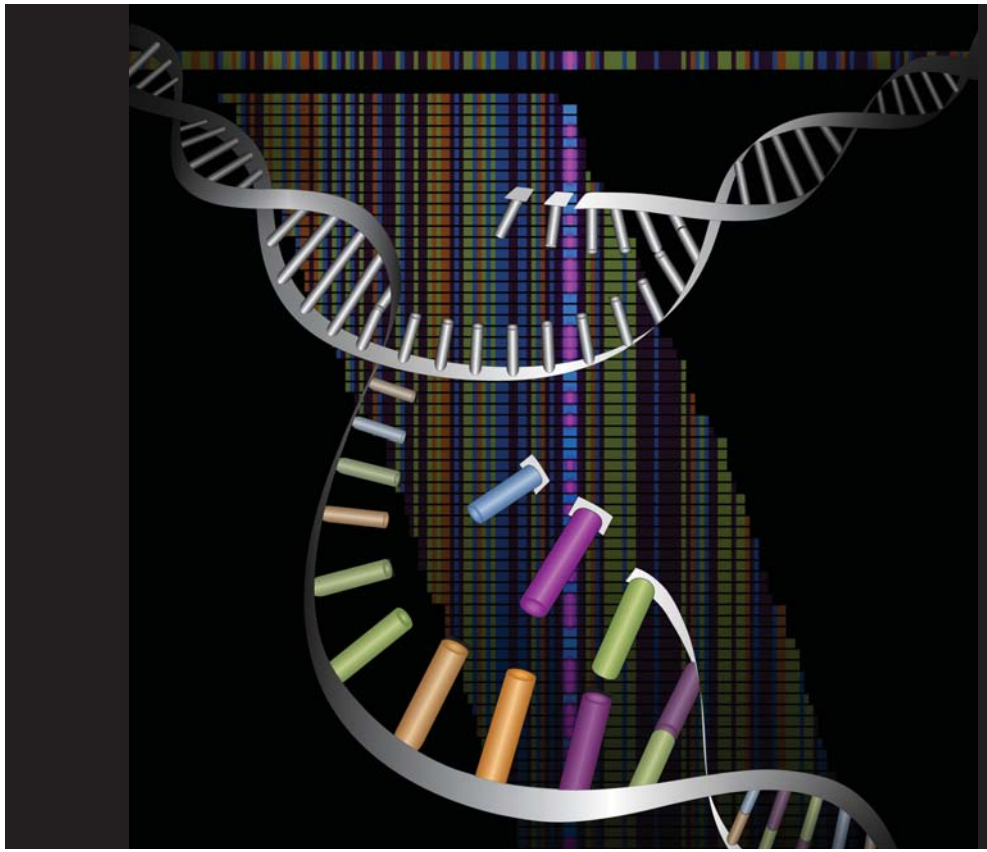
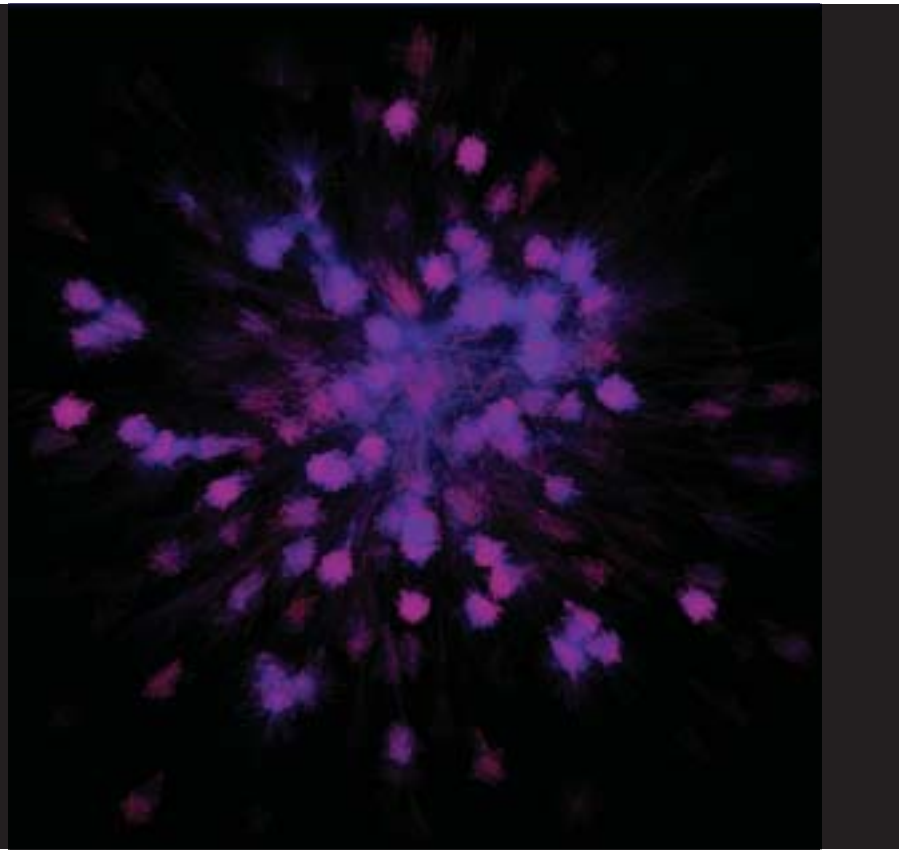
UCSF
University of California
San Francisco

Autism Spectrum Disorder Part III: Genetics and Autism





No conflicts of interest to declare

Funding sources:

Learning objectives

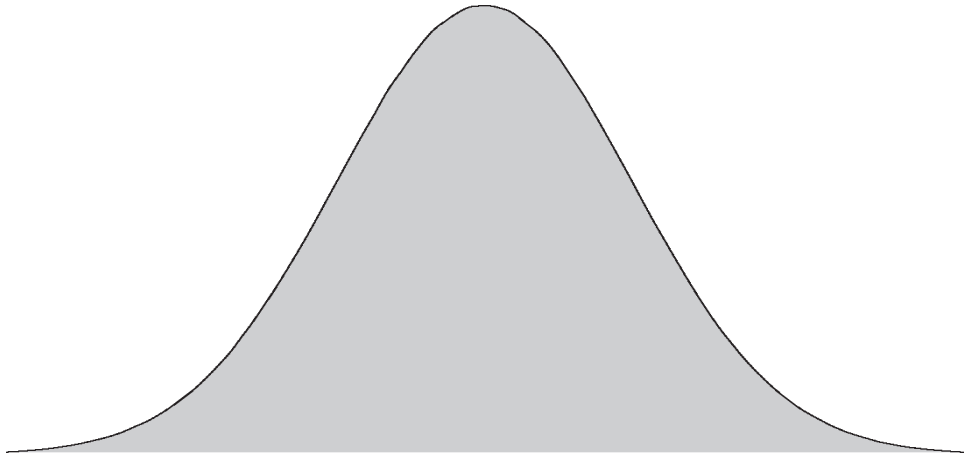
- Discuss the role of genetic and environmental factors that contribute to ASD
- Identify the wide range of genetic variation that is involved in ASD including gene-gene and gene-environment interactions
- Review the current status and key findings of genetics research in ASD

Autism Spectrum Disorder (ASD) is a developmental neurological impairment

- CDC estimates incidence of 1 in 60 (1.7%)
  - More common in males (2:1 to 4:1)
- Individuals with ASD have fewer children – Power *et al.* 2013

Genes vs. “The Environment”

- Trait: A characteristic of individuals in a population
  - E.g. milk production in cows
  - Height in humans
  - Sociability in humans
  - Autism spectrum disorder diagnosis



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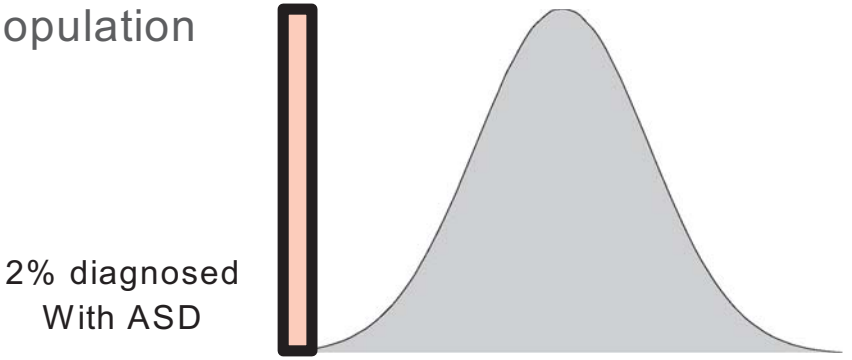


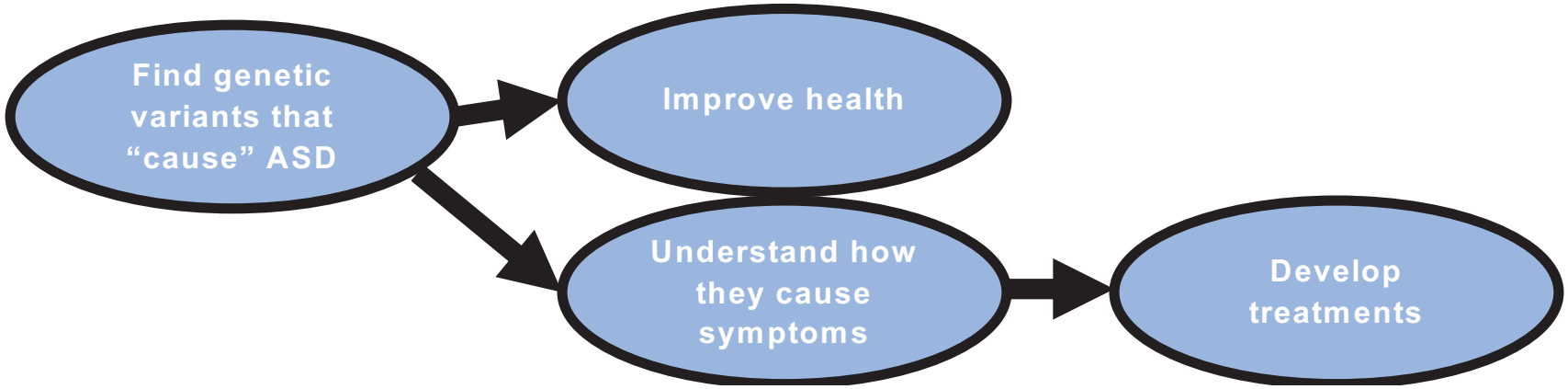
- Genes: The variance in a trait explained by heritable factors

- Environment: The variance in a trait explained by everything else
  - ”Unaccounted” would be a better phrase
  - Environmental factors are one contributor to this term

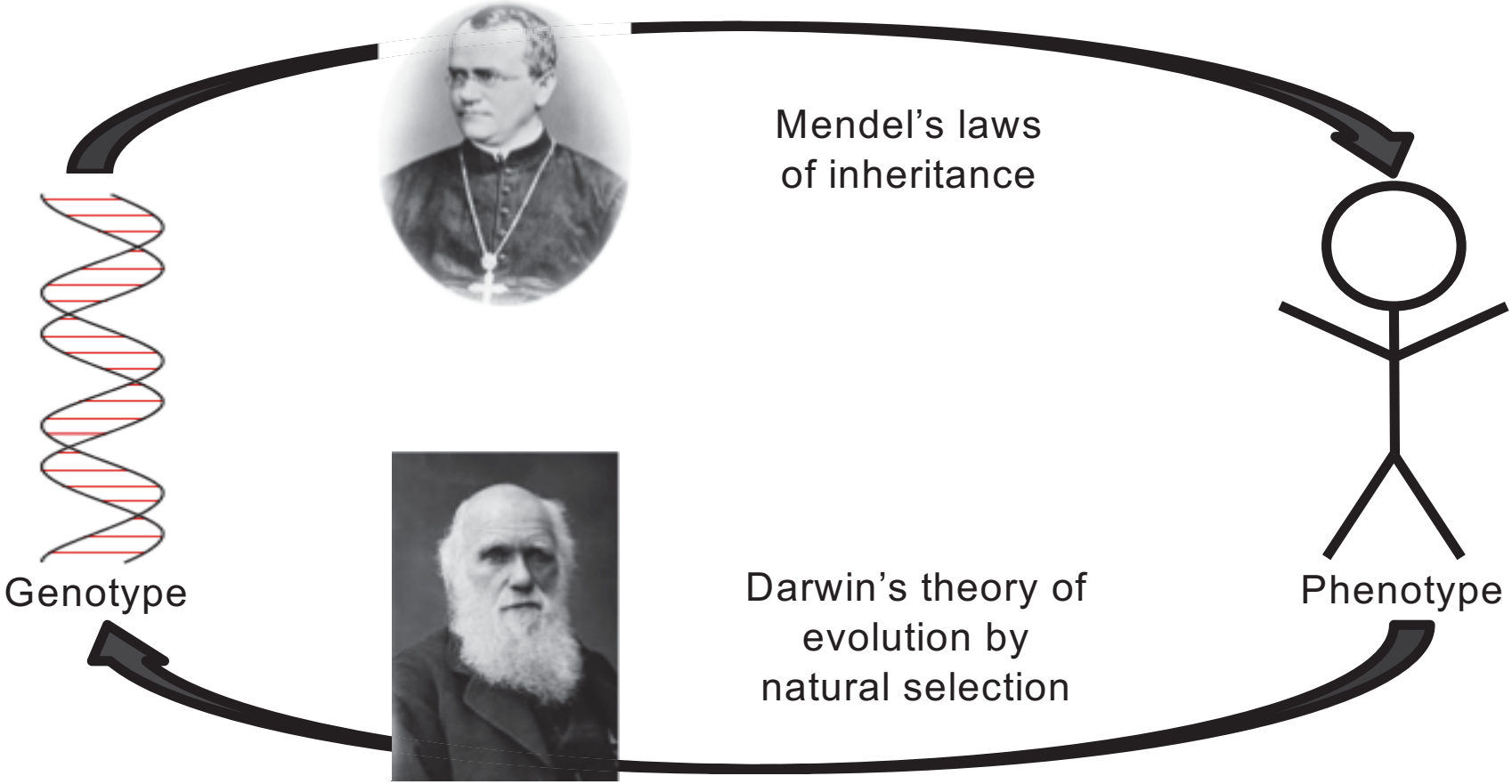
What are researchers trying to achieve?

- **Genetics**

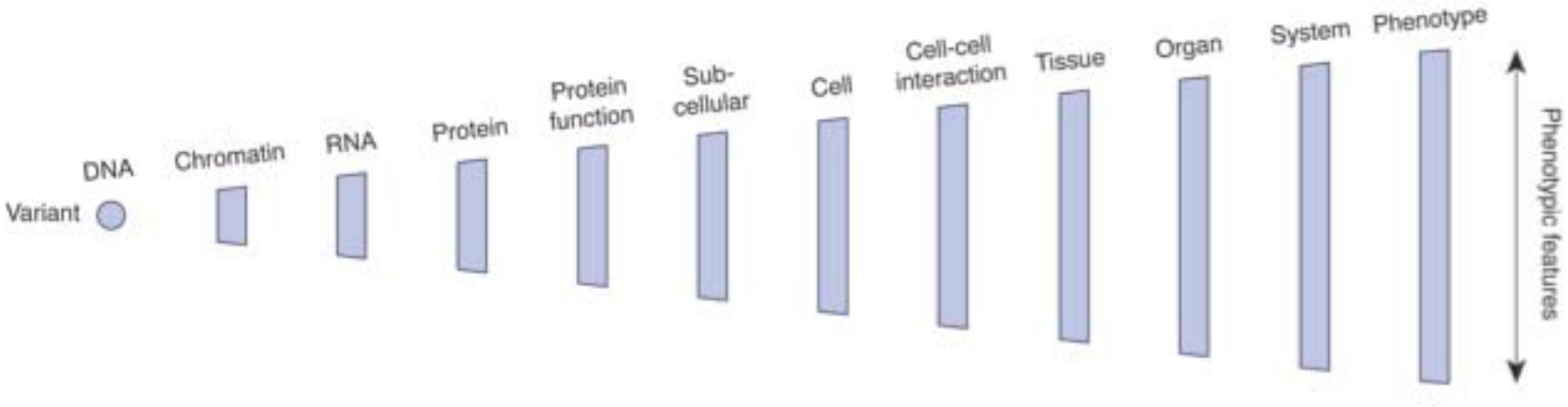
- **Environmental factors**



Genetics and heritability analyses assess the flow of information in biological systems



Genotypes are amplified to produce observable phenotypes



Sanders SJ, Curr Opin Genet Dev, 2015

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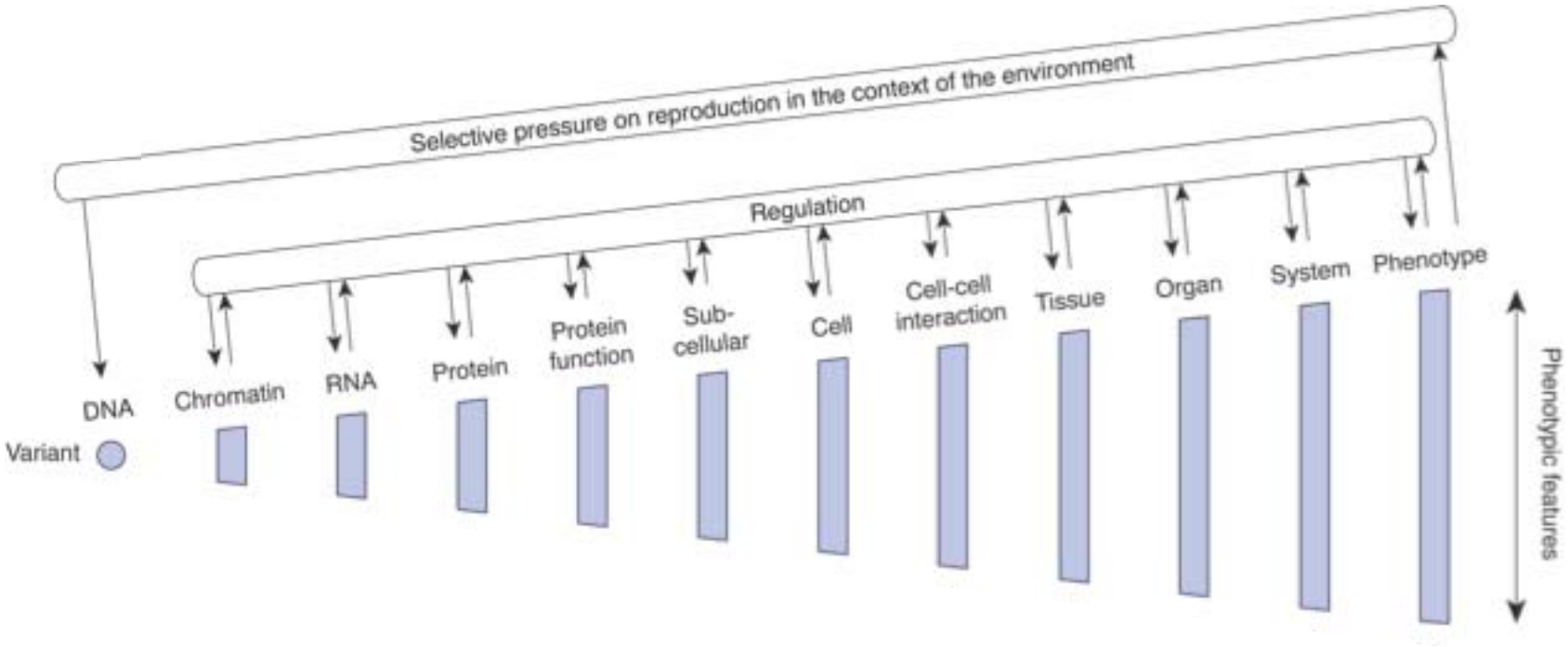

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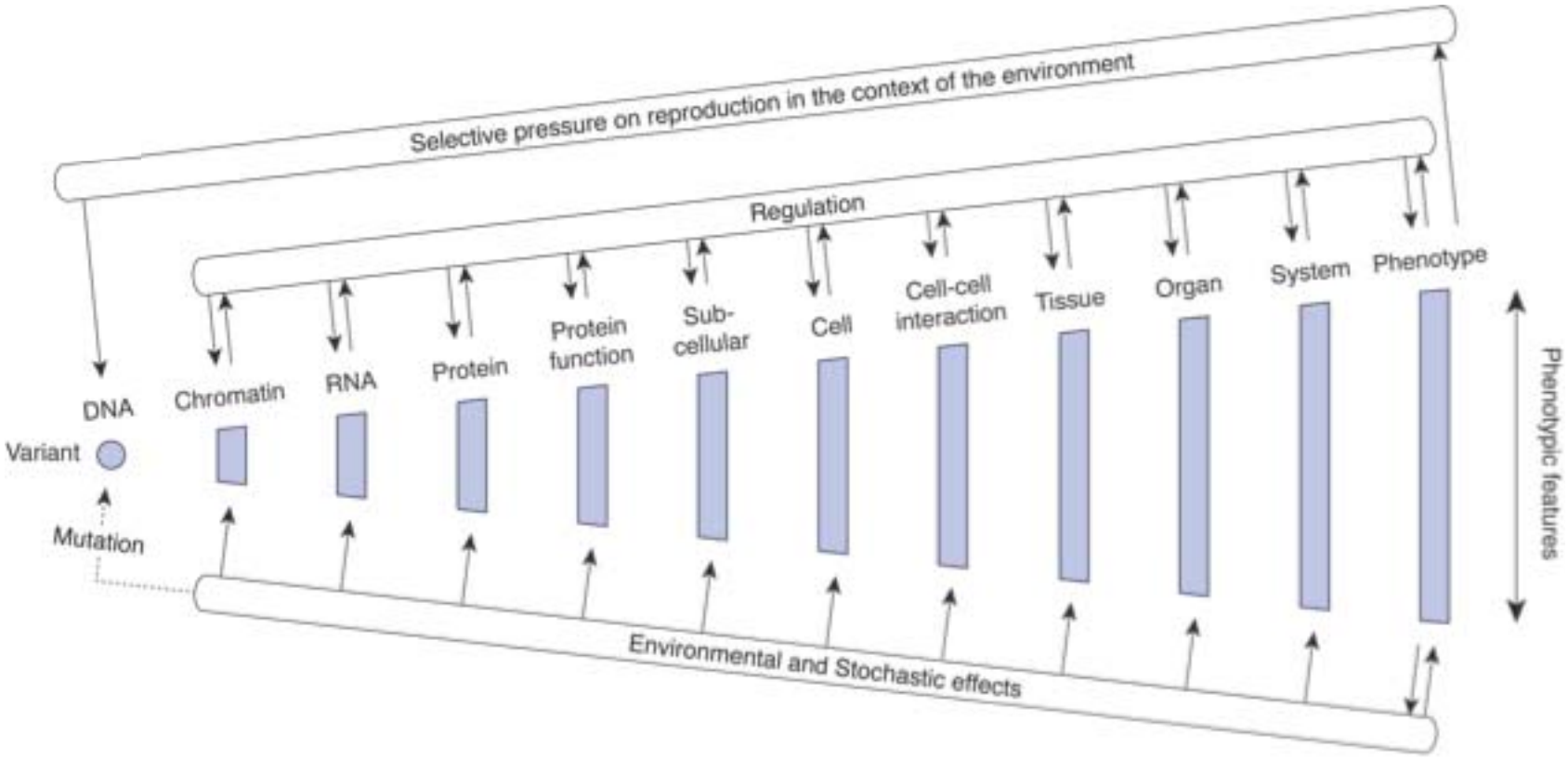

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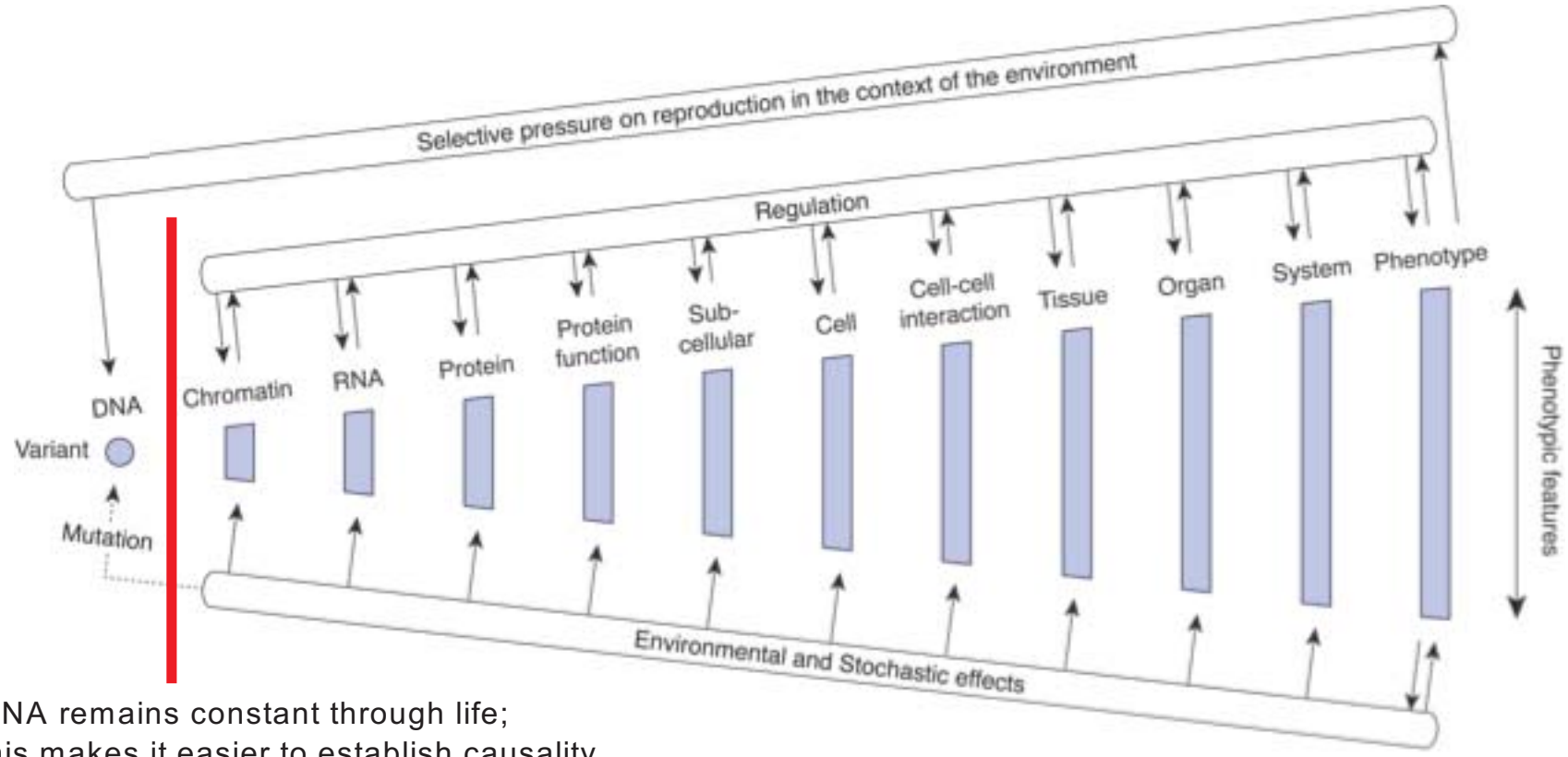
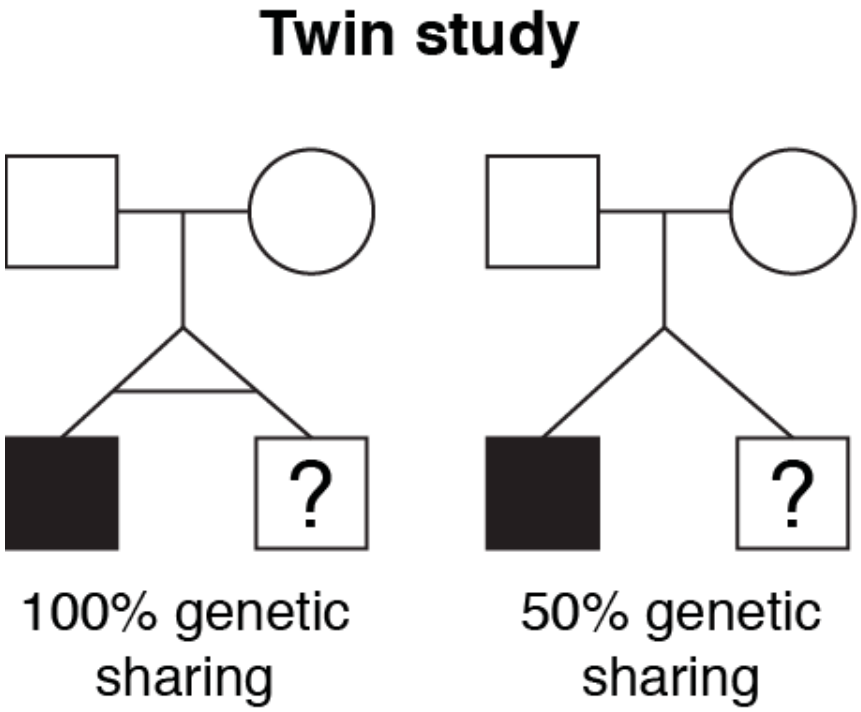


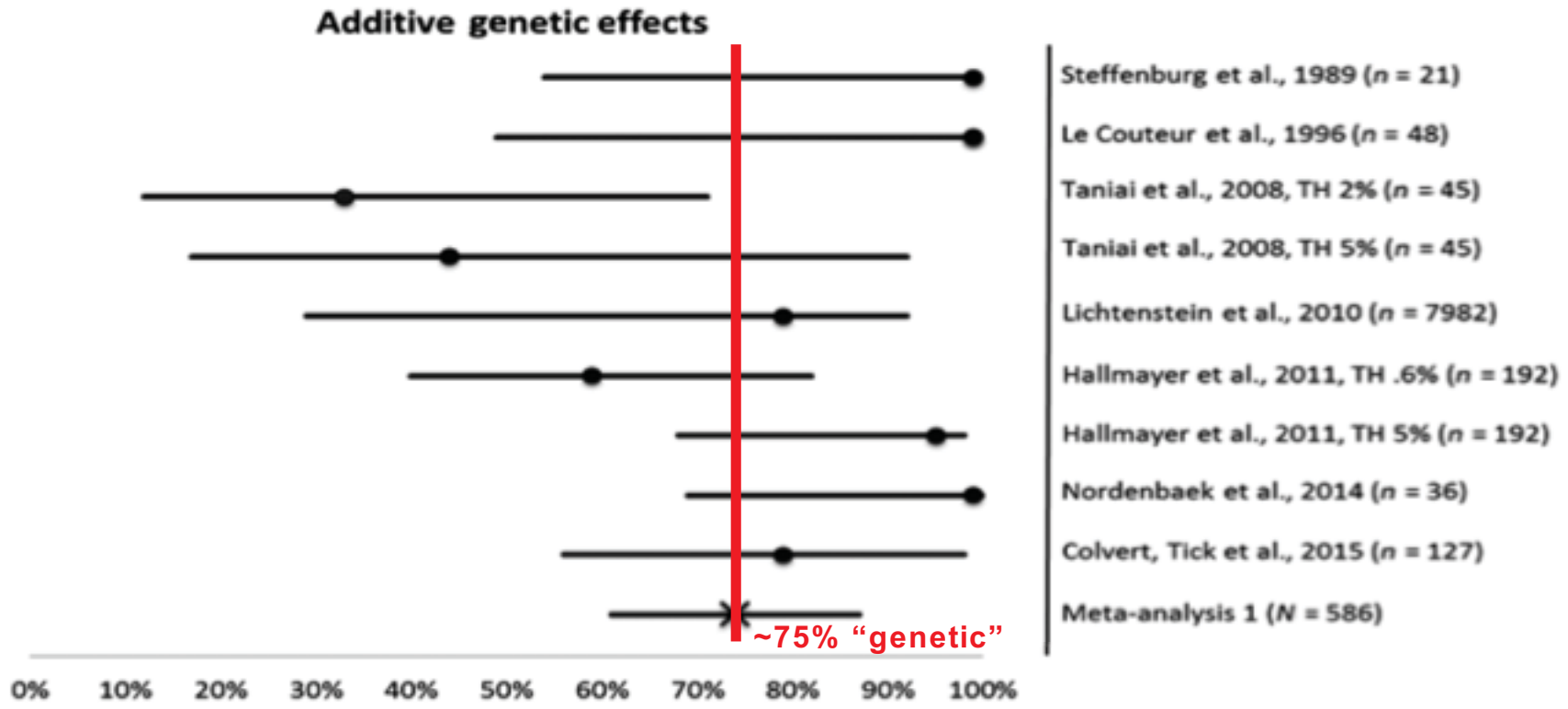
DNA remains constant through life;
this makes it easier to establish causality

Sanders SJ, Curr Opin Genet Dev, 2015

Twin studies compare identical (monozygotic) to non-identical (dizygotic) twins



Twin study: Combining data across 7 twin studies



Tick *et al. Journal of Child Psychology and Psychiatry* 2016

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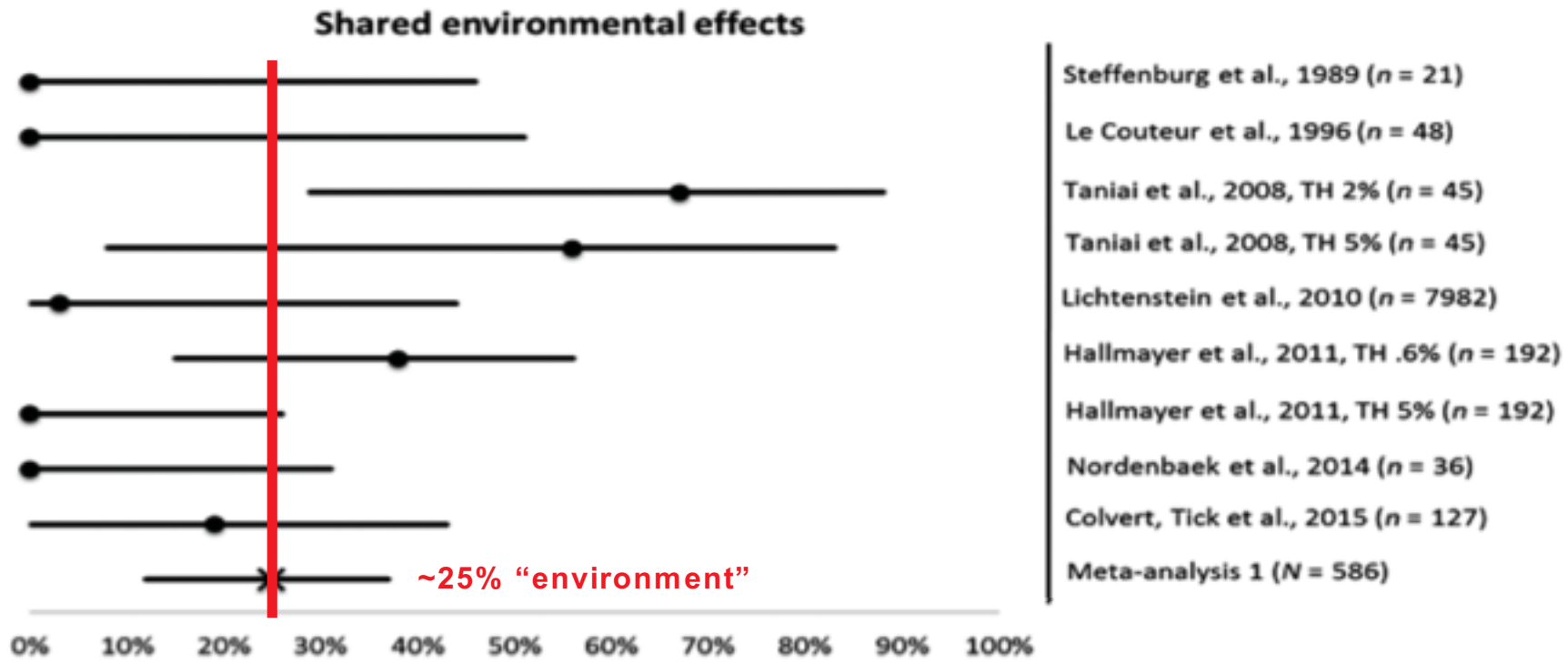

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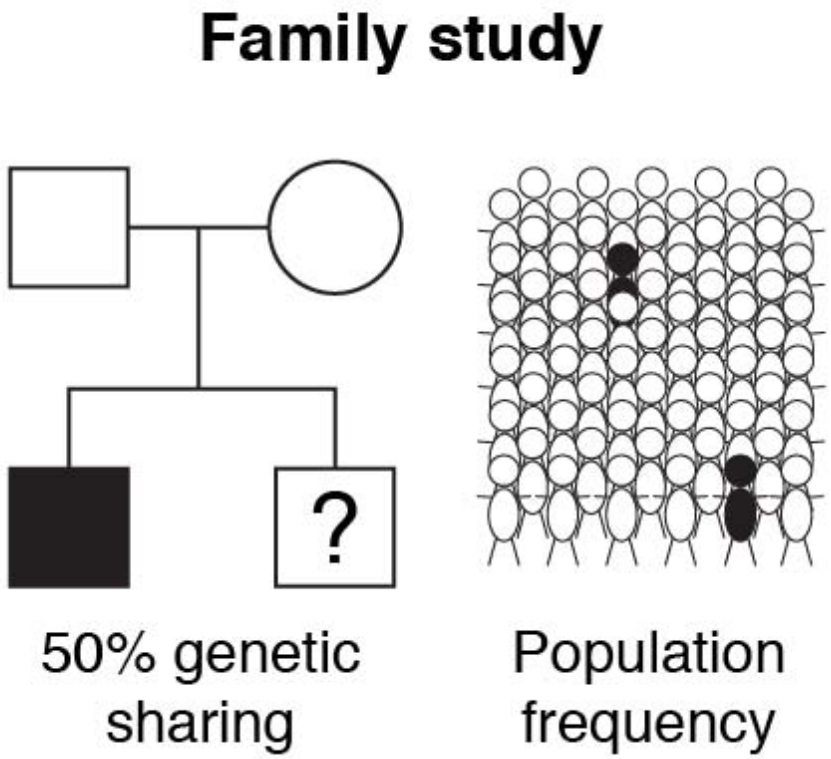
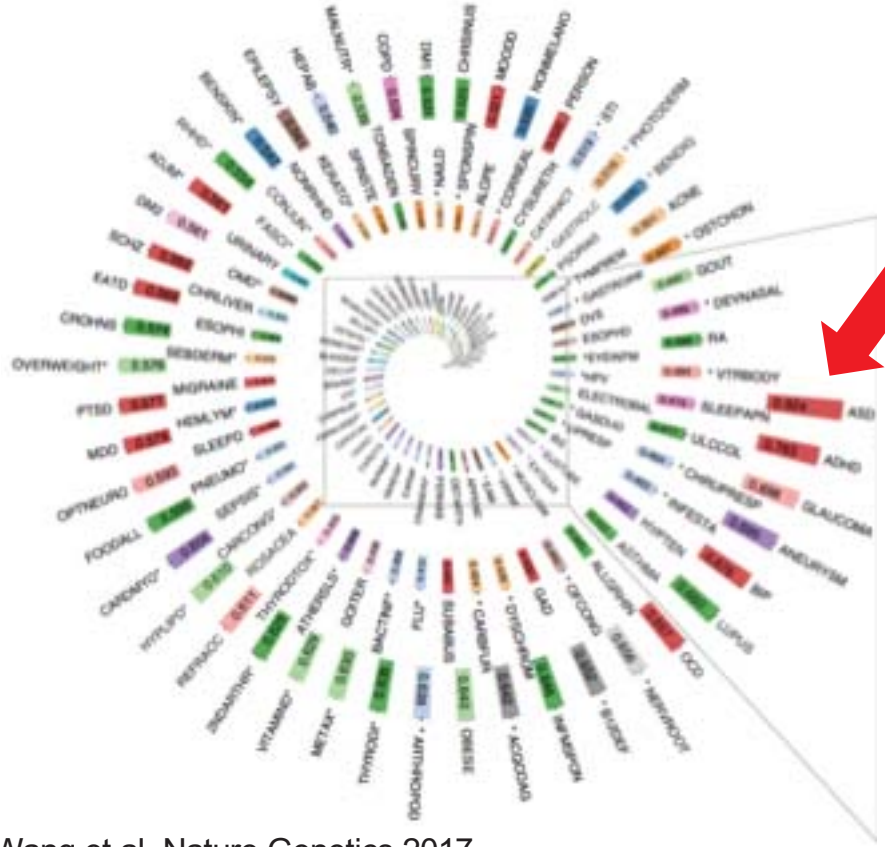
Family/sibling studies compare relatives (e.g. siblings) to the general population



Analysis of heritability across ~130 complex human disorders; ASD is the most heritable



ASD: 92% "genetic"

Many other family studies of ASD alone; most estimate ~75% heritability

Wang et al. Nature Genetics 2017

Neuropsychiatric disorders, like ASD, are frequently genetic

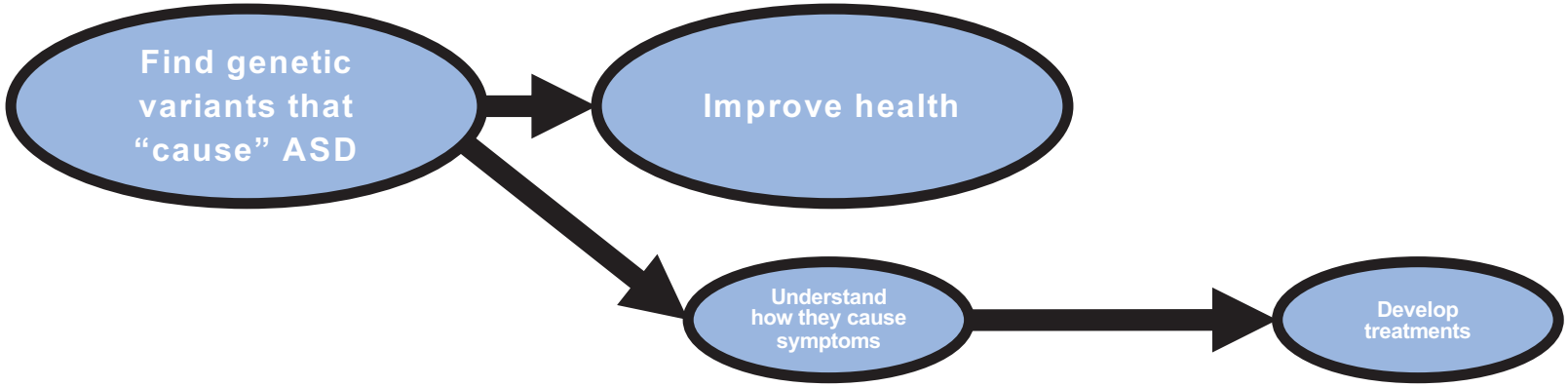
Disorder	Clinical utility	Heritability
ASD	Parental counseling	70 - 95%
Schizophrenia	None at present	60 - 90%
Alzheimer's	APOE4 testing?	60 - 80%
Height	Mid-parental height used to estimate expectation	55 - 81%
Multiple Sclerosis	None at present	64%
Migraine	Commonly assessed in the evaluation of headaches	53%
IQ	None at present	50%
Personality	None at present	50%
Breast cancer	Family history guides genetic testing and counseling	25 - 56%
Coronary Heart Disease	Used to inform models of cardiac risk	49%
Type 2 Diabetes	None at present	26%

Source: SNPedia

What are we trying to achieve?

- **Genetics** – strong contributor, causality can be established; 20,000 genes

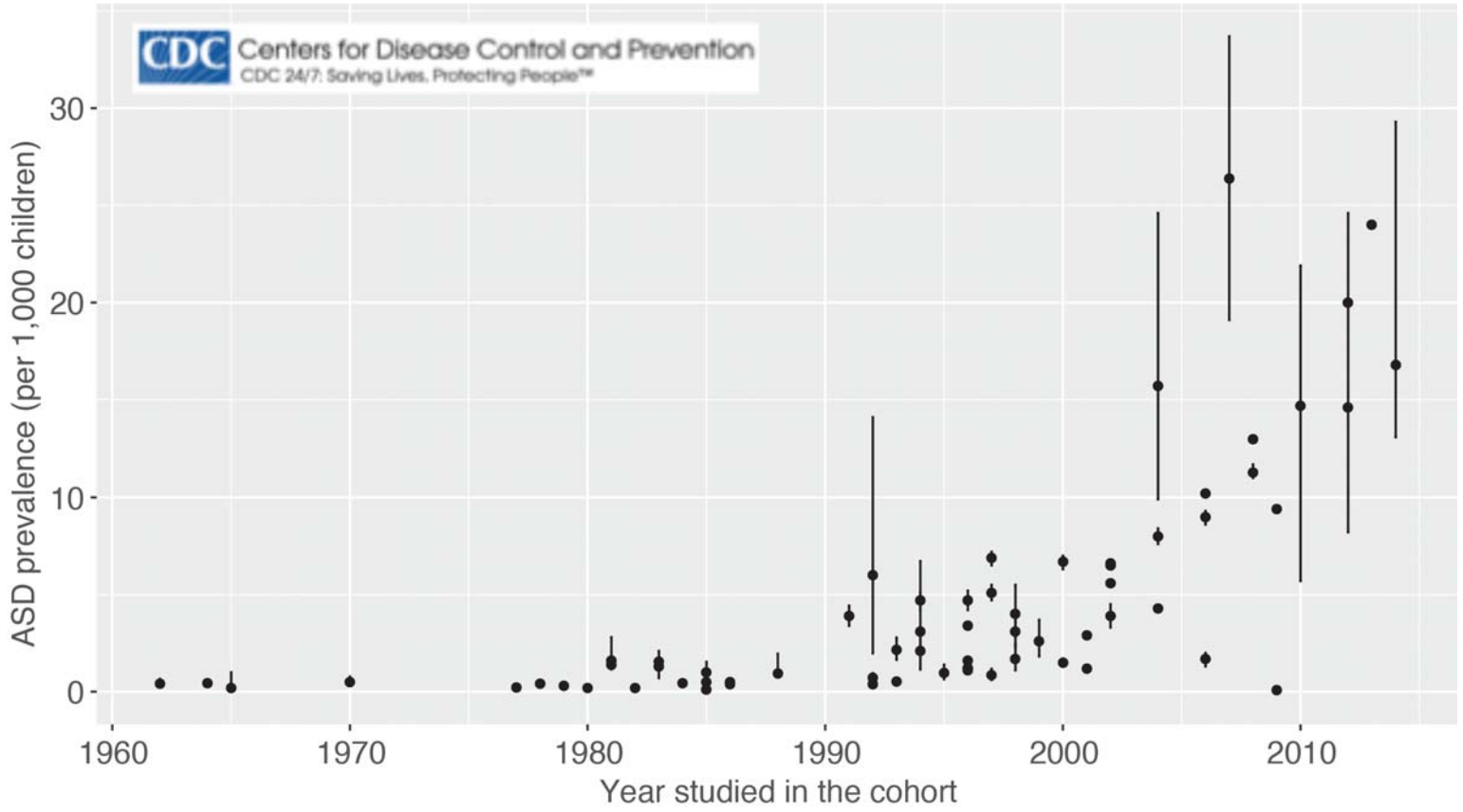
- **Environmental factors** – weaker contributor; causality is hard to establish; 100,000s of factors to assess



Environmental factors associated with ASD

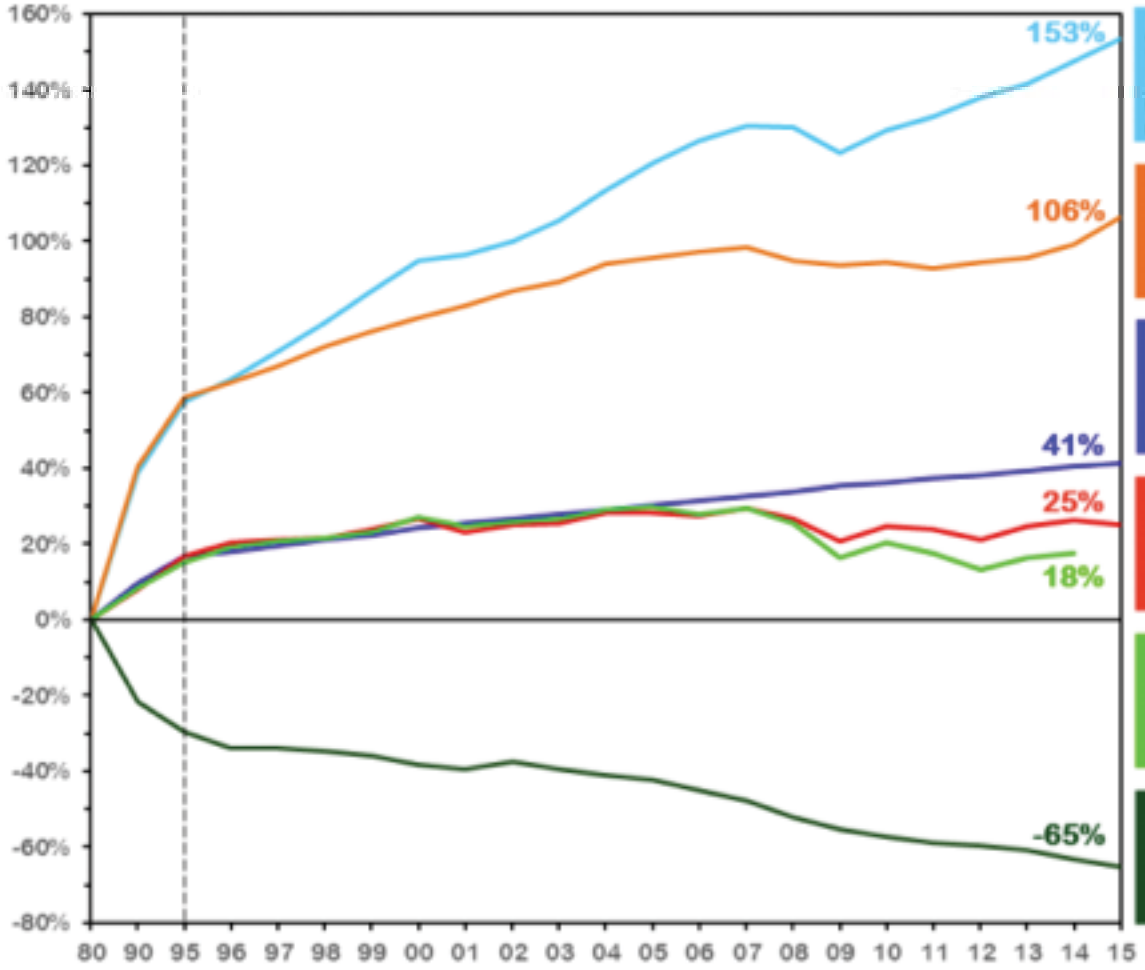
- Maternal valproate use for epilepsy during pregnancy
  - 2-fold increase in risk Christensen *et al. JAMA* 2013
  - Does epilepsy carry genetic risk for ASD, or valproate carry environmental risk?
    - 1,623 mothers with epilepsy (22 had children with ASD, 1.4%)
    - 388 mothers on valproate (12 had children with ASD, 3.1%)
    - But was there an additional reason the mothers were on valproate vs. other treatments?
  - Causality is hard to establish
- Many other factors considered, but few thoroughly assessed:
  - Congenital infection, pollutants, pesticides, heavy metals
- Very hard to “guess” what to study without knowing the biology

The prevalence of ASD has risen over the last few decades



Comparison of Growth Areas and Emissions, 1980-2015

EPA United States Environmental Protection Agency



Gross Domestic Product

Vehicle Miles Traveled

Population

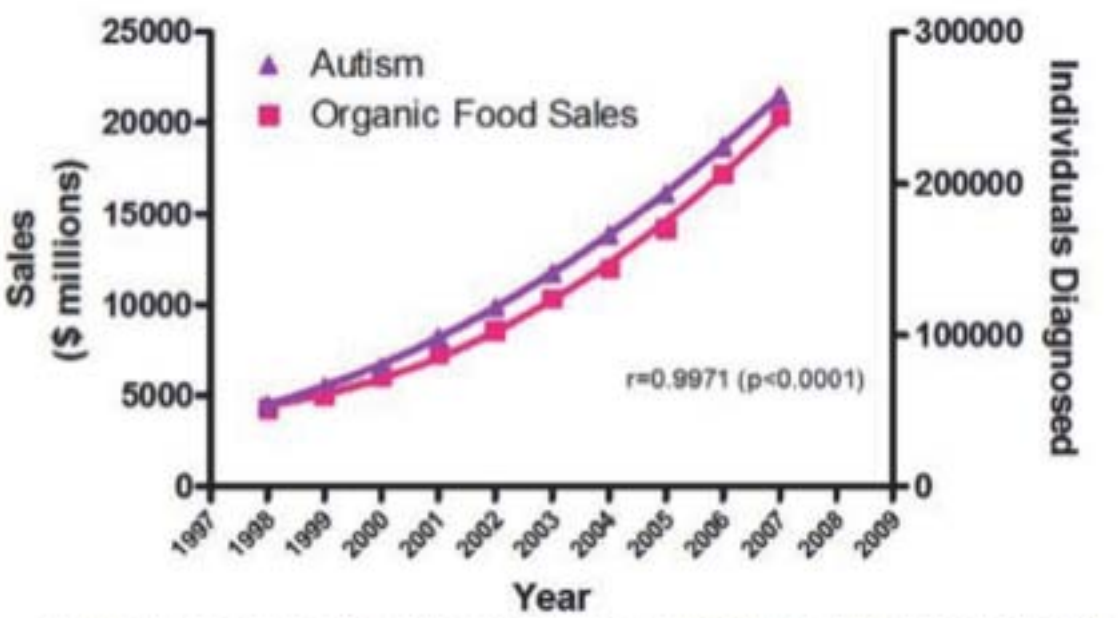
Energy Consumption

CO_2 Emissions

Aggregate Emissions (Six Common Pollutants)

There are many explanations for rising prevalence; simply looking for “new” factors is a risky strategy

- Changing diagnostic methods
- Replacing other diagnoses
  - Developmental delay
  - Intellectual disability
- Increased surveillance
- Rising parental age
- New ways to find a partner
- Environmental factors

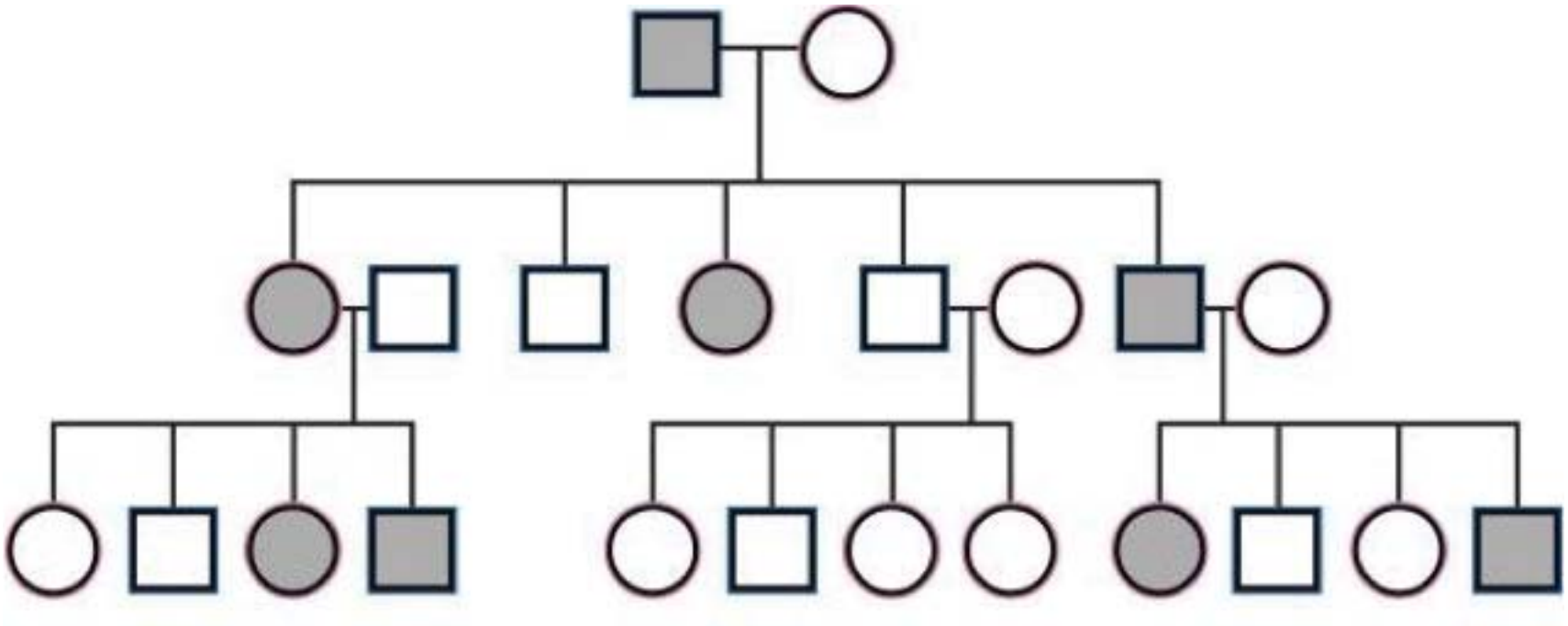


Sources: Organic Trade Association, 2011 Organic Industry Survey; U.S. Department of Education, Office of Special Education Programs, Data Analysis System (DANS), OMB# 1820-0043: "Children with Disabilities Receiving Special Education Under Part B of the Individuals with Disabilities Education Act

Genetic factors associated with ASD

- Several genetic syndromes have ASD as a feature
  - Fragile X, Rett's, TSC1, TSC2, NF1, NF2, PTEN, CACNA1C

Example of a family with neurofibromatosis (NF1) and autosomal dominant inheritance



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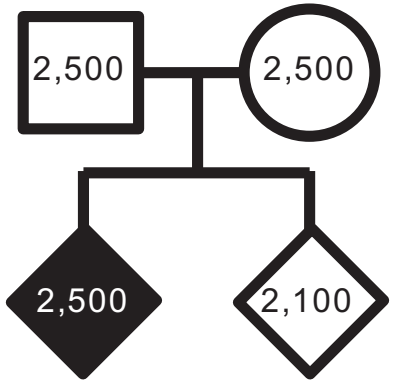
- Using exome sequencing, ASD-associated *de novo* loss of function mutations are found in ~7% of children with ASD (>10-fold increase in risk)
  - Sanders *et al. Nature* 2012, replicated in Iossifov *et al. Neuron* 2012, De Rubeis *et al. Nature* 2014, Iossifov *et al. Nature* 2014, Sanders *et al. Neuron* 2015, and many others

Large cohorts, new technologies, and new statistical approaches have revolutionized genetics

~2,500 ASD families in Simons Simplex Collection



SFARI SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE

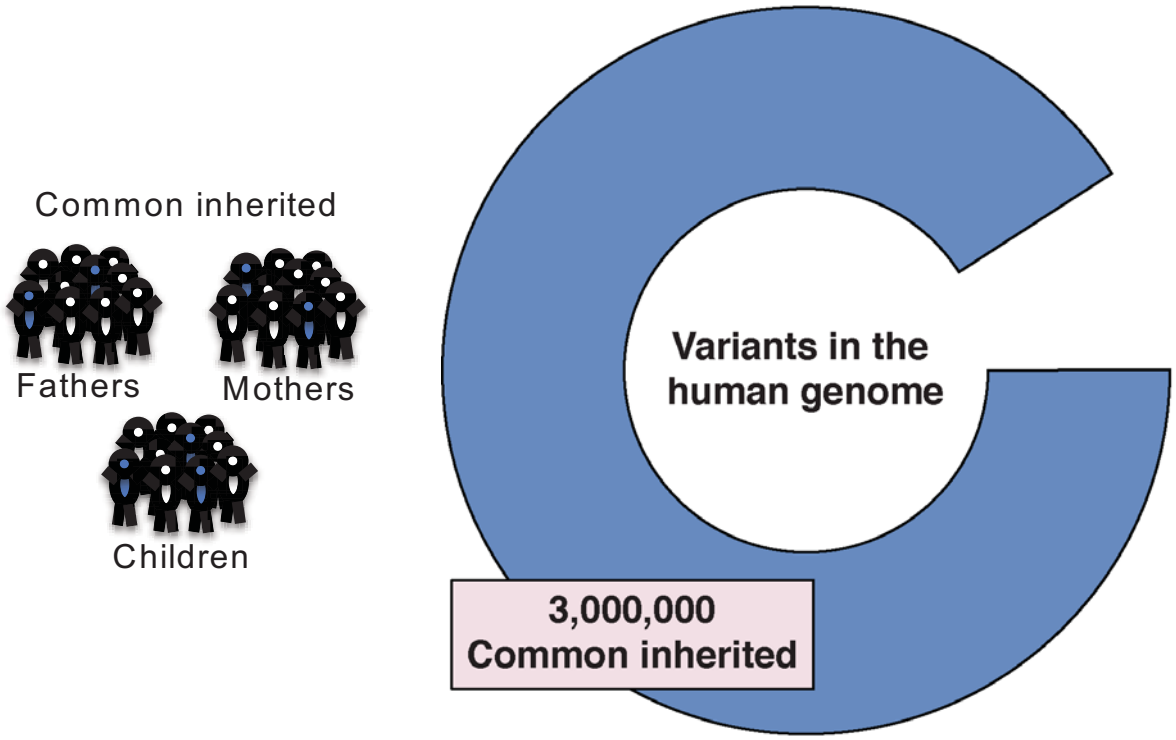
Microarray

illumina

Exome sequencing

illumina

The human genome has 3.2 billion base pairs and 3.3 million variants



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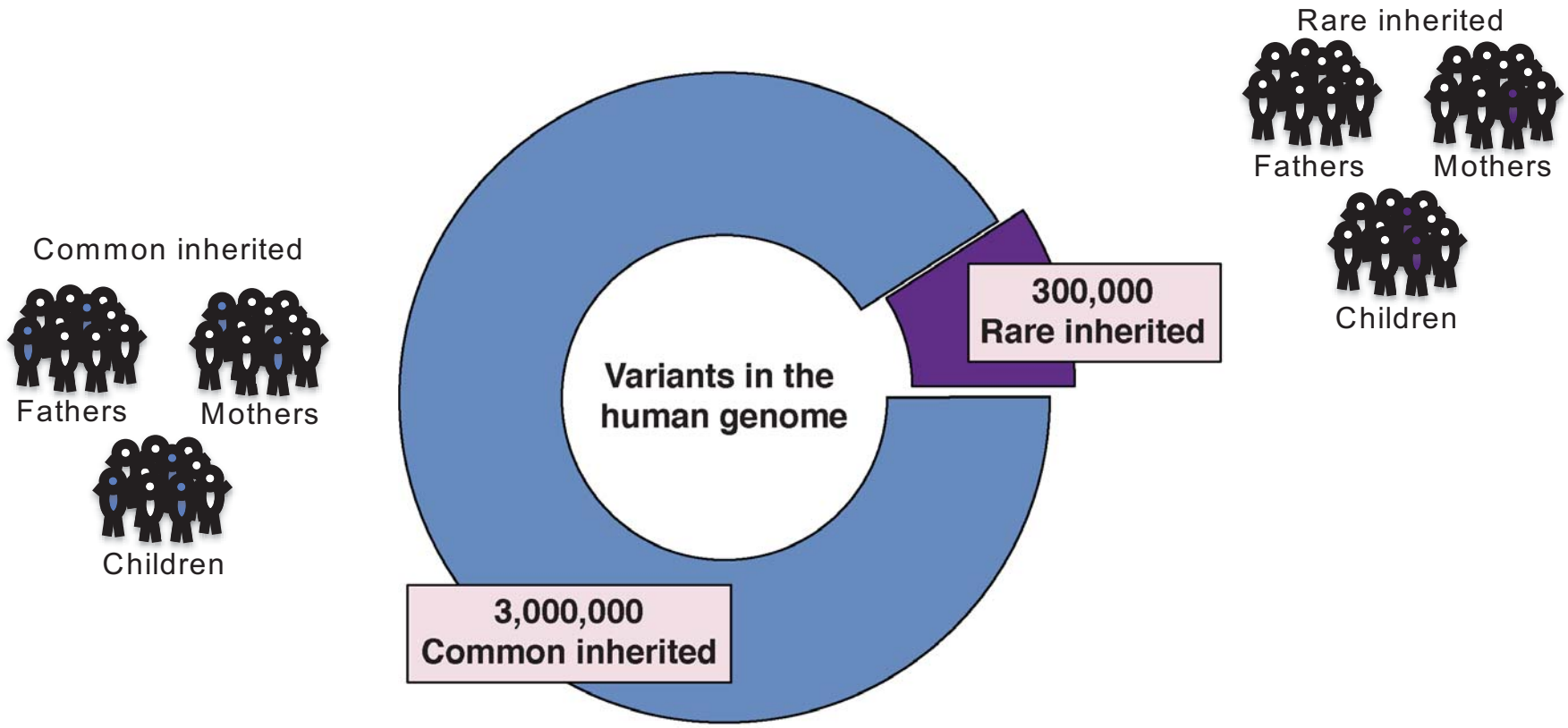

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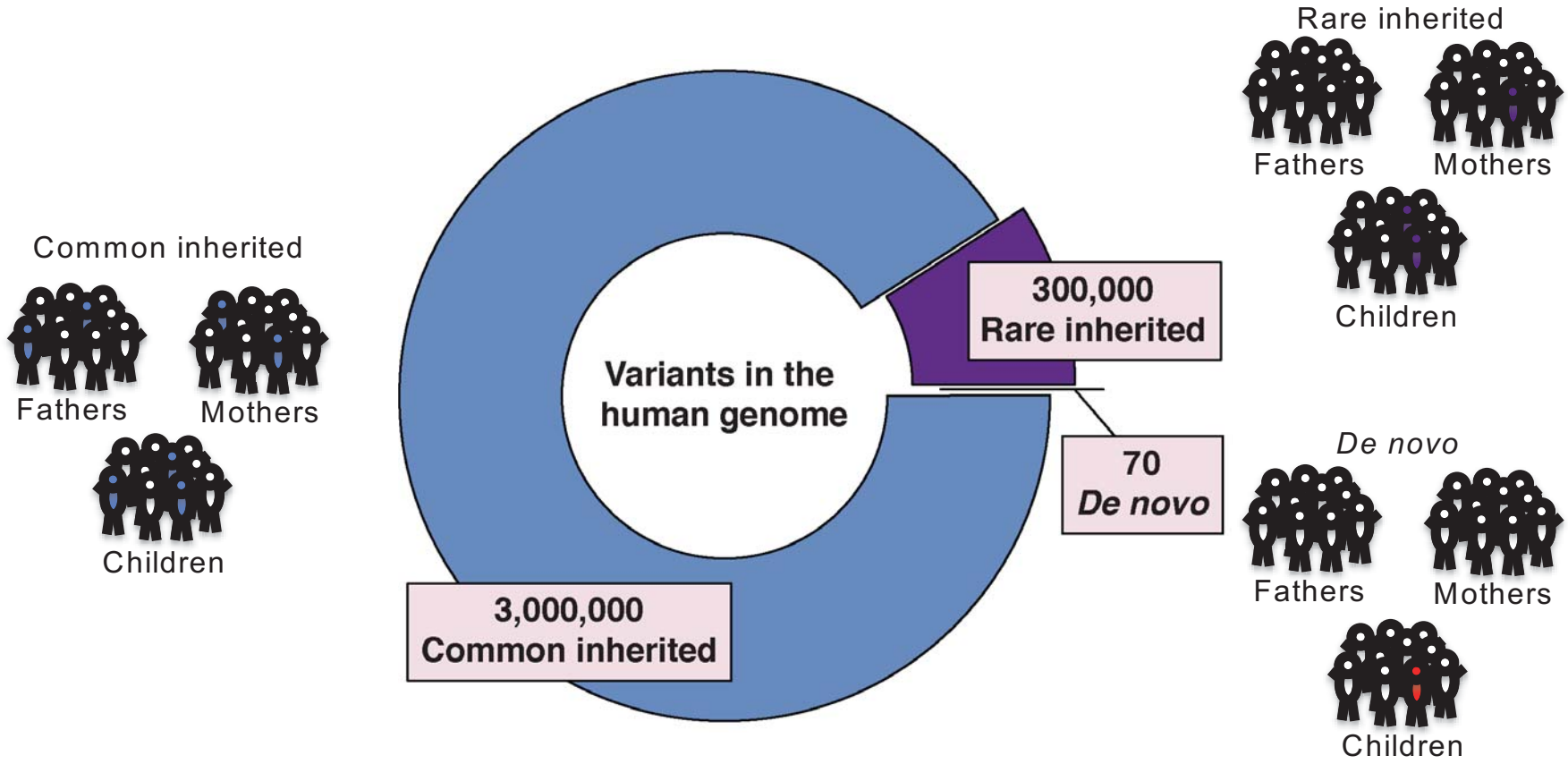
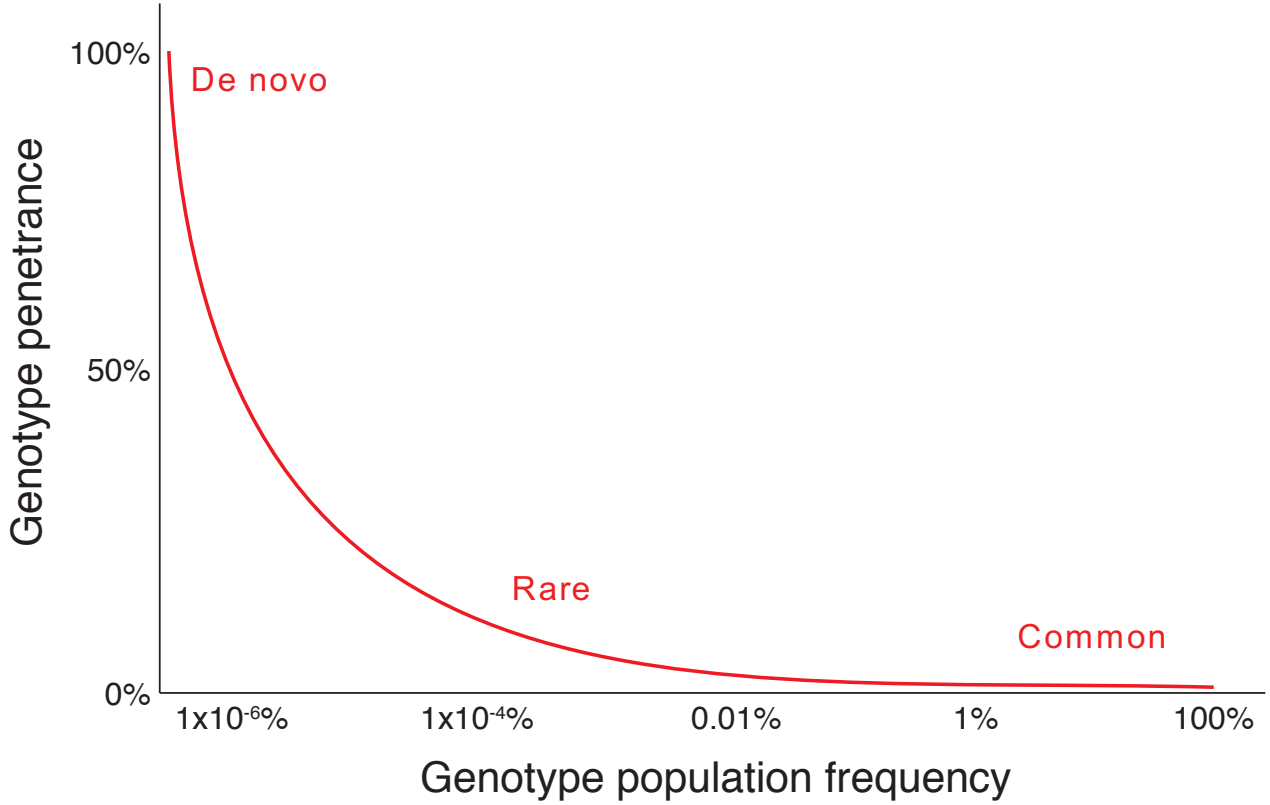
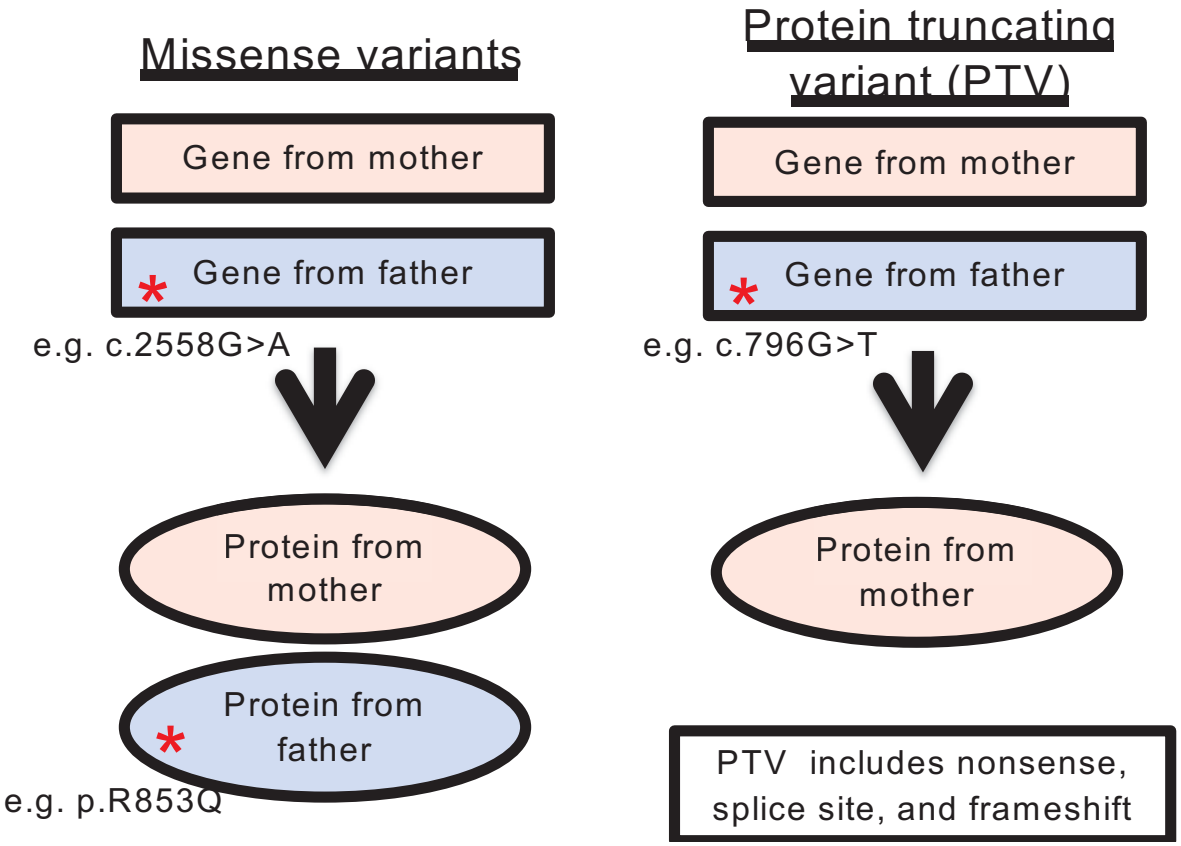


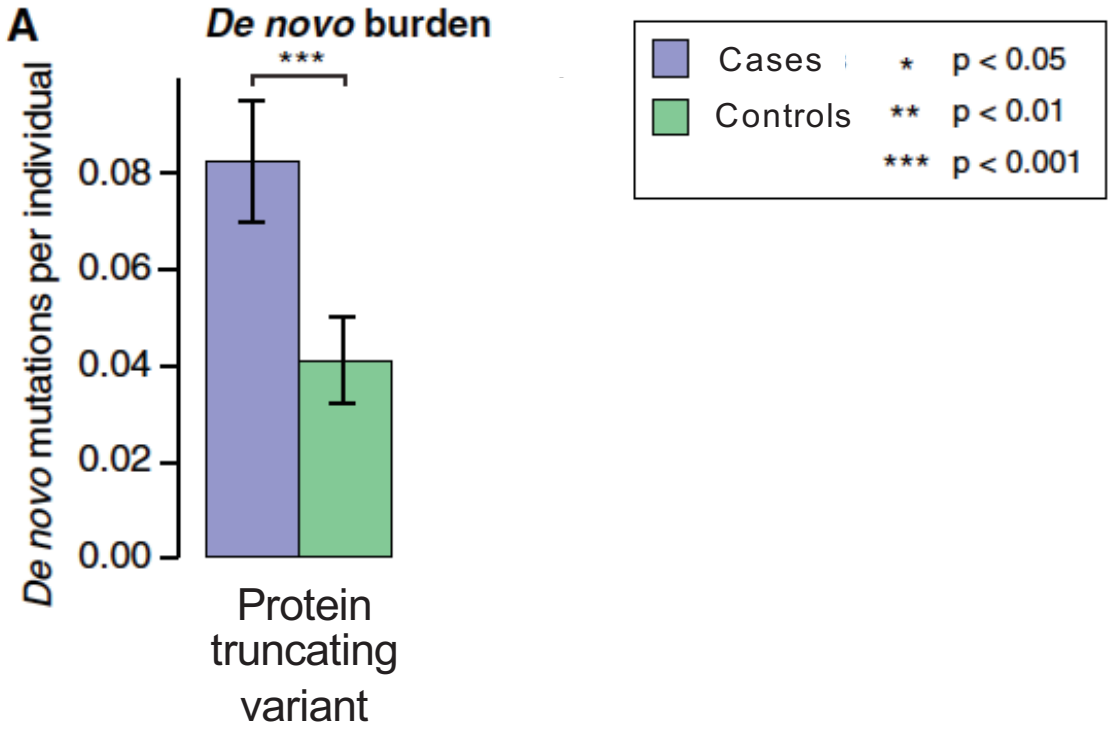
Rarer variants have greater potential to transmit ASD risk due to natural selection



Missense variants **alter** one copy of a protein, LoFs **disrupt** one copy of a protein



An excess of *de novo* protein truncating variants in ASD cases shows they contribute to ASD risk



Sanders et al, Neuron, 2015

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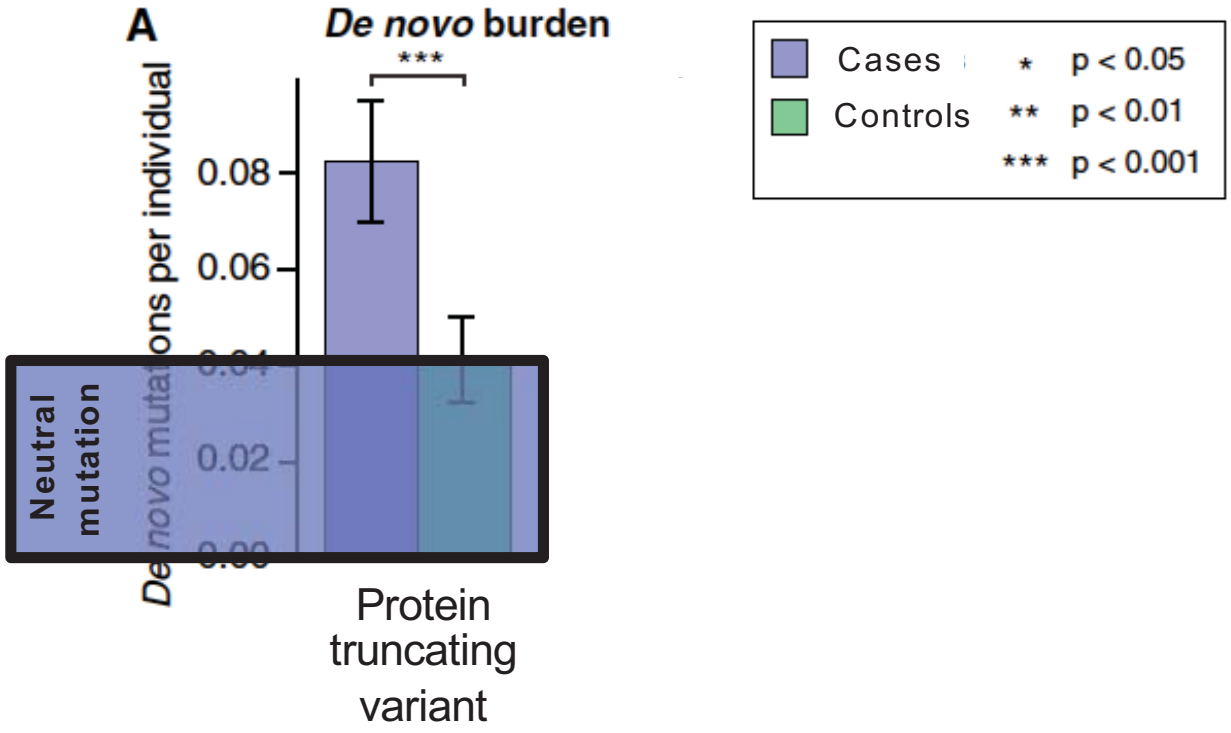

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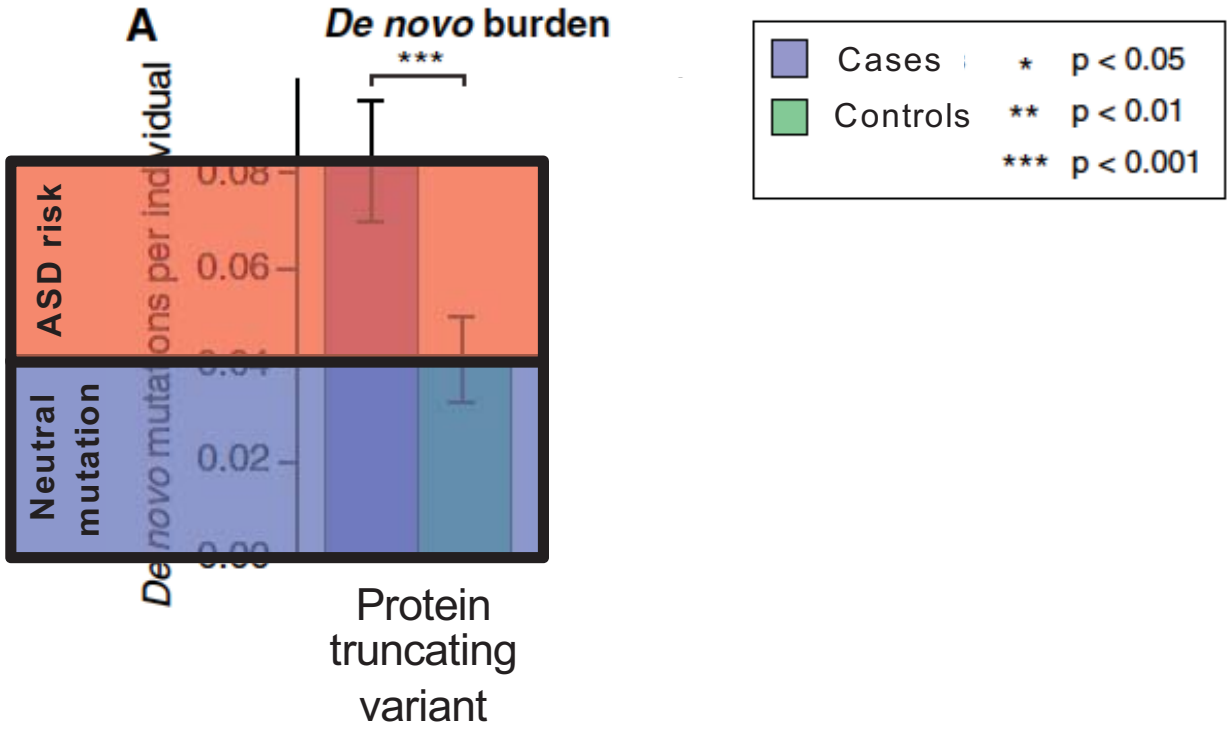

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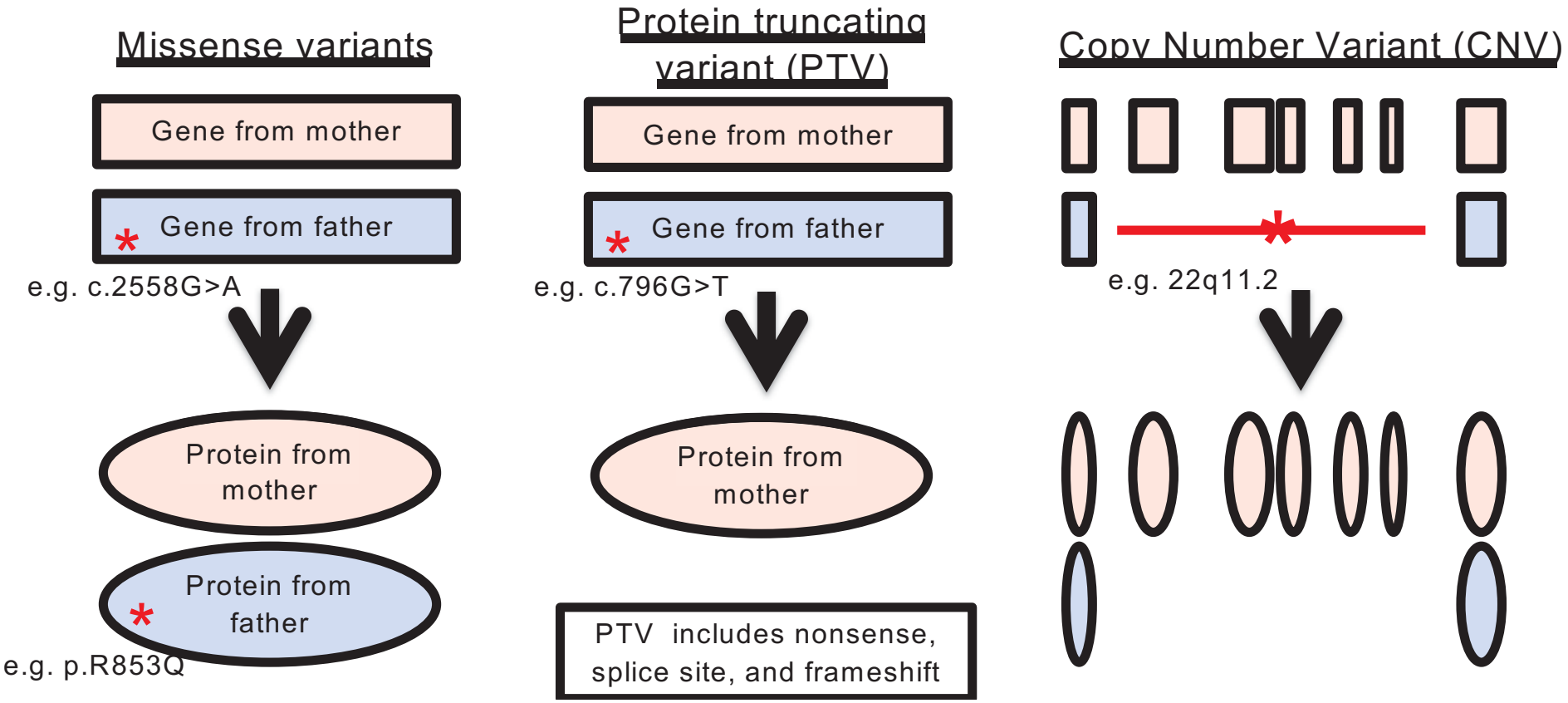
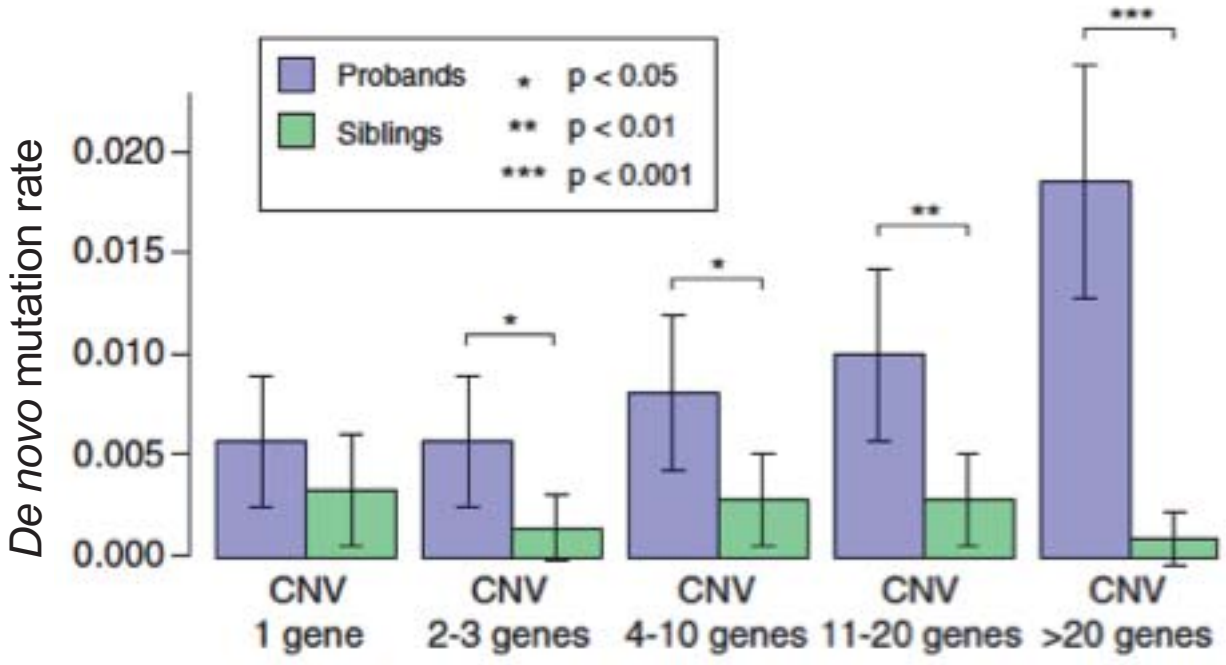
- Using gene microarrays, ASD-associated *de novo* CNVs are found in ~3% of children with ASD (>10-fold increase in risk)
  - Sebat *et al. Science* 2007, replicated in Pinto *et al. Nature* 2010, Sanders *et al. Neuron* 2011, Pinto *et al. AJHG* 2014, Sanders *et al. Neuron* 2015 (over 4,000 cases), and many others

Missense variants **alter** one copy of a protein, LoFs **disrupt** one copy of a protein, copy number variants (CNVs) **disrupt** one copy of multiple proteins



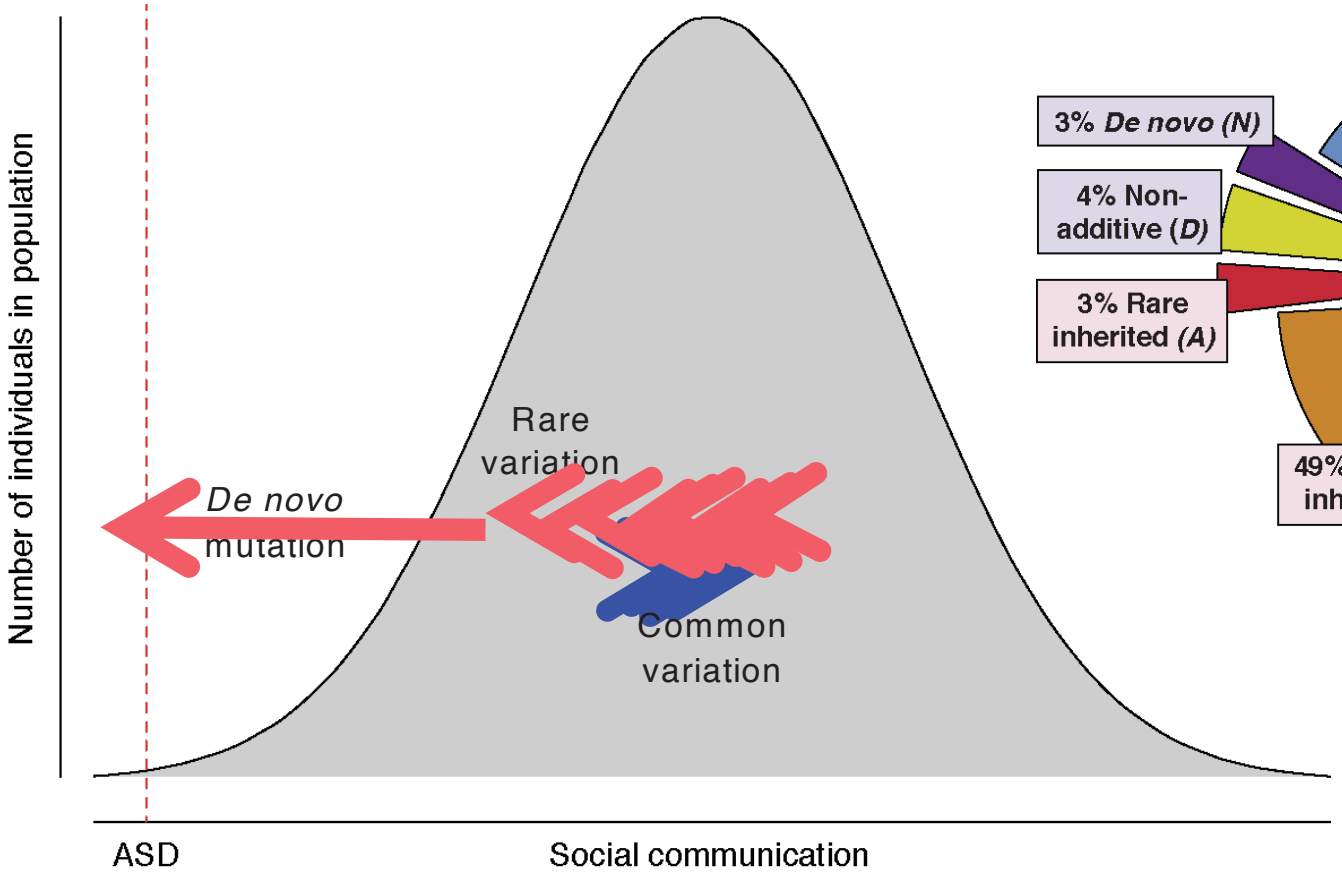
Individuals with a diagnosis of ASD have more *de novo* CNVs than unaffected controls

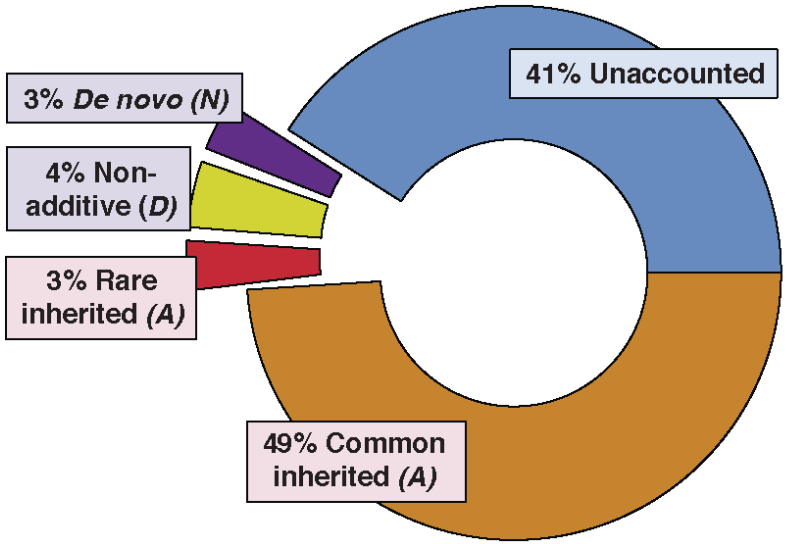
1,991 ASD cases and 1,991 unaffected sibling controls



Sanders *et al. Neuron* 2015

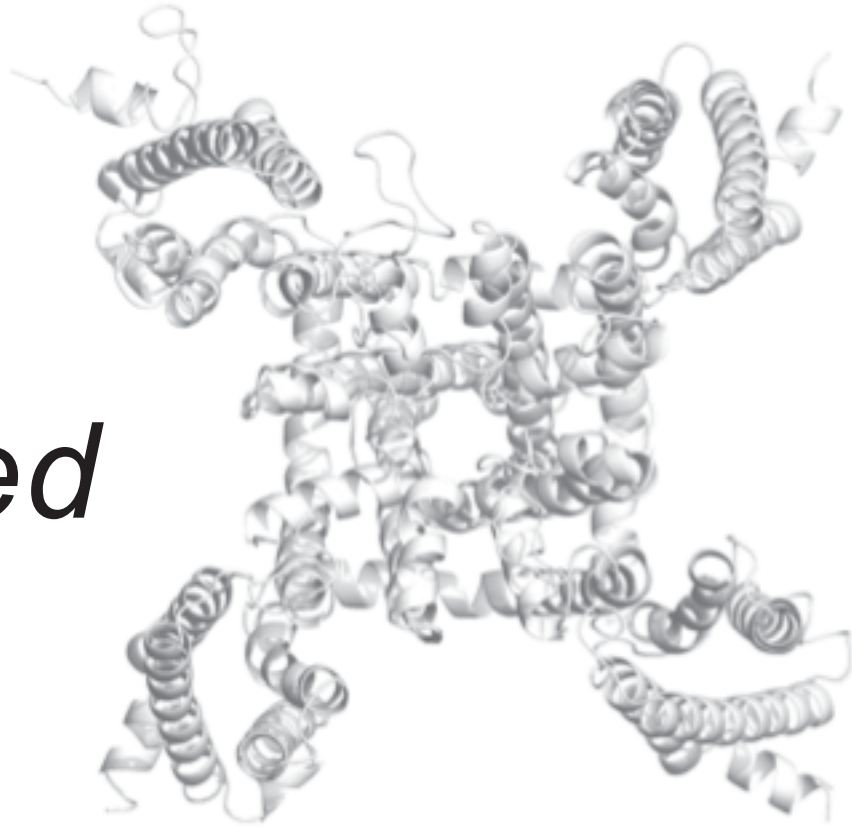
ASD is a frequently a combination of *de novo*, rare, and common variants





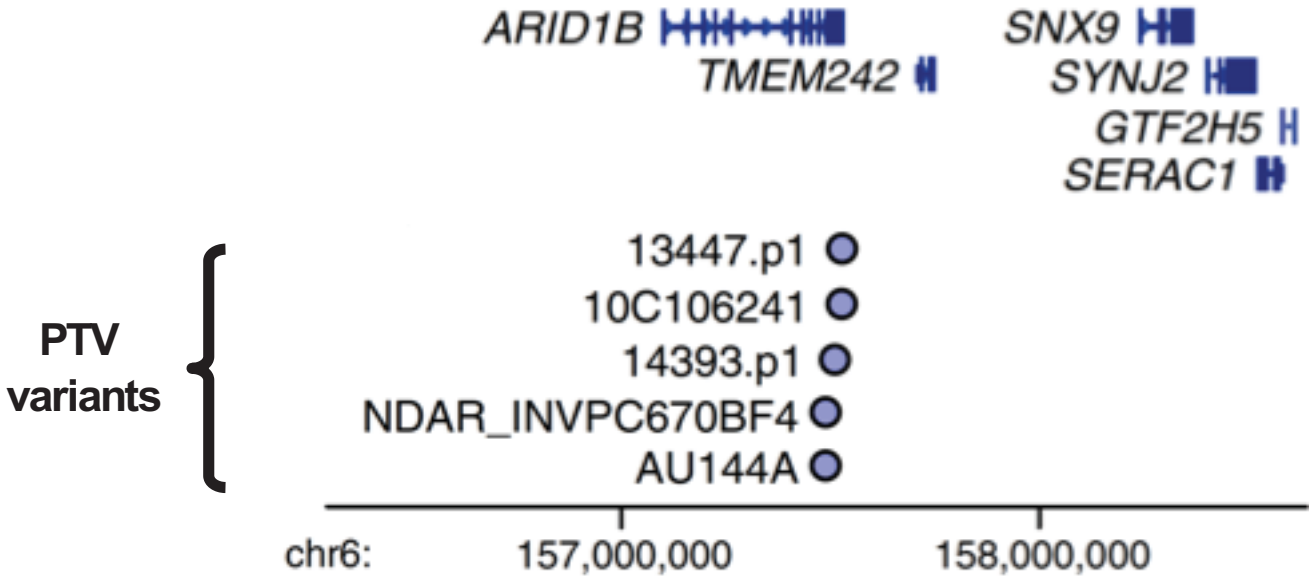
Gaulger *et al.* 2014

Finding genes linked to ASD



Nav1.2
structure from
AJ Campbell,
Broad

To distinguish risk mutations from neutral mutations, we identify genes with clusters of mutations



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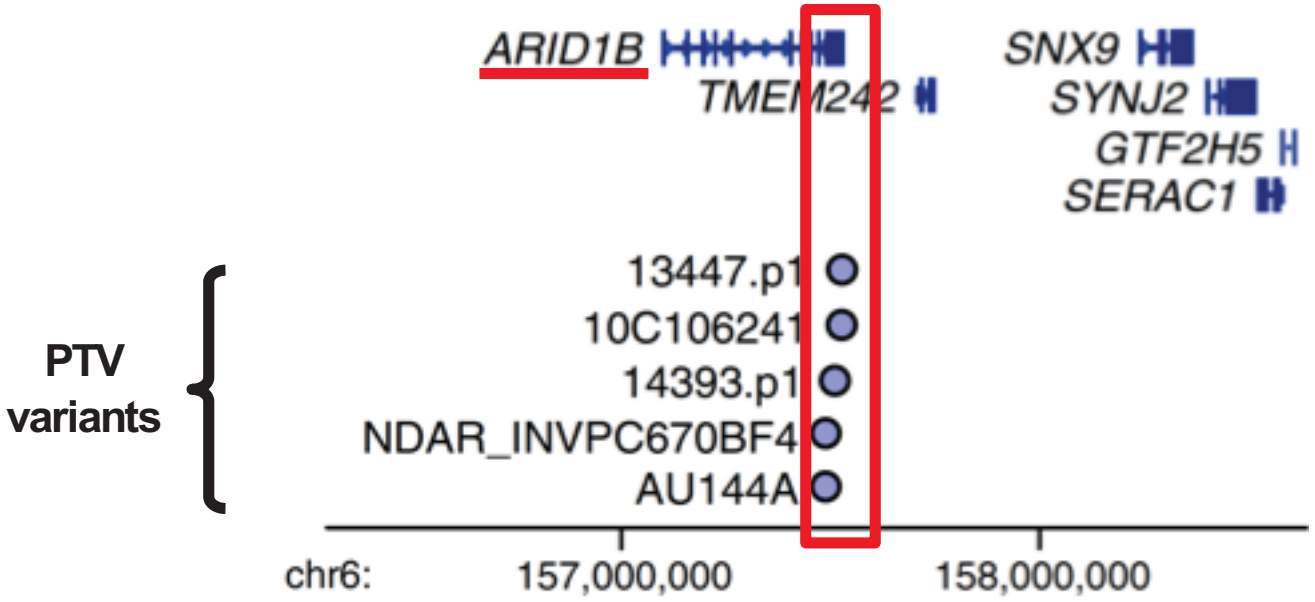

Sanders et al. *Neuron* 2015

Assessment of ~8,000 ASD cases has identified 65 ASD risk genes: a constellation of cryptic syndromes

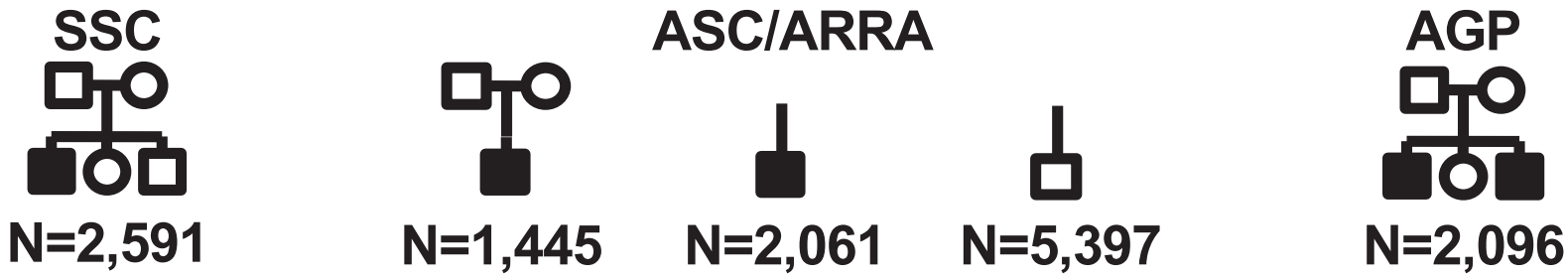

Table 4. Integrating Small De Novo Deletions in TADA Identified 65 ASD Genes

dnLoF Count	FDR ≤ 0.01	0.01 < FDR ≤ 0.05	0.05 < FDR ≤ 0.1
≥2	*ADNP*, *ANK2*, ***ARID1B***, *ASH1L*, ***CHD2***, *CHD8*, *CUL3*, *DSCAM*, *DYRK1A*, *GRIN2B*, *KATNAL2*, *KDM5B*, ***KMT2C***, *NCKAP1*, *POGZ*, *SCN2A*, *SUV420H1*, ***SYNGAP1***, *TBR1*, ***TCF7L2***, ***TNRC6B***, *WAC*	*BCL11A*, *FOXP1*, *GIGYF1*, *ILF2*, *KDM6B*, *PHF2*, *RANBP17*, *SPAST*, *WDFY3*	*DIP2A*, *KMT2E*
1	***NRXN1***, *PTEN*, ***SETD5***, ***SHANK2***, ***SHANK3***, ***TRIP12***	*DNMT3A*, *GABRB3*, ***KAT2B***, *MFRP*, *MYT1L*, *P2RX5*	*AKAP9*, *APH1A*, *CTTNBP2*, *ERBB2IP*, *ETFB*, *INTS6*, *IRF2BPL*, ***MBD5***, *NAA15*, *NINL*, *OR52M1*, *PTK7*, *TRIO*, *USP45*
0	–	*MIB1*, *SLC6A1*, *ZNF559*	*ACHE*, *CAPN12*, ***NLGN3***

Genes with a small de novo deletion are in bold. FDR, false discovery rate.

Sanders *et al.* Neuron, 2015

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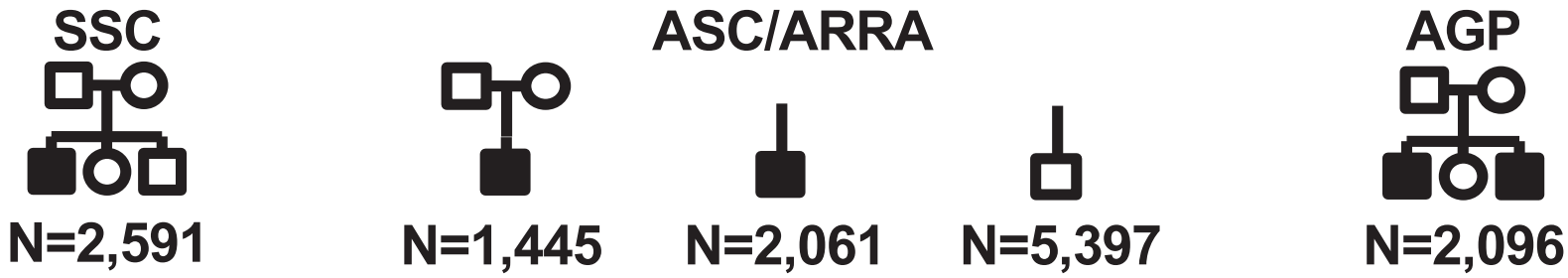

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1	***NRXN1****, PTEN,* ***SETD5****,* ***SHANK2****,* ***SHANK3****,* ***TRIP12***	*DNMT3A, GABRB3,* ***KAT2B****, MFRP, MYT1L, P2RX5*	*AKAP9, APH1A, CTTNBP2, ERBB2IP, ETFB, INTS6, IRF2BPL,* ***MBD5****, NAA15, NINL, OR52M1, PTK7, TRIO, USP45*
0	–	*MIB1, SLC6A1, ZNF559*	*ACHE, CAPN12,* ***NLGN3***

Genes with a small de novo deletion are in bold. FDR, false discovery rate.

Sanders *et al.* Neuron, 2015

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Review

Progress in Understanding and Treating *SCN2A*-Mediated Disorders

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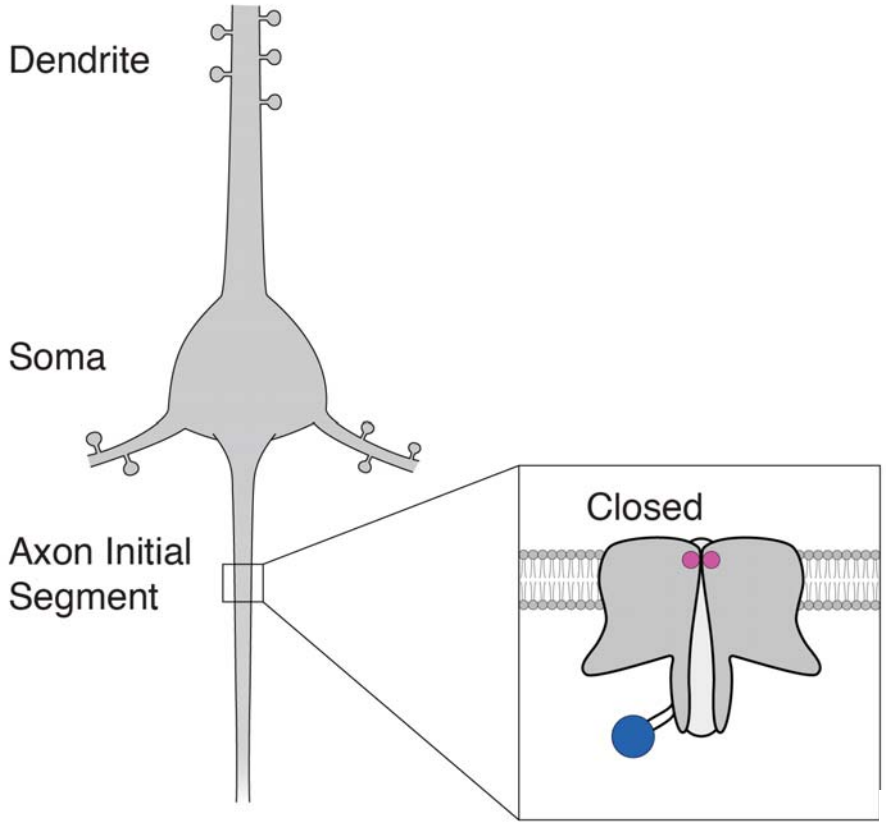
FAMILIESCN2A FOUNDATION

***SCN2A* in Neurodevelopmental Disorders**

CellPress REVIEWS

SIMONS VIP
Simons Variation in Individuals Project

SCN2A/$Na_V1.2$ initiates the action potential at the axon initial segment



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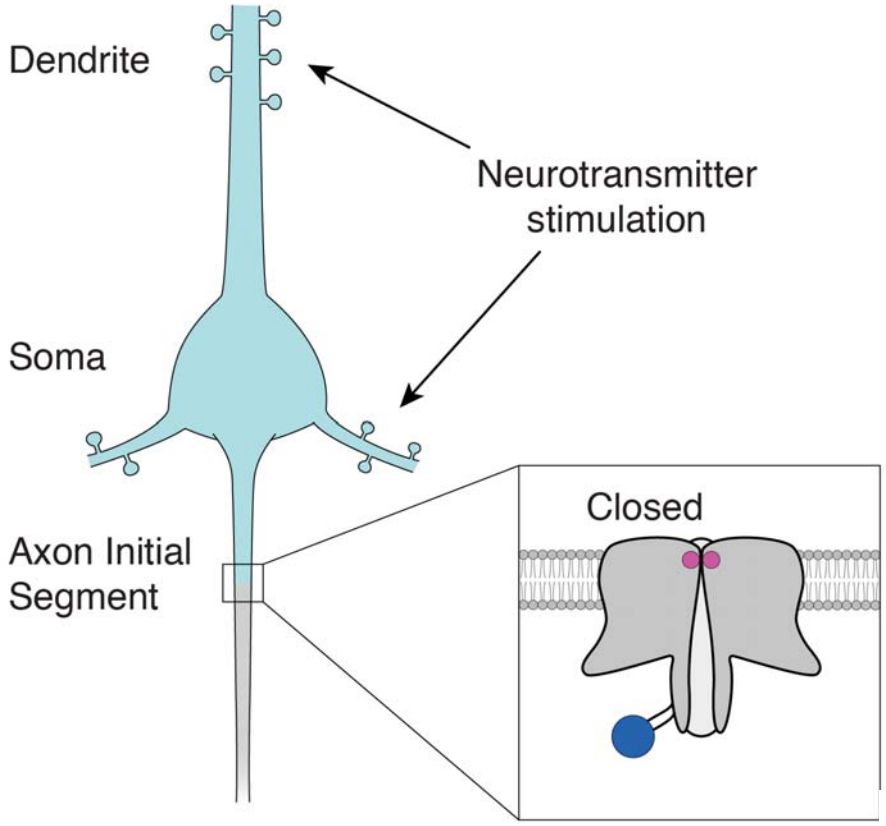

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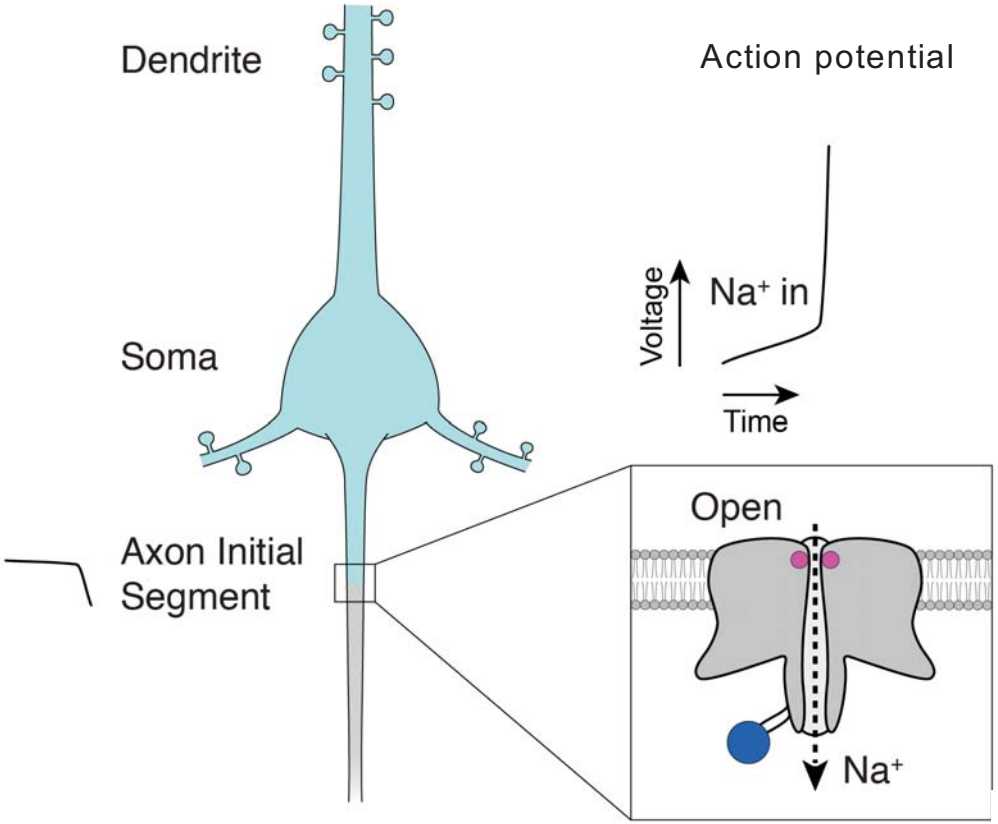

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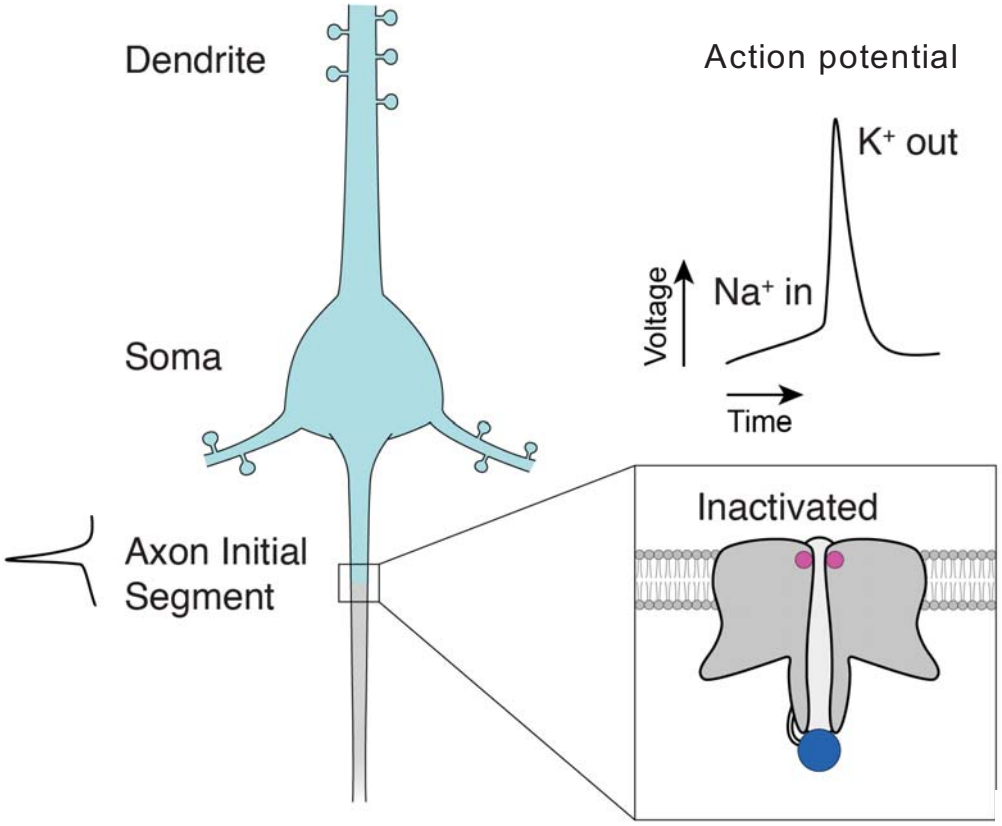

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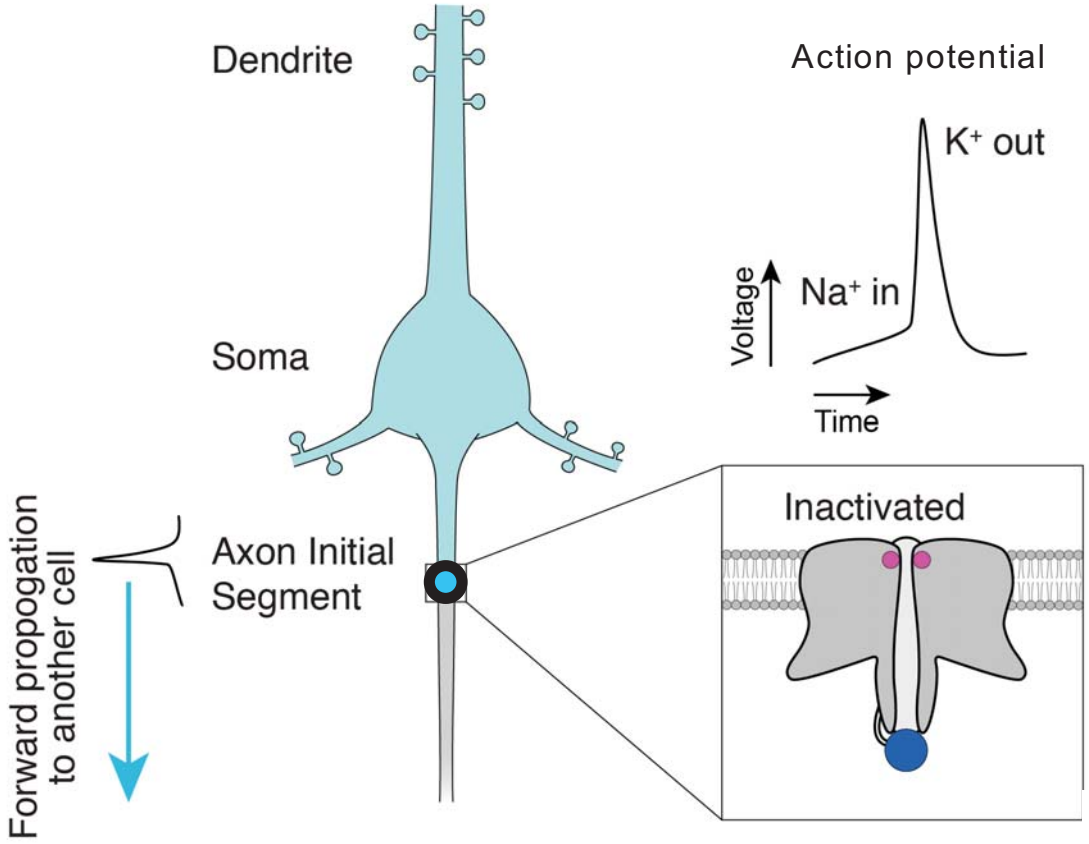

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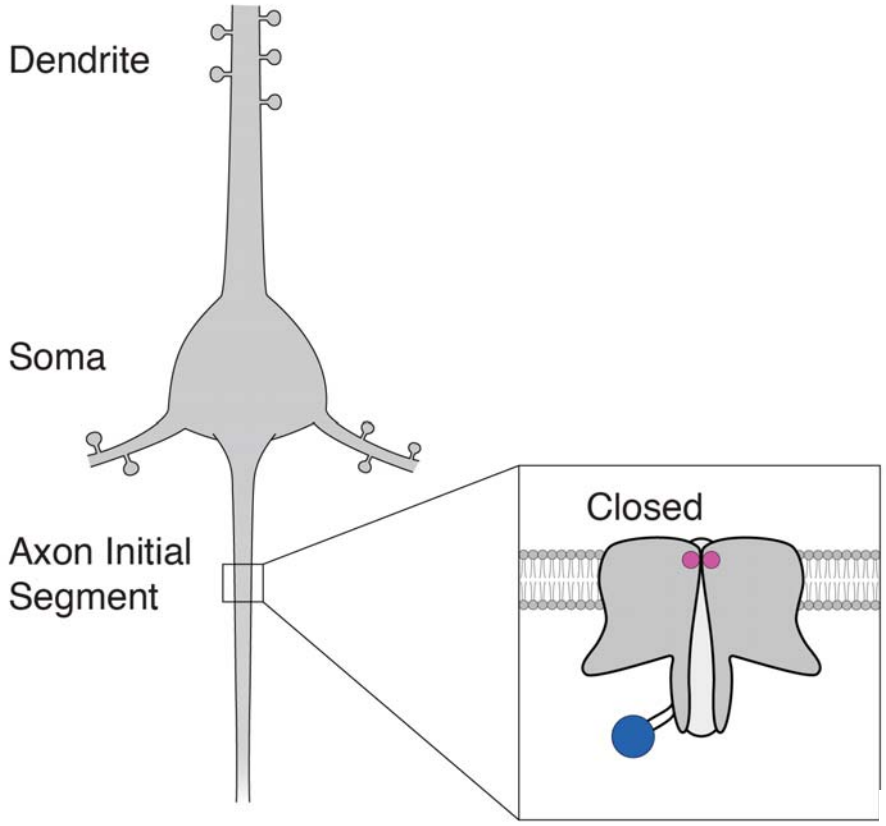

Along with ASD, genetic variants in *SCN2A* are associated with three disorders

	Disorder name	Infantile Seizures (<12mths)	Ongoing seizures (>2yrs)	Developmental delay
BIS	Benign (familial) infantile seizures	Y	N	N
IEE	Infantile epileptic encephalopathy	Y	Y	Y
ASD/ID	Autism Spectrum Disorder/ID	N	~25%	Y

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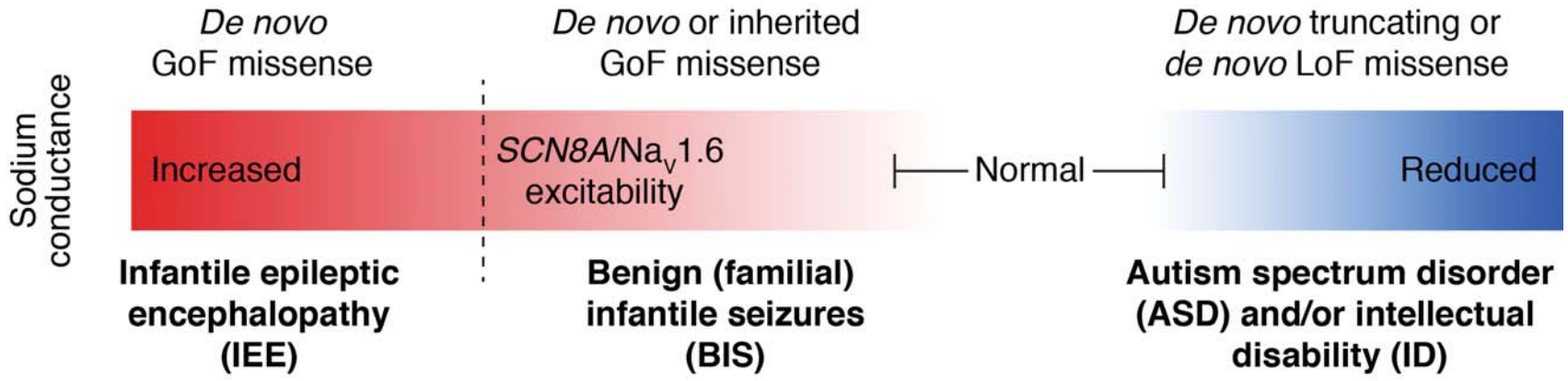
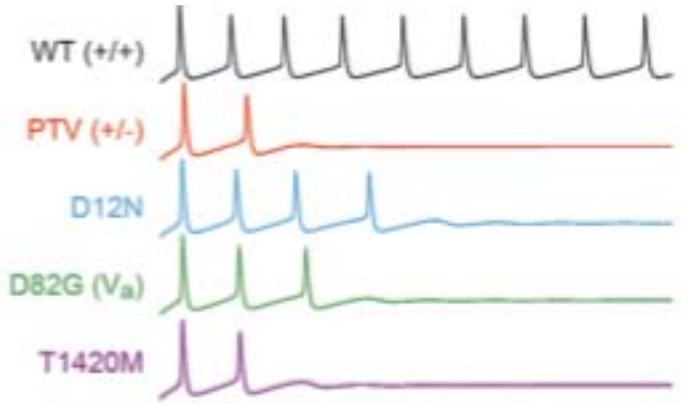
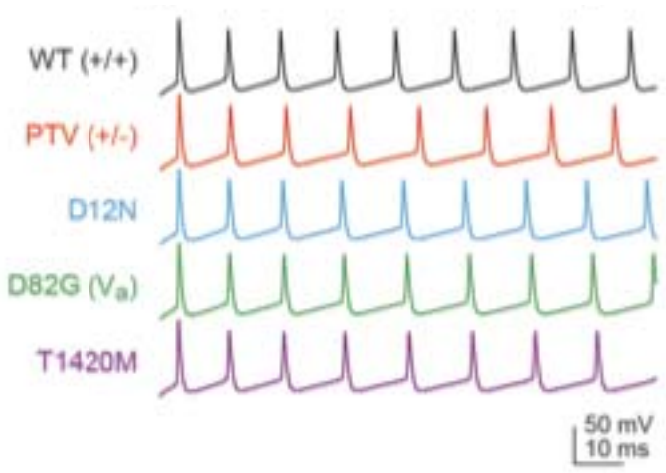


Loss of function in one copy of *SCN2A* makes excitatory neurons less excitable during development

Before 1yr of age



After 1yr of age

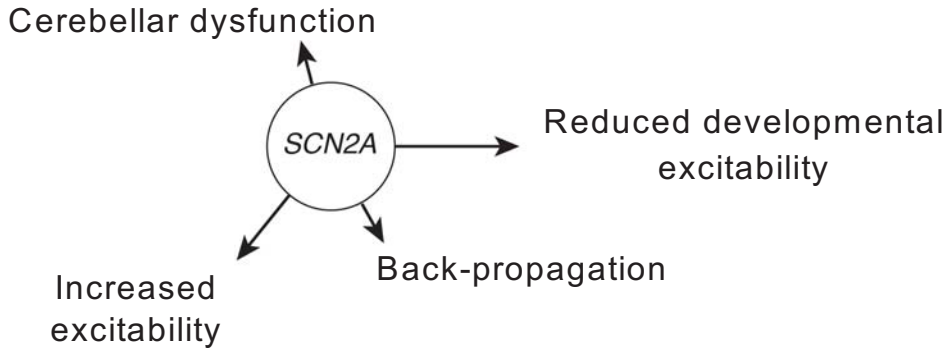


Ben-Shalom et al., 2017

Understanding the role of one gene provides some insight into when and how ASD occurs

- A 50% reduction in *SCN2A* function leads to ASD
- This implicates excitatory neurons in ASD
- It suggests that a reduction in neuron excitability may be involved
- It suggests that the “cause” of ASD occurs before 1yr of age

Since each gene has multiple functions, how do we know which functions lead to specific symptoms?



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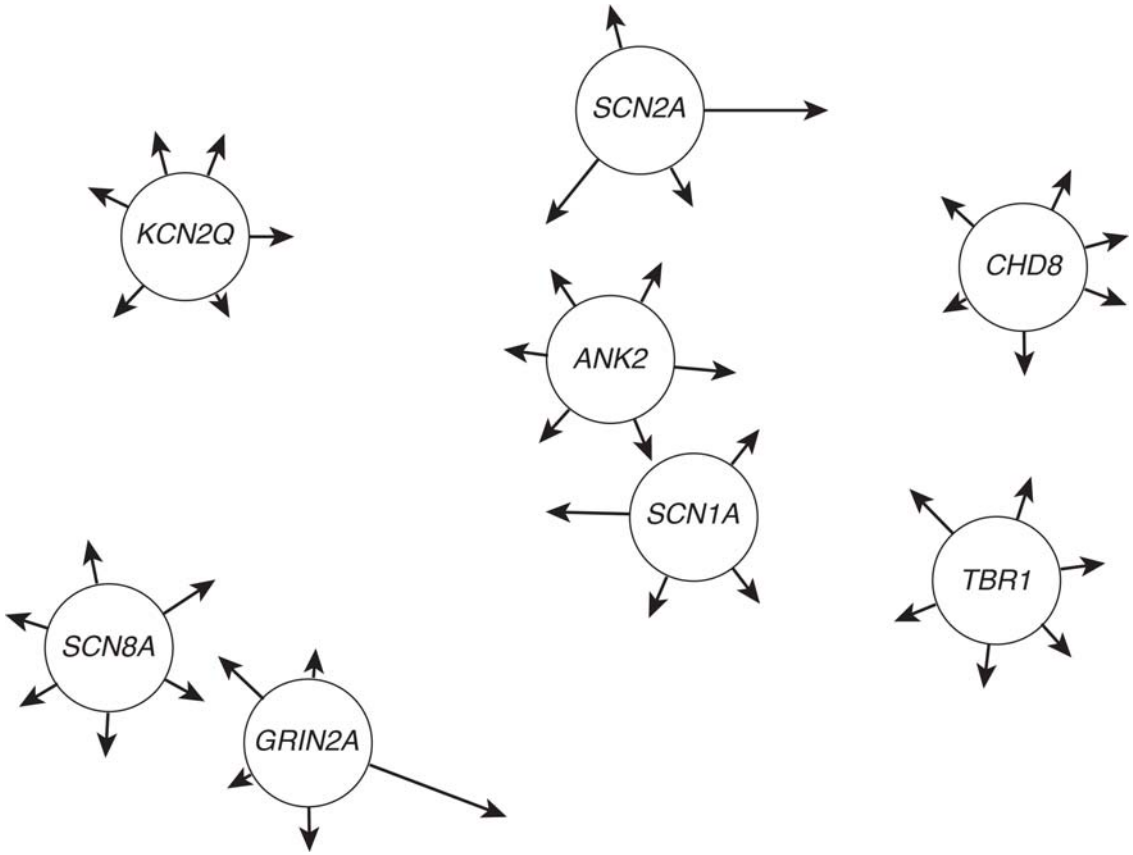

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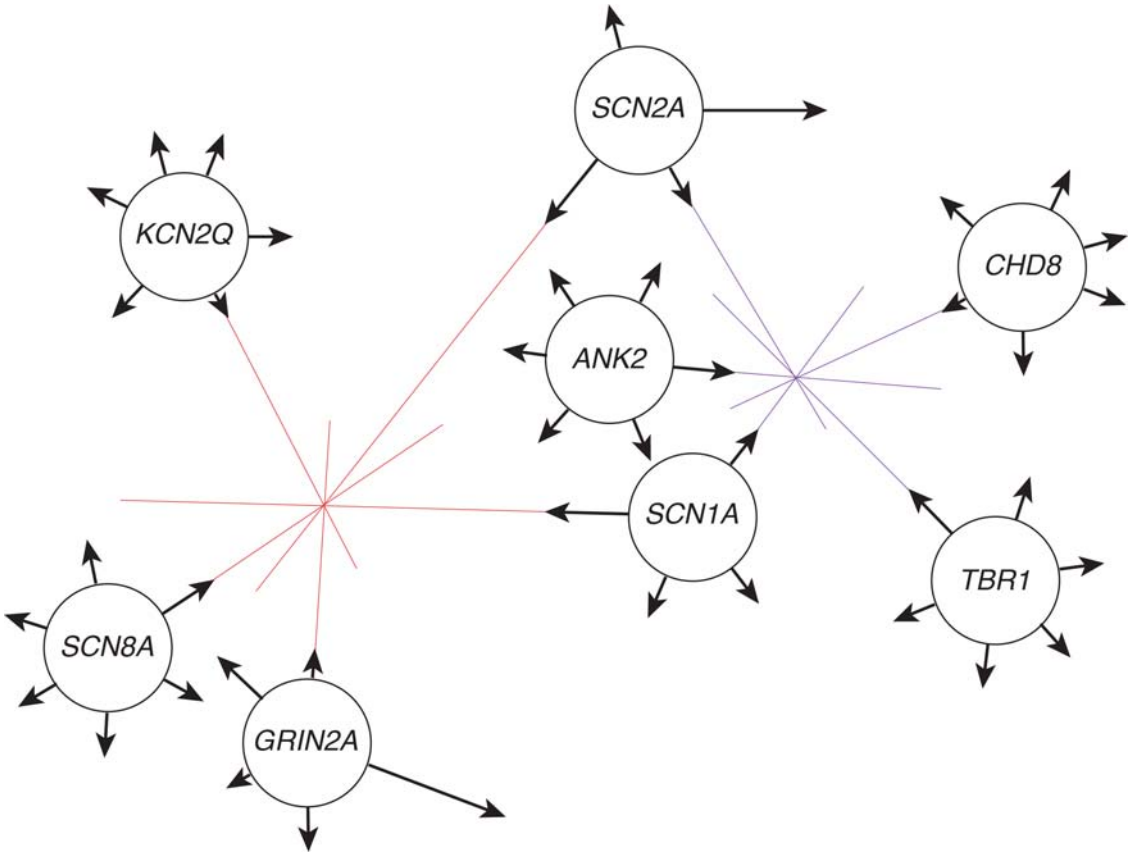

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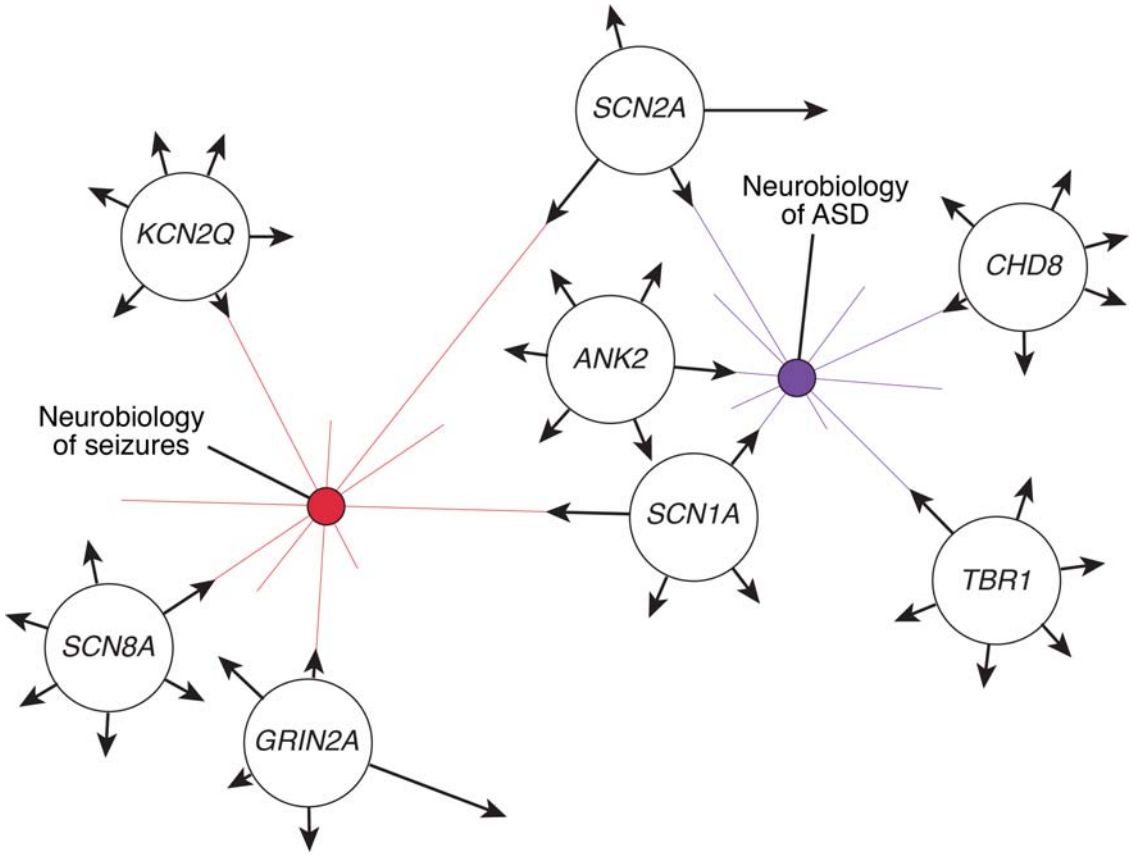


Assessment of ~8,000 ASD cases has identified 65 ASD risk genes: a constellation of cryptic syndromes

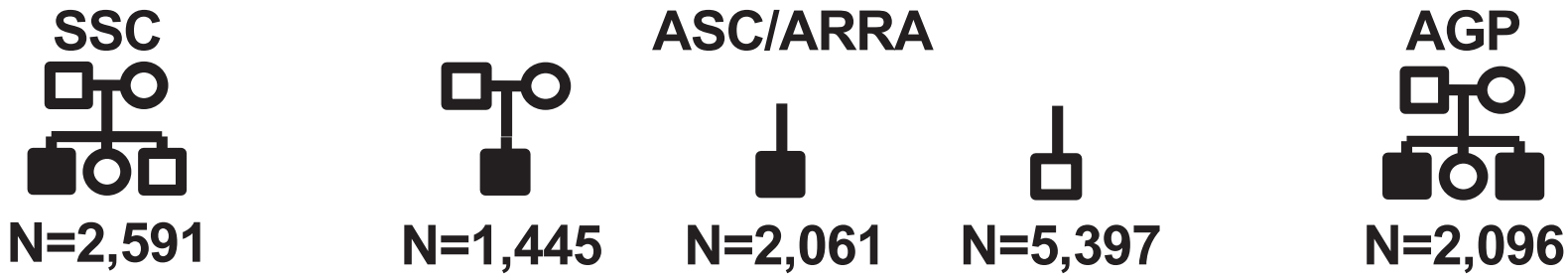


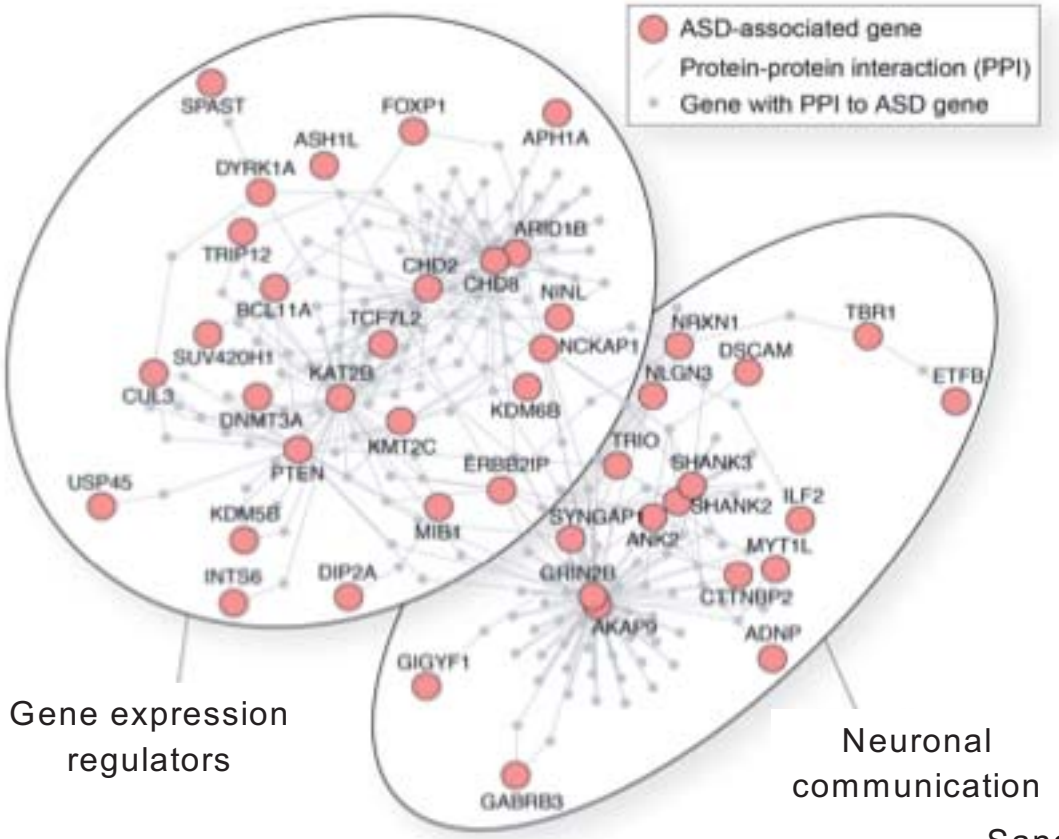
Table 4. Integrating Small De Novo Deletions in TADA Identified 65 ASD Genes

dnLoF Count	FDR ≤ 0.01	0.01 < FDR ≤ 0.05	0.05 < FDR ≤ 0.1
≥2	*ADNP*, *ANK2*, ***ARID1B***, *ASH1L*, ***CHD2***, *CHD8*, *CUL3*, *DSCAM*, *DYRK1A*, *GRIN2B*, *KATNAL2*, *KDM5B*, ***KMT2C***, *NCKAP1*, *POGZ*, *SCN2A*, *SUV420H1*, ***SYNGAP1***, *TBR1*, ***TCF7L2***, ***TNRC6B***, *WAC*	*BCL11A*, *FOXP1*, *GIGYF1*, *ILF2*, *KDM6B*, *PHF2*, *RANBP17*, *SPAST*, *WDFY3*	*DIP2A*, *KMT2E*
1	***NRXN1***, *PTEN*, ***SETD5***, ***SHANK2***, ***SHANK3***, ***TRIP12***	*DNMT3A*, *GABRB3*, ***KAT2B***, *MFRP*, *MYT1L*, *P2RX5*	*AKAP9*, *APH1A*, *CTTNBP2*, *ERBB2IP*, *ETFB*, *INTS6*, *IRF2BPL*, ***MBD5***, *NAA15*, *NINL*, *OR52M1*, *PTK7*, *TRIO*, *USP45*
0	–	*MIB1*, *SLC6A1*, *ZNF559*	*ACHE*, *CAPN12*, ***NLGN3***

Genes with a small de novo deletion are in bold. FDR, false discovery rate.

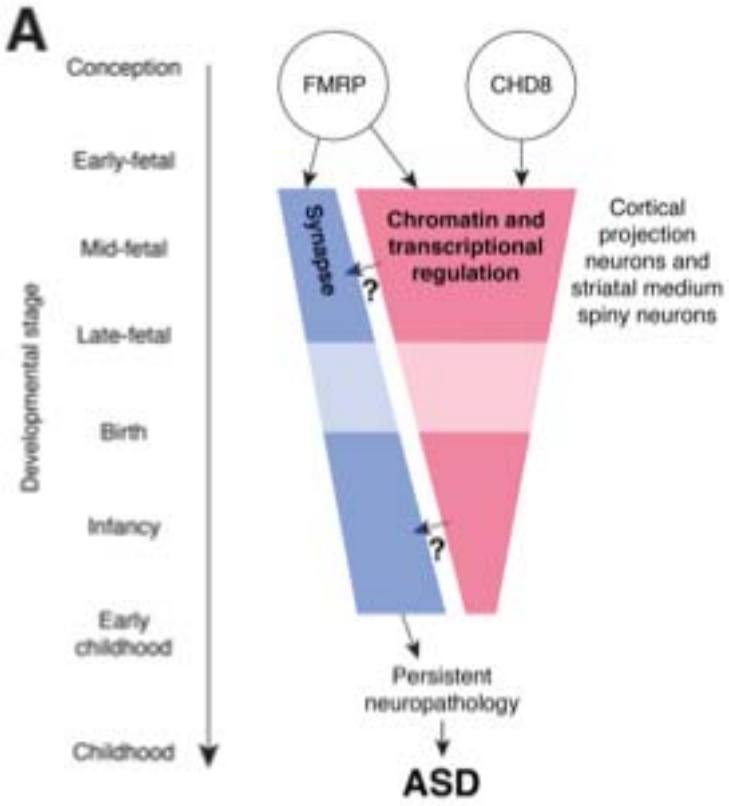
Sanders *et al.* Neuron, 2015

The 65 ASD risk genes converge on chromatin and synaptic networks



Sanders *et al.* Neuron, 2015

By considering the ASD-associated genes alongside other datasets, we can start to understand the 20,000ft view



BRAINSPAN
ATLAS OF THE DEVELOPING HUMAN BRAIN

PsychENCODE Knowledge Portal

GTEx Portal

ENCODE: Encyclopedia of DNA Elements

Sanders SJ *Curr Opin Genet Dev* 2015

Considering multiple genes in together also provides insight into when and how ASD occurs

- Two main groups of ASD-associated genes involved in:
  - Gene expression regulation
  - Neuronal communication
- Implicates prefrontal cortex in mid-fetal development
- Enriched for excitatory neurons and striatal neurons

Summary

- There is strong evidence that genetic factors play a role in ASD
  - Twin studies
  - Family studies
  - Syndromes
  - *De novo* mutations
- *De novo* mutations have identified ~65 genes associated with ASD
- These genes are providing insight into ASD etiology
  - Gene expression and neuronal communication
  - Early development (<1yr)
  - Excitatory neurons in the cortex
- Environmental factors are likely to be involved
  - Harder to search for than genetic factors

Useful information sources of ASD information

- Spectrum: https://www.spectrumnews.org
- Autism Science Foundation: https://autismsciencefoundation.org
- Autism Society: http://www.autism-society.org
- International Society for Autism Research: https://www.autism-insar.org