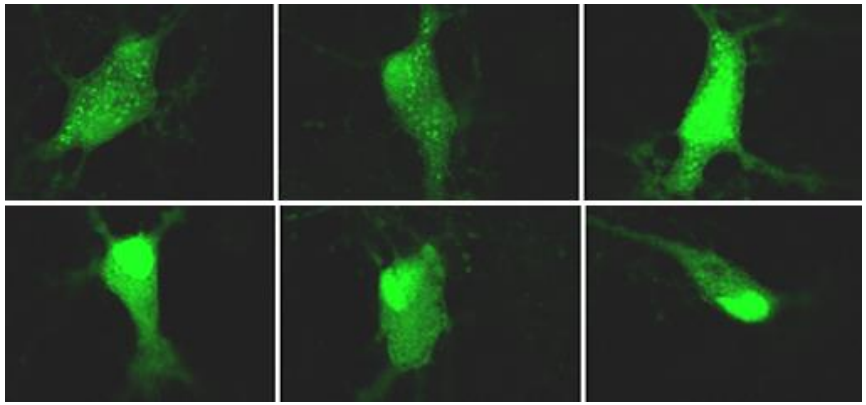

Dendritic transport:




BDNF val

BDNF met

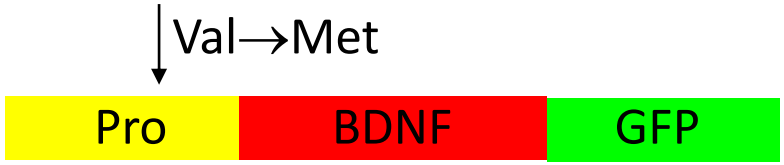
Peri-nuclear packaging:

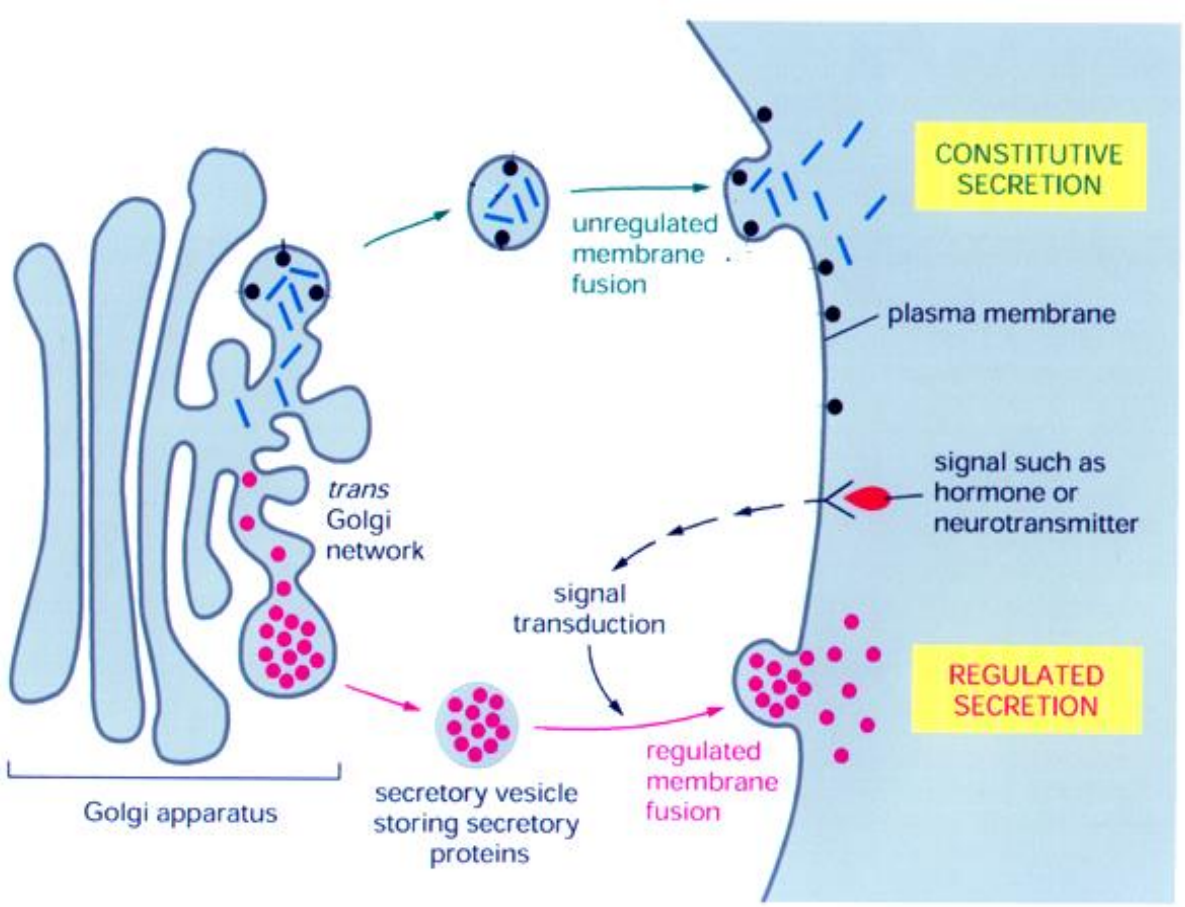


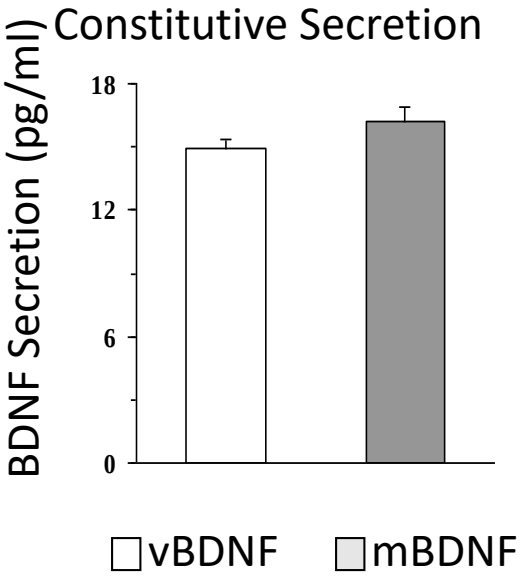
BDNF val

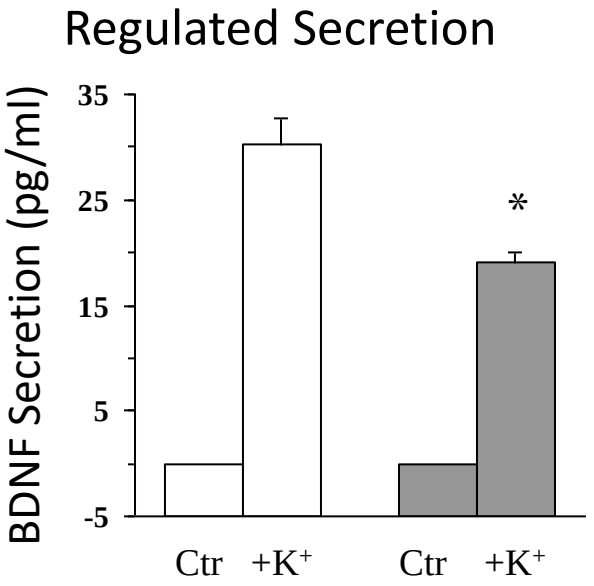
BDNF met

# Differential secretion of BDNF alleles



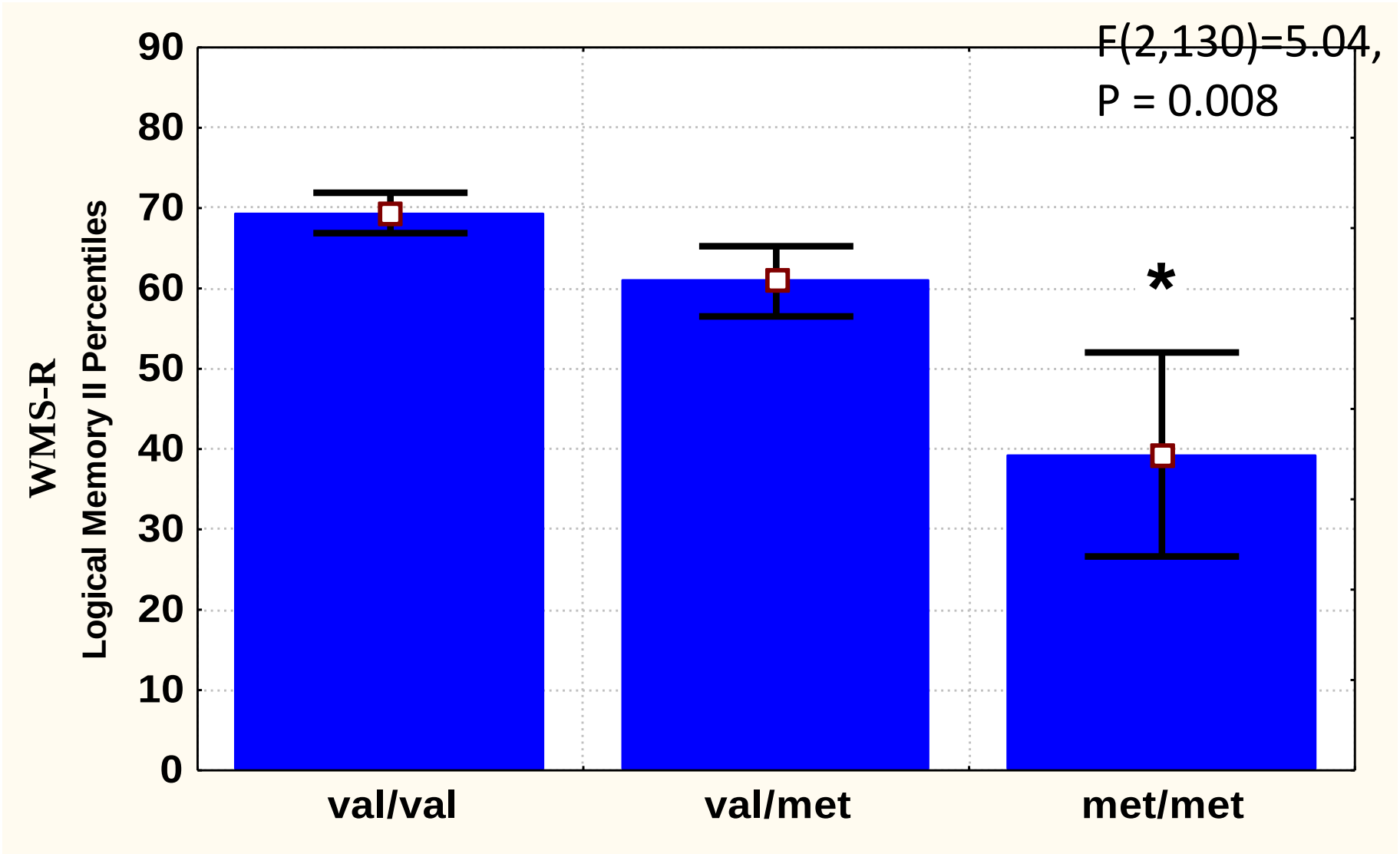




Egan et al *Cell* (2003)

# BDNF val$^{66}$met associated with reduction in episodic memory



# Incidental declarative memory engages the hippocampus

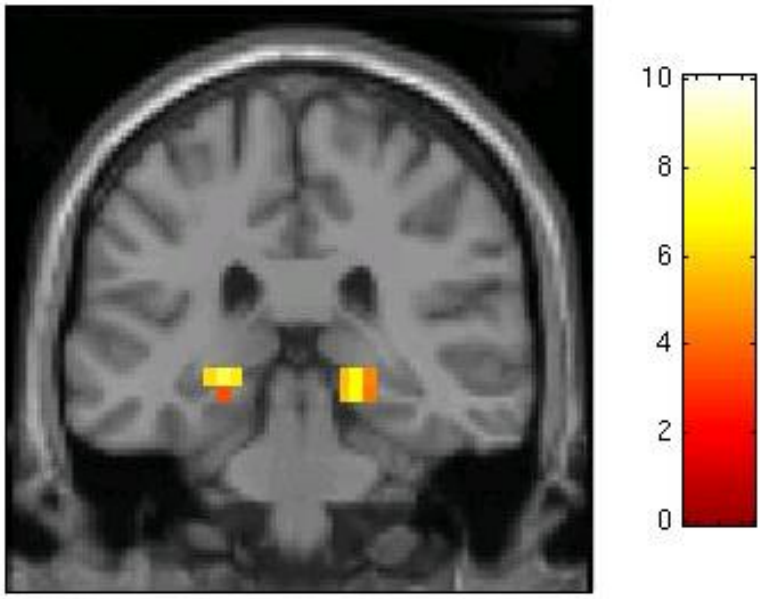
**Incidental Picture Encoding Task**

**"Indoor or Outdoor?"**

"Indoor or Outdoor?"

"New or Old?"

**Hippocampal Activation**




**y = -30**

# BDNF val$^{66}$met genotype and hippocampal function during declarative memory

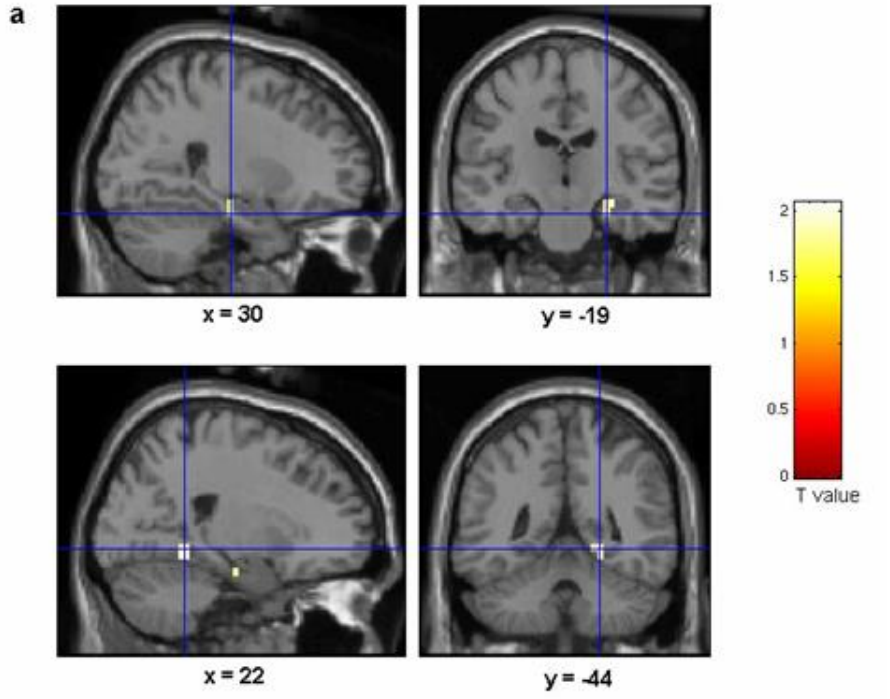
Subjects:

| 14 val/val individuals | 14 met carriers (12 val/met) |
| --- | --- |
| 6 females | 6 females |
| mean age: 30 | age: 30 |
| mean IQ: 110 ±1.5 | IQ: 100 ±2.1 |

# BDNF $^{66}$*met* is associated with reduced hippocampal engagement during memory processing

AnantLife

Encoding



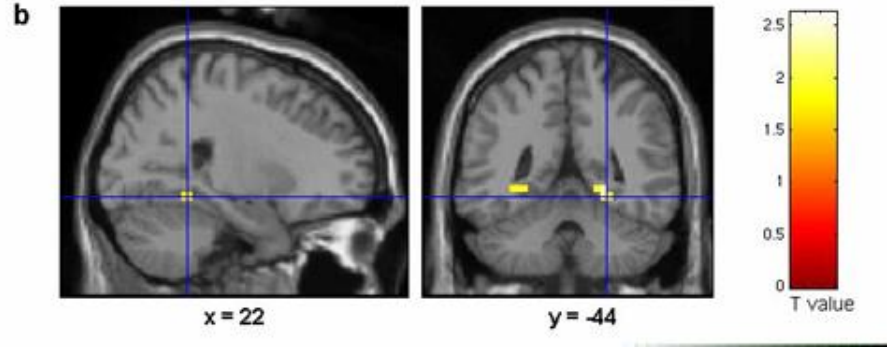

val/val (N=14)

>

val/met (N=14)

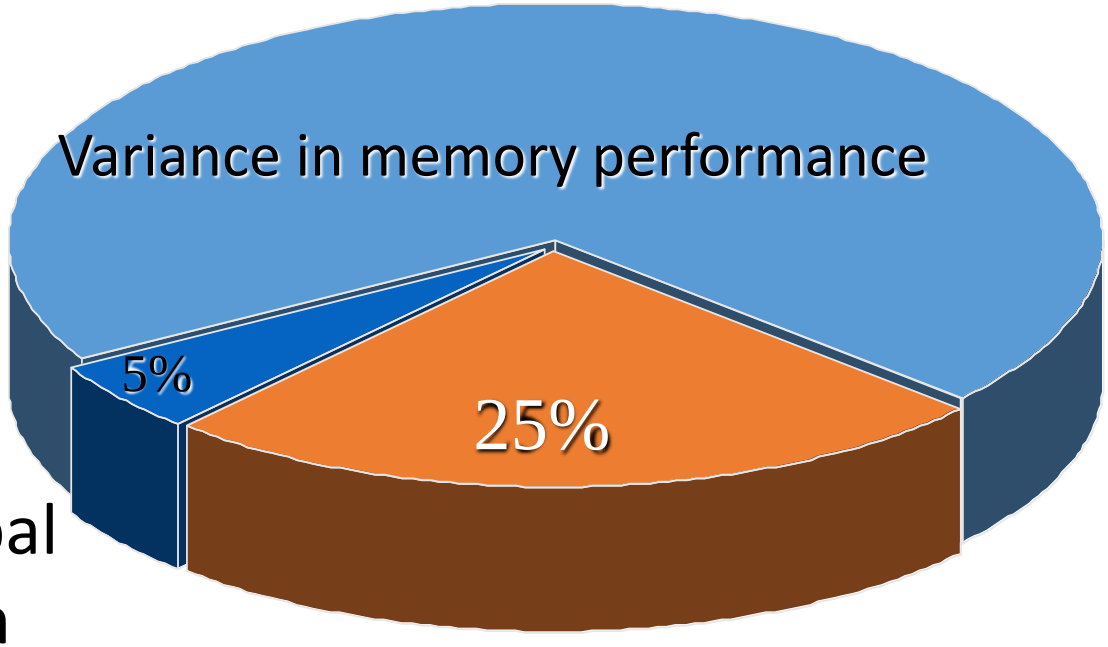
Groups matched for age,
gender, IQ, education,
apo ε4

Retrieval



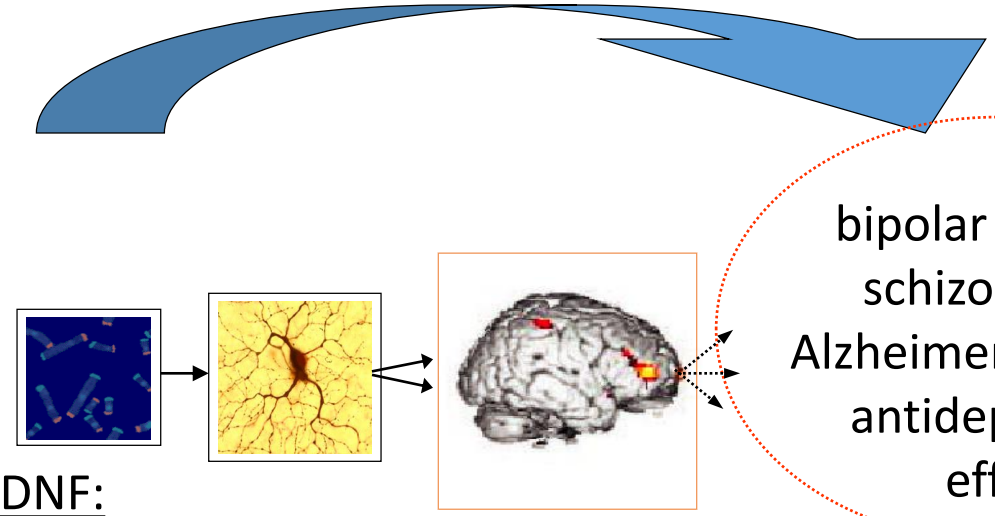

SPM 99, p<.05, corrected

# BDNF val/met genotype, hippocampal activation and prediction of recognition accuracy




hippocampal activation during retrieval

interaction of BDNF genotype and hippocampal activation during encoding




**BDNF val$^{66}$met genotype affects hippocampal neuronal function and memory processing.**