The Amazing Brain Webinar Series: Select Topics in Neuroscience and Child Development for the Clinician

Part VII – Recent Advances in the Genetics of Autism Spectrum Disorders

Abha R. Gupta, MD, PhD

Jointly sponsored by the Association of University Centers on Disabilities, the Maternal and Child Health Bureau/Health Resources and Services Administration and Yale School of Medicine, Section of Developmental-Behavioral Pediatrics

Webinar Overview

- Webinar Recording
  - Visit www.aucd.org/webinars
- Q & A
  - Please submit your questions throughout the webinar via the “question box” on your webinar dashboard. Questions will be answered following the presentation.
- Survey
  - Please complete the short survey at the end of the webinar!

Introductions

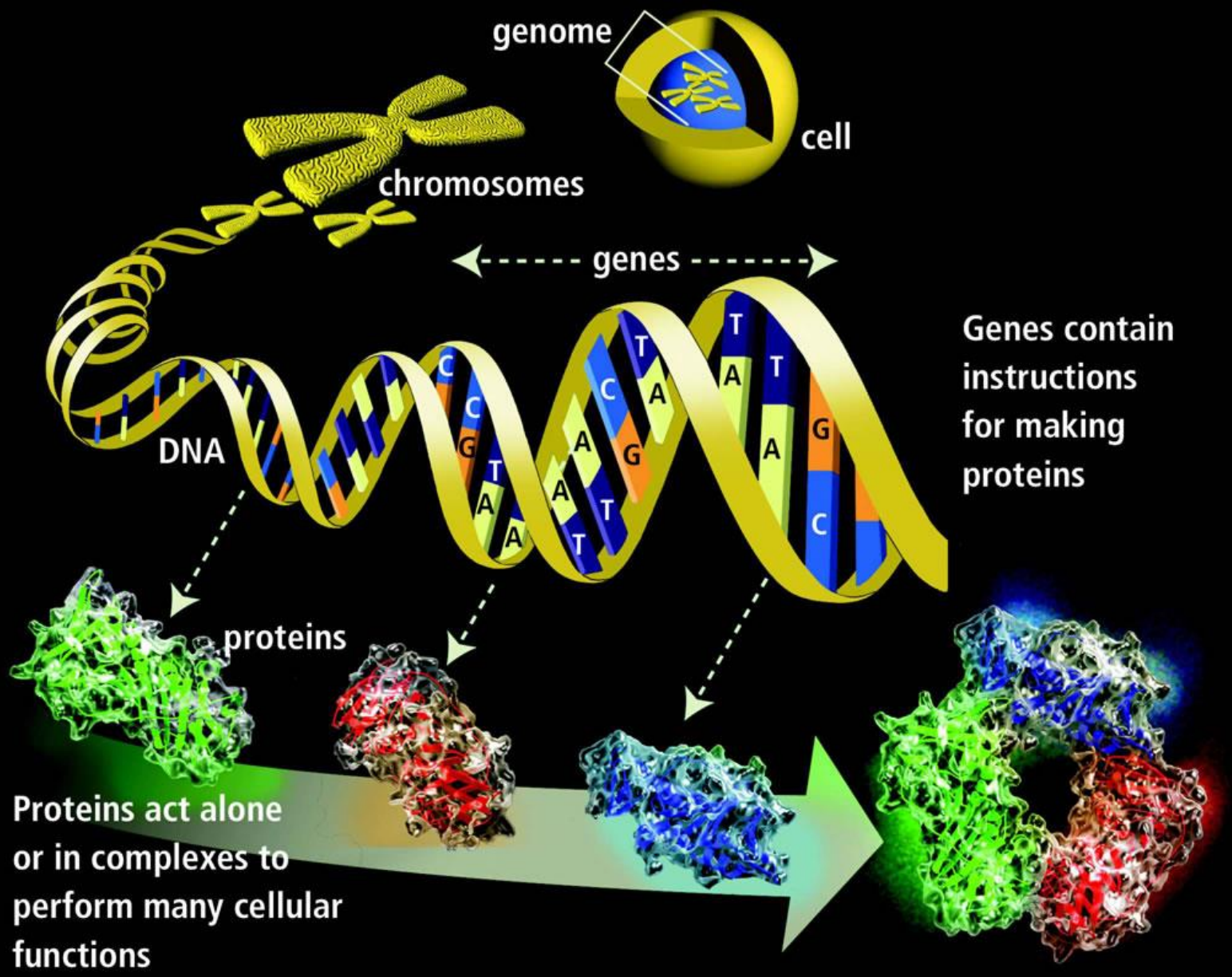
Carol Weitzman, MD, Professor of Pediatrics and the Child Study Center, Yale School of Medicine

Abha R. Gupta, MD, PhD, Associate Research Scientist in Pediatrics and the Child Study Center, Yale School of Medicine

The Genetics of Autism Spectrum Disorders

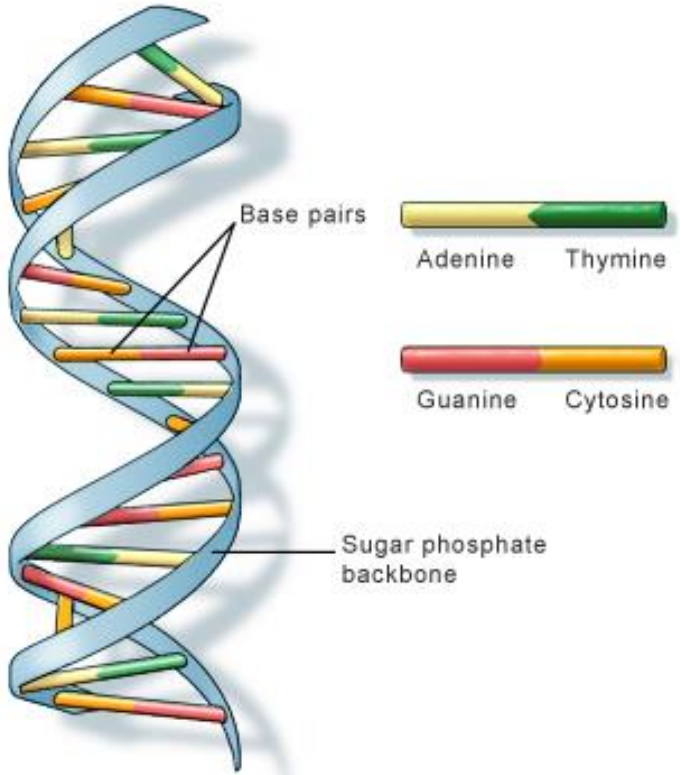
Abha R. Gupta, MD, PhD
Developmental-Behavioral Pediatrics
Department of Pediatrics and Child Study Center

Yale SCHOOL OF MEDICINE

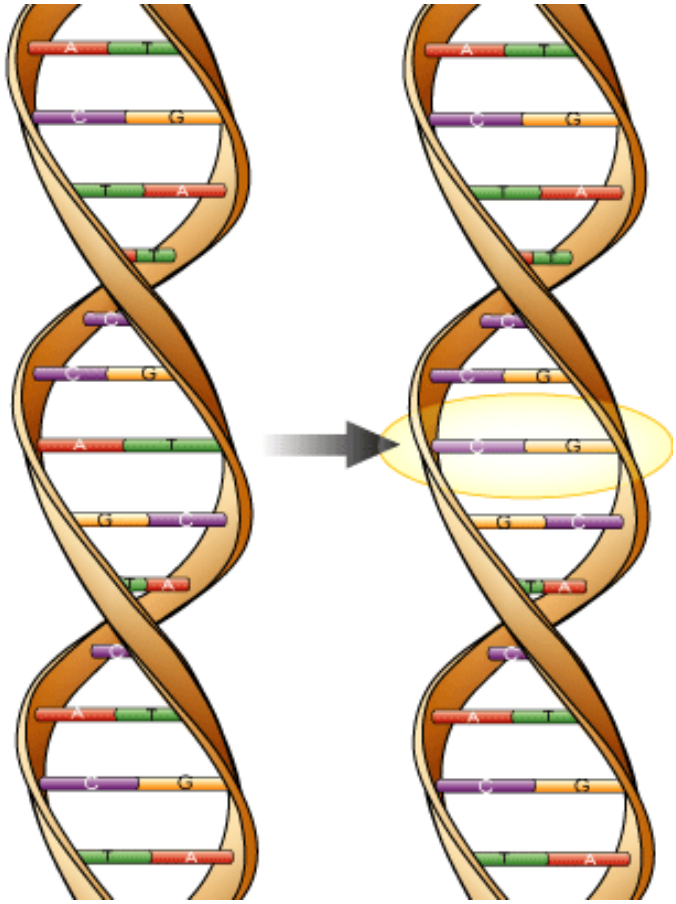


U.S. DEPARTMENT OF ENERGY

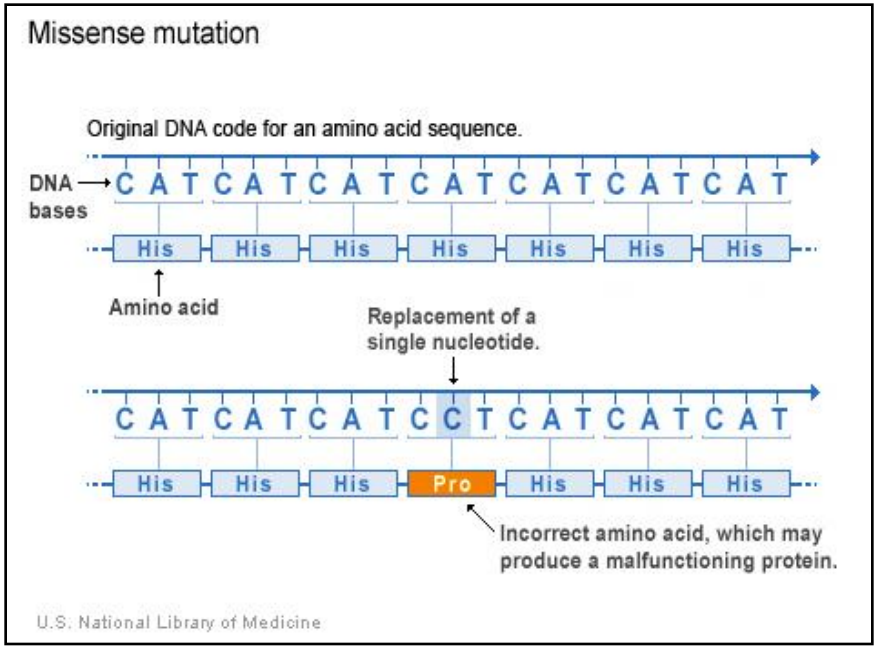
DNA sequence changes

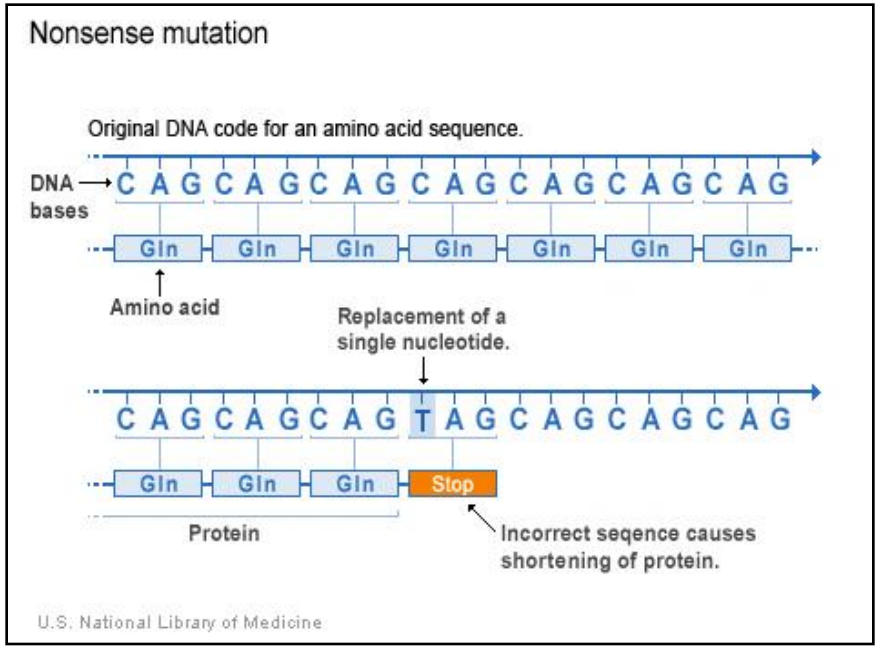


U.S. National Library of Medicine

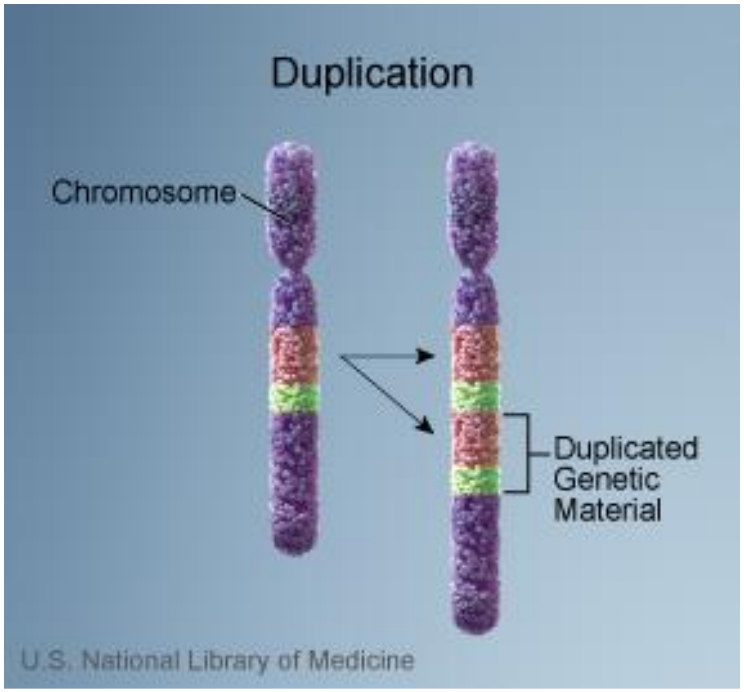


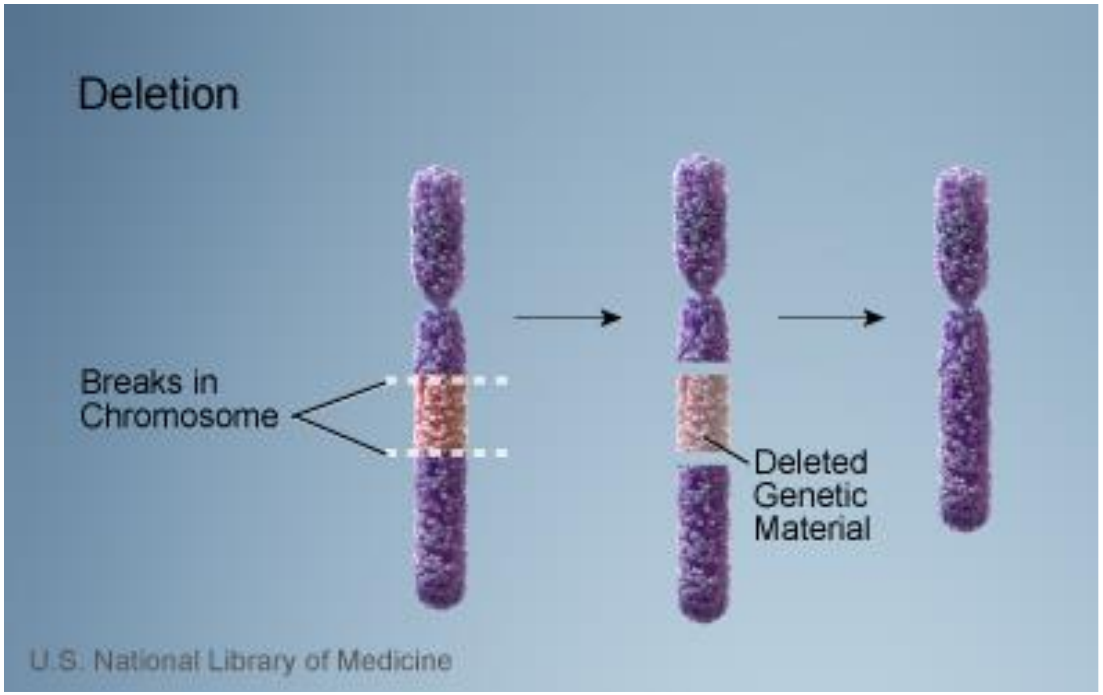
DNA sequence changes





DNA copy number variation (CNV)





Evidence for genetic basis of ASD

Sibling recurrence risk: 18.7% (Ozonoff *et al* 2011)

Twin studies (Bailey *et al* 1995, Hallmayer *et al* 2011)

- Identical twins share 100% of their genetic info
- Fraternal twins share 50% of their genetic info
- Identical twins share an ASD diagnosis more frequently than fraternal twins
- Identical twins are not always concordant

10% of cases also have a genetic syndrome

Table 1 | **ASD-related syndromes**

Syndrome	Gene(s) associated with the syndrome	Proportion of patients with the syndrome that have an ASD	Proportion of patients with an ASD that have the syndrome	Refs
15q duplication	Unknown	High	1–2%	101
Angelman syndrome	*UBE3A* (and others)	>40%	Rare	102, 103
16p11 deletion	Unknown	High	~1%	20, 35, 44
22q deletion	*SHANK3*	High	~1%	21, 22, 104
Cortical dysplasia-focal epilepsy syndrome	*CNTNAP2*	~70%	Rare	37
Fragile X syndrome	*FMR1*	25% of males; 6% of females	1–2%	105
Joubert syndrome	Several loci	25%	Rare	106
Potocki–Lupski syndrome	Chromosome position 17p11	~90%	Unknown	107
Smith–Lemli–Optiz syndrome	*DHCR7*	50%	Rare	108
Rett syndrome	*MECP2*	All individuals have Rett syndrome	~0.5%	109
Timothy syndrome	*CACNA1C*	60–80%	Unknown	24
Tuberous sclerosis	*TSC1* and *TSC2*	20%	~1%	110

Abrahams and Geschwind 2008

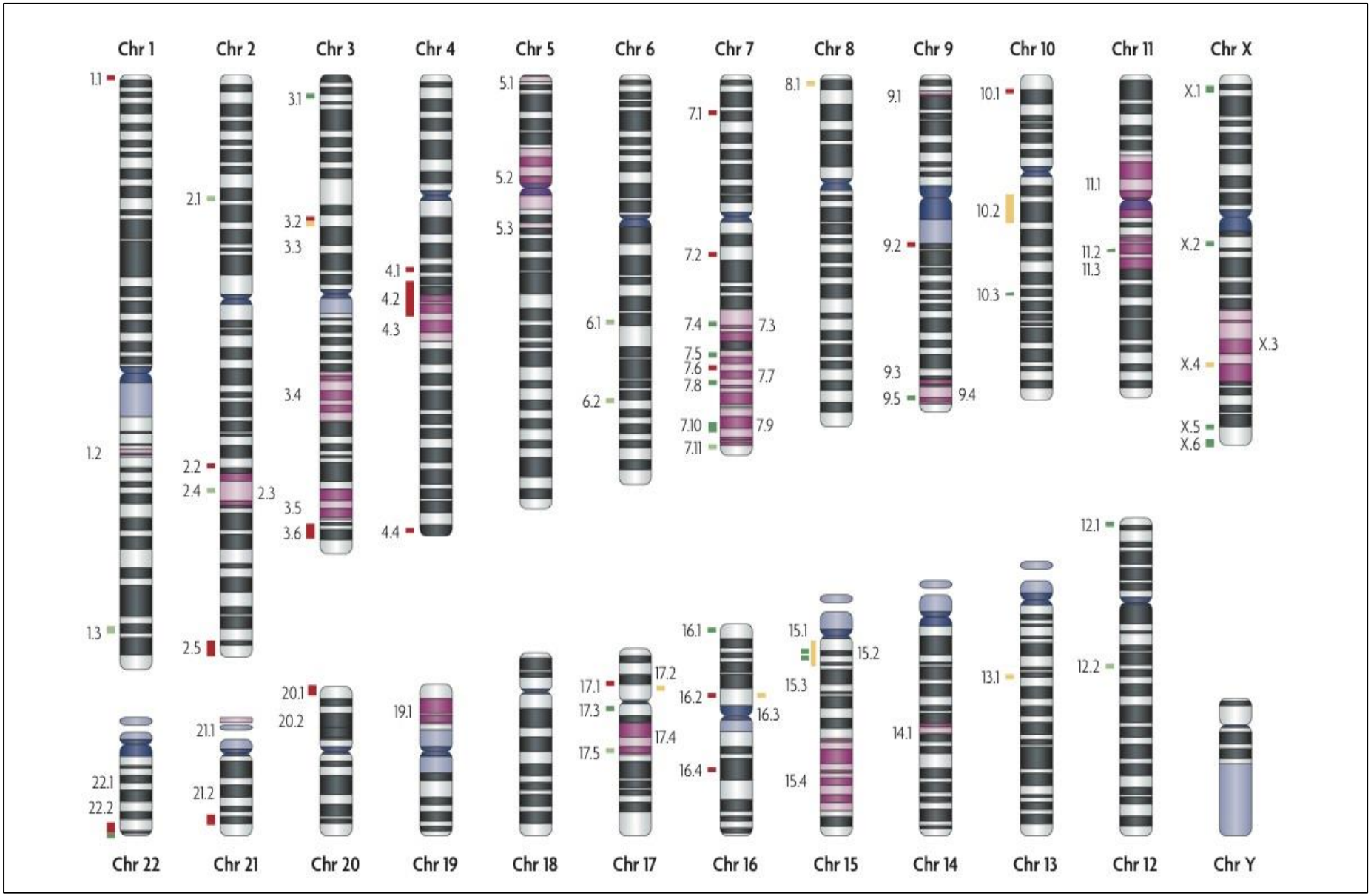
Genetics of ASDs

ASDs are complex disorders with:

- Wide phenotypic variability
- Multiple etiological factors
- A great deal of genetic heterogeneity

There is no one gene for autism, not even a few. There are likely hundreds.

Chromosomal regions implicated in ASD etiology



Abrahams and Geschwind 2008

Disease gene identification

Positional: genome-wide, unbiased

Candidate genes

Funtional: hypothesis driven

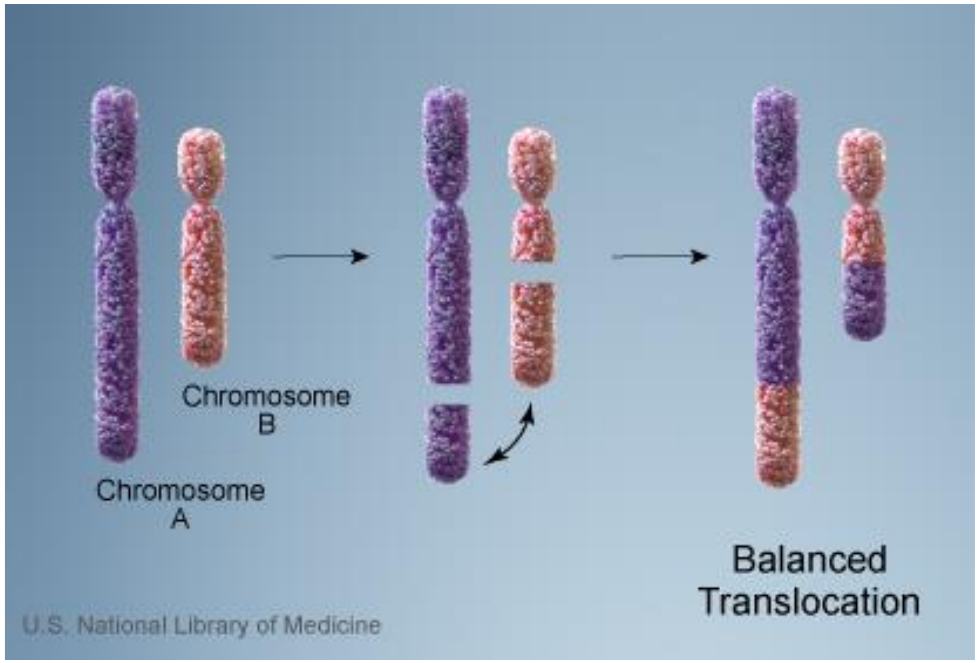
Mutation screening, functional analysis

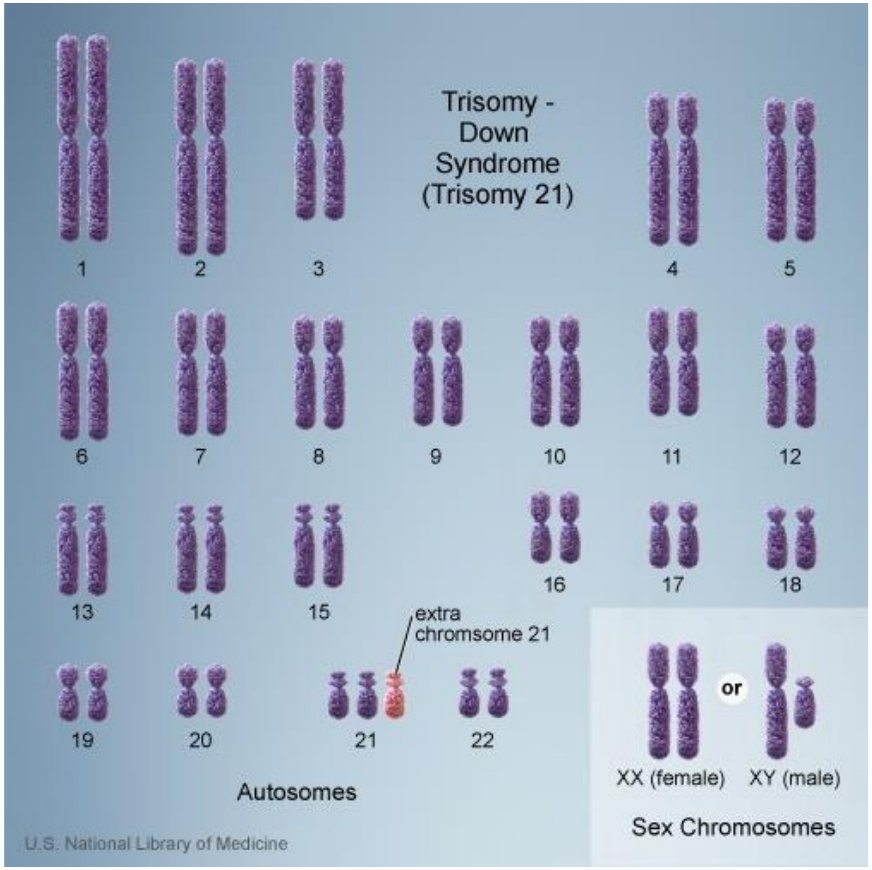
Disease genes

Cytogenetic analyses

Chromosomal abnormalities

- Balanced: inversion, translocation
- Unbalanced: duplication, deletion, aneuploidy





Genome-wide CNVs

Glessner *et al* 2009: CNVs are significantly enriched in:

- Neuronal cell adhesion molecules (*NRXN1, CNTN4, NLGN1, ASTN2)*
- Ubiquitin pathway (*UBE3A, PARK2, RFWD2, FBXO40)*

Pinto *et al* 2010: Higher rate of rare, genic CNVs

- *SHANK2, SYNGAP1, DLGAP2, DDX53-PTCHD1*

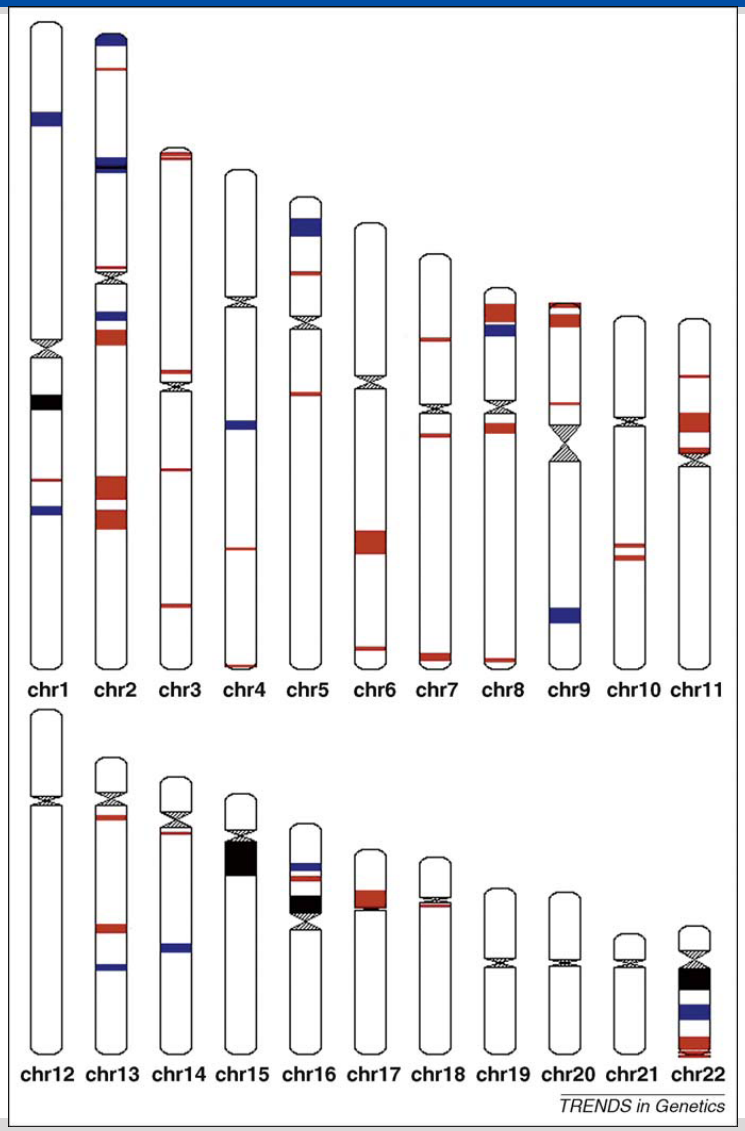
Sanders *et al* 2011: Rare *de novo* CNVs

- chr7q11.23, chr16p13.2 (*USP7*, *c16orf72*), *CDH13,* chr15q11.2-13.1, chr16p11.2, *NRXN1*

Individually rare but implicate important gene networks

CNVs in ASD and schizophrenia

Red: ASD
Blue: schizophrenia
Black: both



Merikangas *et al* 2009

Genome-wide association studies (GWAS)

- Wang *et al* 2009
  - 5p14.1, between *CDH9* and *CDH10*, which mediate neuronal cell adhesion
  - Perhaps contributes to cortical underconnectivity in ASD
- Weiss *et al* 2009
  - 5p15, between *SEMA5A* and *TAS2R1*
  - SEMA5A has been implicated in axonal guidance
  - Reduced SEMA5A expression in postmortem brains from autism patients

Whole-exome sequencing

Obtain the DNA sequence for the entire coding region (exome), which is 1% of the human genome

```
    Sense  5'-CCTCTCAACATTGAGGTCCCCAAAATCAGCCTCCACAGCCTCATTCTCGACTTTTCAGCAGTGTCCTTTCTTGATGTTTCTTCAGTGAGGGGCCTTAAA-3'
Antisense  3'-GGAGAGTTGTAACTCCAGGGGTTTTAGTCGGAGGTGTCGGAGTAAGAGCTGAAAAGTCGTCACAGGAAAGAACTACAAAGAAGTCACTCCCCGGAATTT-5'
                                                              *
           3'-GGAGCGTTGTAACTCCAGGGGTTTTAGTCGGAGGTGTCGGAGTAAGAGTT-5'
                3'-GTTGTAACTCCAGGGTTTTTAGTCGGAGGTGTCGGAGTAAGAGTTGAAAA-5'
                     3'-AACTCCAGGGTTTTTCGTCGGAGGGGTCGGAGTAAGAGTTGAAAAGTCGT-5'
                       5'-ctccaggggttttagtcggaggtgtcggagtaagagttgaaaagtcgtca-3'
                         3'-CCAGGGGTTTTAGTCGGAGGTGTCGGAGTAAGAGTTGAAAAGTCGTCACA-5'
                            5'-ggggttttagtcggaggtgtcggagtaagagttgaaaagtcgtcacagga-3'
                               3'-TTTTTGGTGGGAGGTGTCGGAGTAAGAGTTGAAAAGTCGTCACAGGAAAG-5'
                                 3'-TTTAGTCGGAGGTGTCGGAGTAAGAGTTGAAAAGTCGTCACAGGAAAGAA-5'
                                      3'-GTCGGAGGCGTCGGAGTAAGAGTTGAAAAGTCGTCACAGGAAAGAACTAC-5'
                                        5'-cggaggtgtcggagtaagagttgaaaagtcgtcacaggaaagaactacaa-3'
                                         3'-GGGGGGGTCGGAGTAAGAGTTGAAAAGTCGTCACAGGAAAGAACTACAAA-5'
                                          5'-gaggtgtcggagtaagagatgaaaagtcgtcacaggaaagaactacaaag-3'
                                             3'-GGGTCGGAGTAAGAGTTGAAAAGTCGTCACAGGAAAGAACTACAAAGAAG-5'
                                                5'-tcggagtaagagttgaaaagtcgtcacaggaaagaactacaaagaagtca-3'
                                                   3'-GAGTAAGAGTAGAAAAGTCGTCACAGGAAAGAACTACAAAGAAGTCACTC-5'
                                                        5'-agagttgaaaagtcgtcacaggaaagaactacaaagaagtcactccccgg-3'
                                                           3'-GTTGAAAAGTCGTCACAGGAAAGAACTACAAAGAAGTCACTCCCCGGAAT-5'
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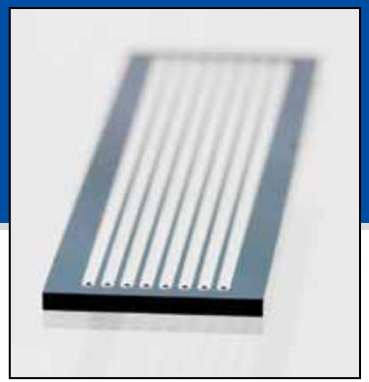
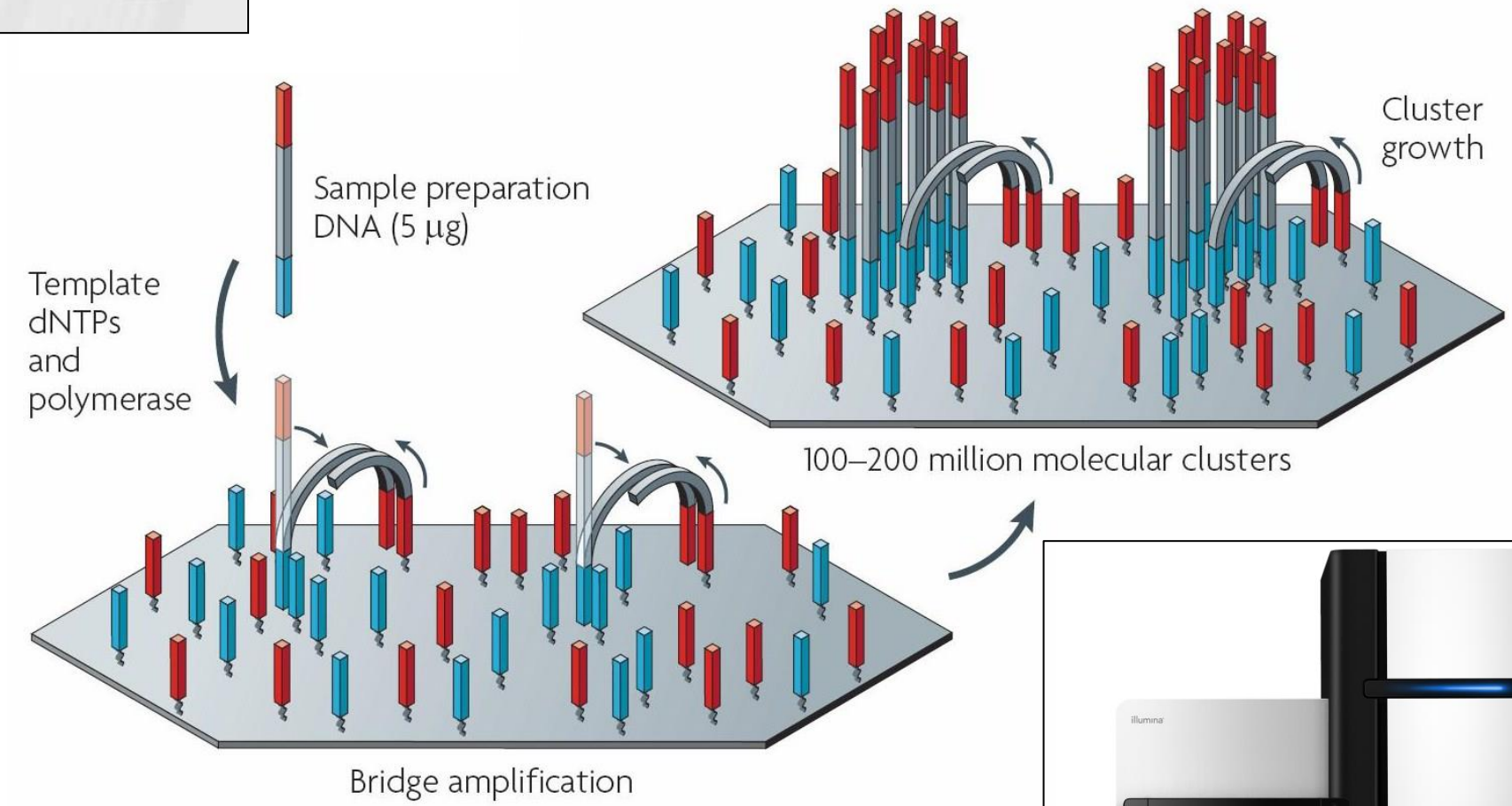


Whole-exome sequencing

Illumina HiSeq

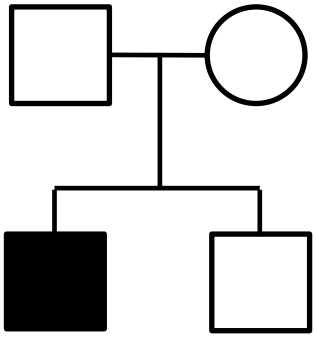


Metzker 2010

Whole-exome sequencing

Experimental design

DNA studied for 200 quartets from the Simons Simplex Collection to investigate the rate of *de novo* variants in patients versus unaffected siblings



Whole-exome capture: 32 million base pair target

Sequencing yields 100 million reads, each 75 base pairs long

Analysis on high performance cluster

Whole-exome sequencing

Results

Brain-expressed, protein-changing *de novo* mutations

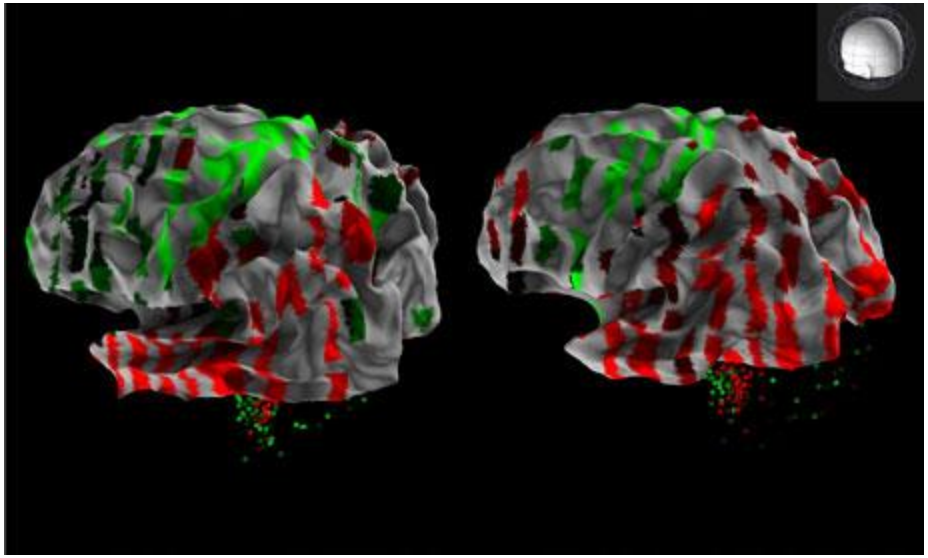
- 114 in 200 patients vs. 67 in 200 siblings
- p=0.001, OR 2.22 (95% CI 1.19-4.13)

SCN2A (Sodium channel, voltage gated, type II, alpha)

- Previously reported in epilepsy
- 2 *de novo* nonsense mutations in patients, neither has seizures

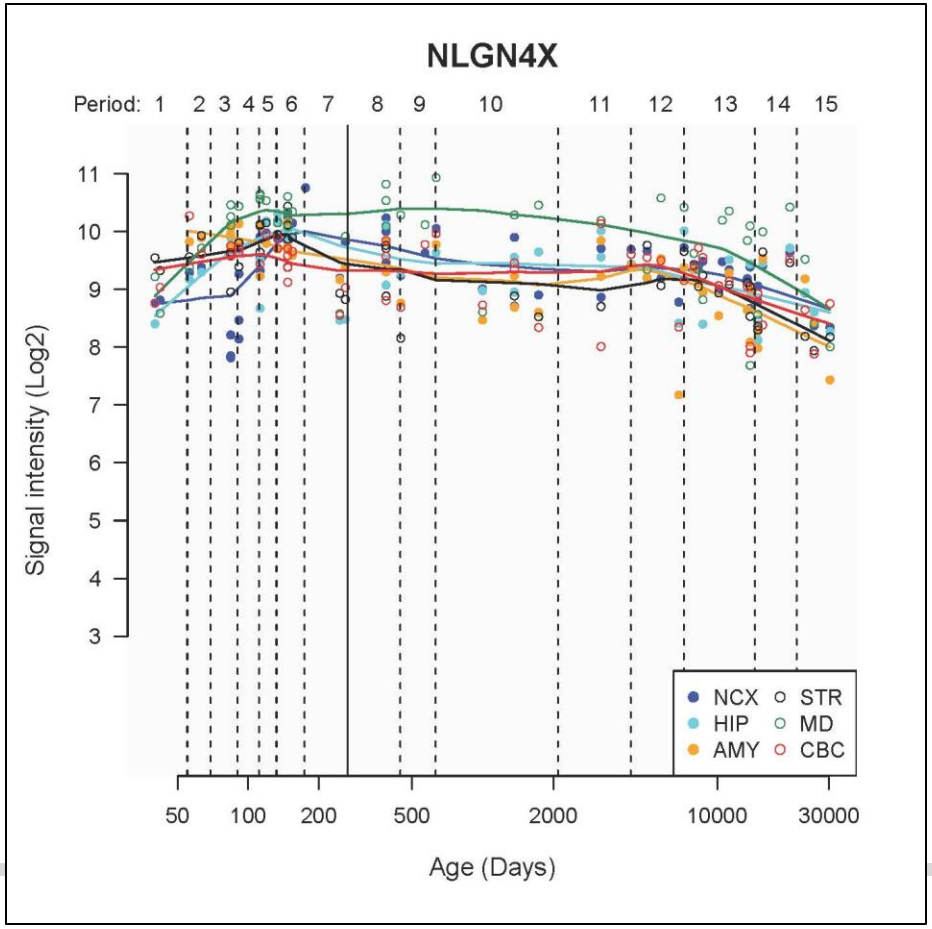
Including 3 other studies: *CHD8, DYRK1A, GRIN2B, KATNAL2, POGZ*

Developmental neurobiology…



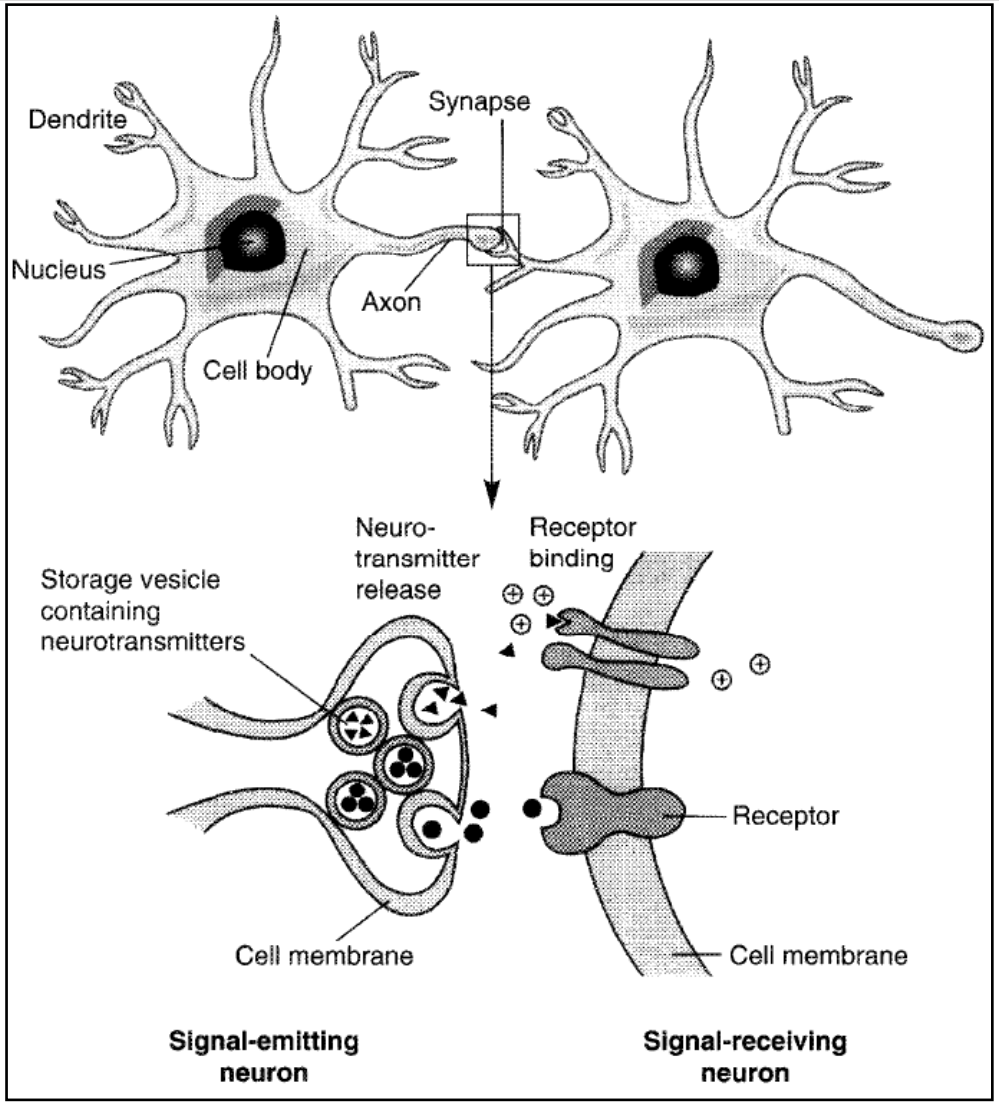
Hawrylycz *et al* 2012

…will inform ASD pathophysiology



Kang *et al* 2011

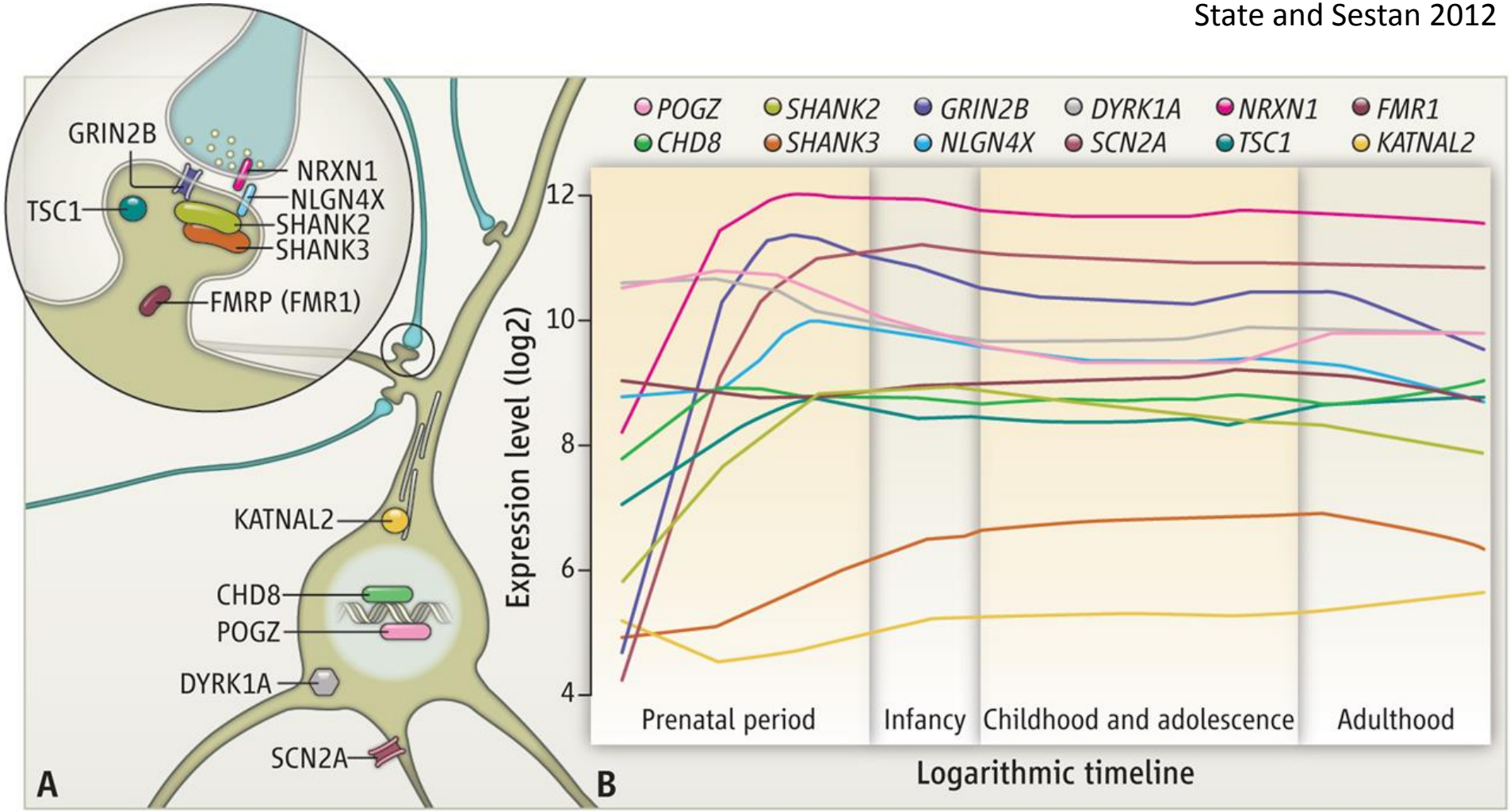
The neural synapse



www.niaaa.nih.go

Candidate genes for ASD

State and Sestan 2012

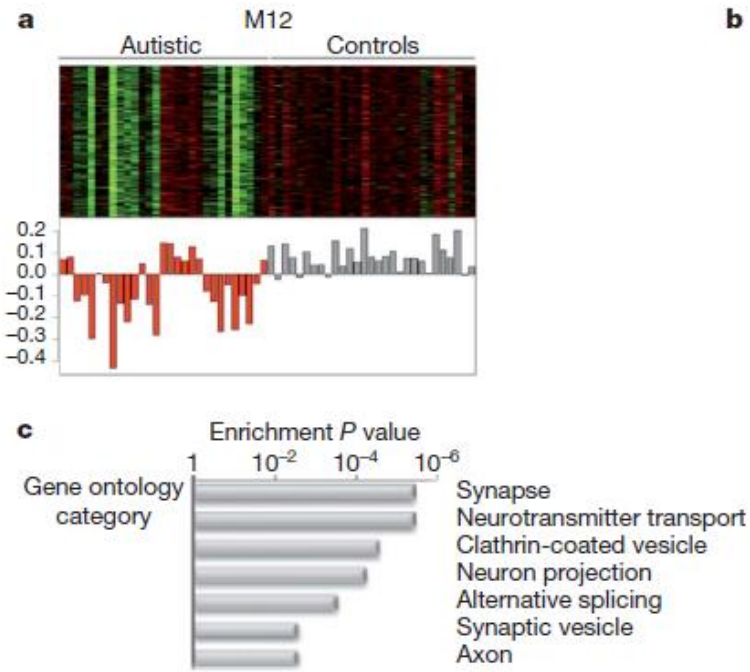


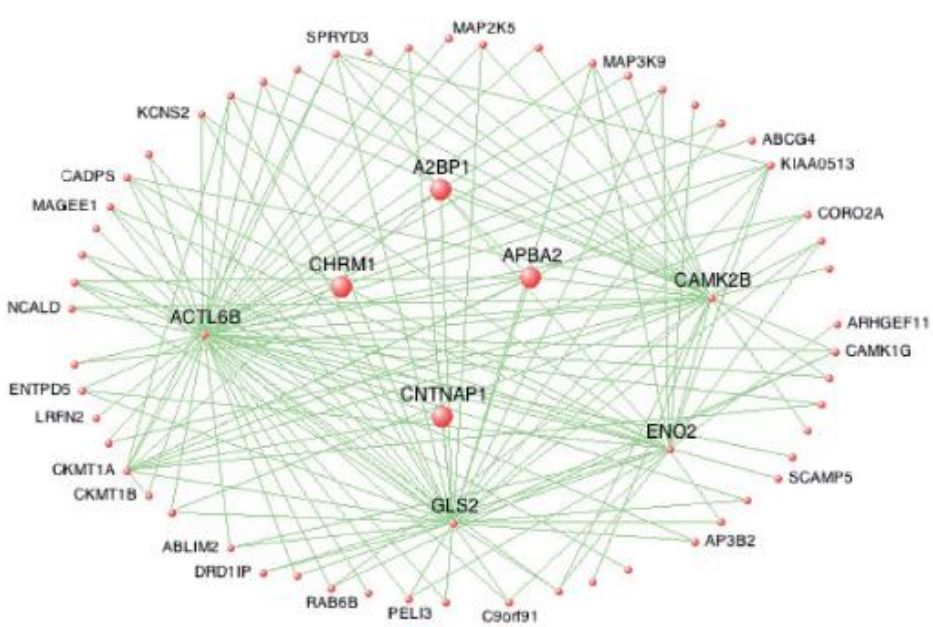
Gene expression analysis in postmortem brain

Differential gene expression in frontal and temporal cortices is attenuated in autism

Discrete set of co-expressed genes in autism is:

- Down-regulated compared to control tissue
- Enriched for synaptic function and known autism risk genes

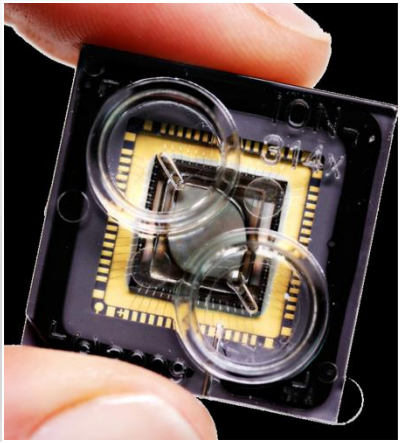




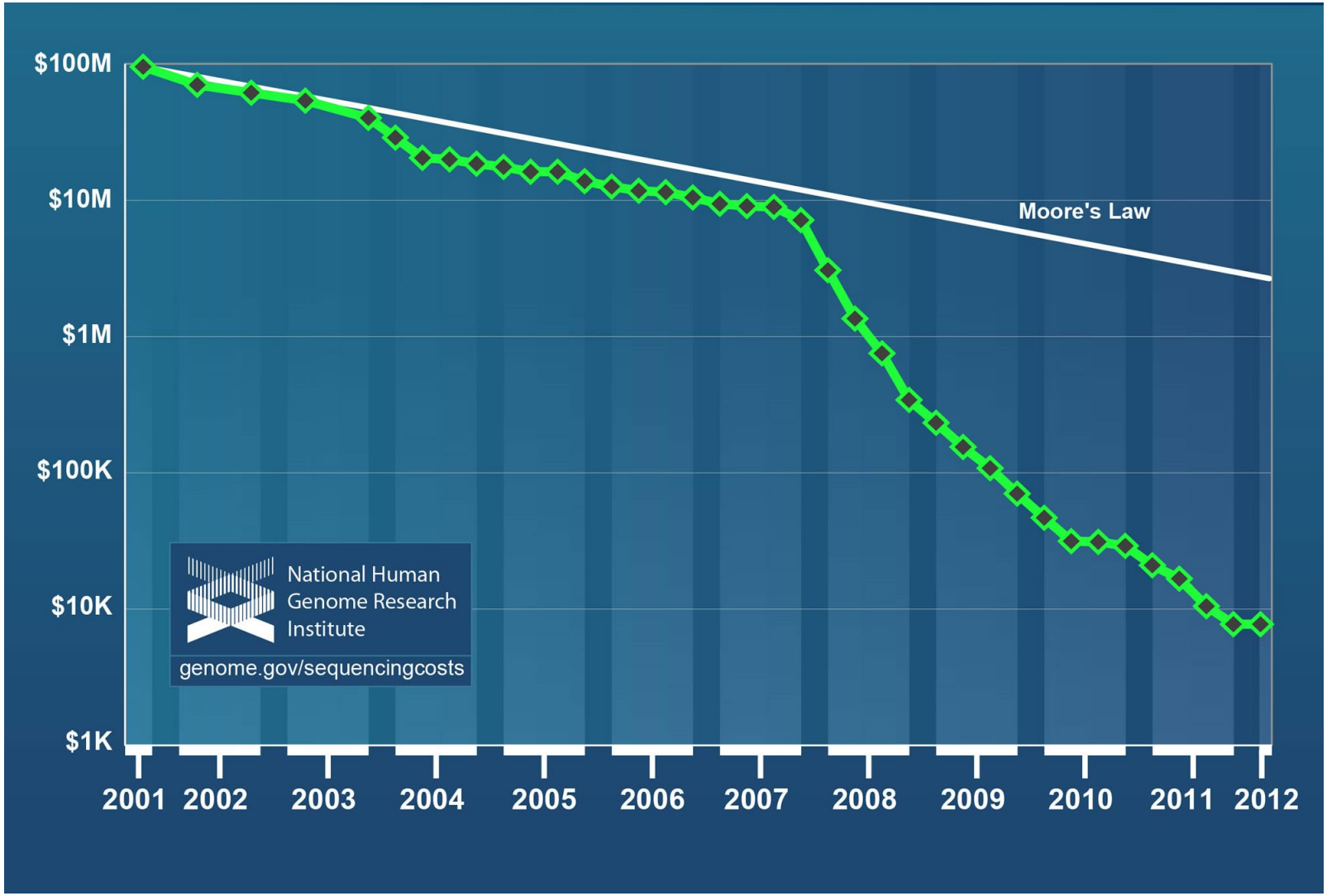
Voineagu *et al* 2011

Whole-genome sequencing is here!

Ion Proton



Cost per human genome



Clinical genetics evaluation in ASD

AACAP practice parameters 1999

- FraX DNA test and Wood's lamp for TS is typically indicated

American Academy of Neurology 2000

- Karyotype and FraX if ID, family history of ID, or dysmorphisms

American Academy of Pediatrics clinical report 2007

- Karyotype and FraX if ID

American College of Medical Genetics practice guidelines 2013

- Tiered evaluation…

Clinical genetics evaluation in ASD

First tier

- 3 generation family history
- Identify known syndromes or associated conditions
  - Examination with special attention to dysmorphisms
  - If specific syndrome suspected, do targeted testing
  - If clinical indicators present, do metabolic/mitochondrial testing
- Chromosomal microarray
- DNA testing for Fragile X
  - Males: routinely
  - Females: if indicators present (family history, phenotype)

Clinical genetics evaluation in ASD

Second tier

- *MECP2* sequencing for all females with ASD
- *MECP2* duplication testing in males if phenotype suggestive
- *PTEN* testing if head circumference >2.5 SDs above average
- Brain MRI for specific indicators (microcephaly, regression, seizures, history of stupor/coma)

Schaefer *et al.* 2013

Clinical genetics evaluation

Projected diagnostic yields:

- CMA: 10%
- Fragile X: 1-5%
- *MECP2*: 4% of females
- *PTEN*: 5% if head circumference >2.5 SDs
- Karyotype: 3%
- Other: 10%

Estimated to result in an identified etiology in 30-40% of individuals

Schaefer *et al.* 2013

Clinical genetics evaluation

Clinical genetics evaluation will become increasingly routine in the medical assessment of ASD, regardless of specific diagnosis:

Abnormal genetics test:

- Autism: 3.1% (2/65)
- PDD-NOS: 5.1% (6/117)

(Challman *et al.* 2003)

Genes to pathophysiology to treatment

Rett syndrome and *MECP2*

- Mutations in *MECP2* cause most cases of Rett syndrome.
- *MECP2* controls the expression of other genes.
- Patients show abnormal neurons but not neuronal death: Can viable but defective neurons be repaired?
- Mutant mice lacking *MECP2* develop neurological symptoms.
- Reactivation of *MECP2* in adult mutant mice reversed symptoms, including deficits in neuronal signaling in the hippocampus (Guy *et al* 2007).
- Absence of *MECP2* doesn't irreversibly damage neurons.

Genes to pathophysiology to treatment

Fragile X syndrome and *FMR1*

- Loss of *FMR1* causes FXS.
- *FMR1* controls the expression of other genes.
- Pathophysiology involves hyperactivity of the glutamate receptor, mGluR5.
- Mutant mice lacking *FMR1* develop neurological symptoms.
- Reducing expression of mGluR5 by 50% showed rescue of symptoms, including alterations in dendritic density and hippocampal protein synthesis (Dolen *et al* 2007).
- GABA agonist R-baclofen corrects increased synaptic protein synthesis and spine density (Henderson *et al* 2012).

Genes to pathophysiology to treatment

Tuberous sclerosis and *TSC*

- Mutations in *TSC1* and *TSC2* cause TS.
- Pathophysiology involves the mammalian target of rapamycin (mTOR) signaling pathway in the hippocampus.
- Mutant mice lacking 1 copy of *TSC2* show cognitive deficits. Treatment of adult mutant mice with rapamycin improved synaptic plasticity and behavioral deficits (Ehninger *et al* 2008).
- Mutant mice lacking 1 or 2 copies of *TSC1* in cerebellum show decreased neuronal activity, abnormal social interaction, and repetitive behavior. Treatment with rapamycin prevented pathological and behavioral deficits (Tsai *et al* 2012).

Future directions

- Increase study populations by ten-fold
- Whole-genome sequencing: regulatory elements
- Pathway analysis
- Epigenetics
- Biomarkers: neuroimaging, eye tracking, ERPs
- Genetic overlap between neuropsychiatric disorders
- Functional analysis of variants: bridging genetics and neuroscience
  - *In vitro* and *in vivo* studies
  - Postmortem brain tissue, induced pluripotent stem cells, animal systems

Thank you for your attention!