Autism Spectrum Disorders

John N. Constantino MD

William Greenleaf Eliot Division of Child and Adolescent Psychiatry
Intellectual and Developmental Disabilities Research Center
Disclosure of Interests

• **Industry Consulting:**
  – *Roche Pharmaceuticals*

• **Stock Equity:** None

• **Royalties:** Western Psychological Services
  The Social Responsiveness Scale (SRS-2)

• **Research Support:**
  – *NICHD*
  – *U.S. CDC*
  – *U.S. Administration for Children and Families*

• **Subsidy of Clinical/Research “Hybrids”:**

  ![Missouri Autism Centers of Excellence](image)

  ![St. Louis County Children’s Service Fund](image)
DSM-V

Autism Spectrum Disorder Must meet criteria 1, 2, and 3:

1. Clinically significant, persistent deficits in social communication and interactions, as manifest by all of the following:
   a. Marked deficits in nonverbal and verbal communication used for social interaction:
   b. Lack of social reciprocity;
   c. Failure to develop and maintain peer relationships appropriate to developmental level

2. Restricted, repetitive patterns of behavior, interests, and activities, as manifested by at least TWO of the following:
   a. Stereotyped motor or verbal behaviors, or unusual sensory behaviors
   b. Excessive adherence to routines and ritualized patterns of behavior
   c. Restricted, fixated interests

3. Symptoms must be present in early childhood (but may not become fully manifest until social demands exceed limited capacities)
Epidemiology of Autism Spectrum Disorders in Adults in the Community in England

Traolach S. Brugha, MD (NUI), FRCPsych; Sally McManus, MSc; John Bankart, MSc, PhD; Fiona Scott, PhD, CPsychol; Susan Purdon, MSc, PhD; Jane Smith, BSc; Paul Bebbington, PhD, FRCPsych; Rachel Jenkins, MD, FRCPsych; Howard Meltzer, PhD

Arch Gen Psychiatry. 2011;68(5):459-466

National survey, 7,461 participants

Figure 2. Predicted values of autism spectrum disorder (ASD) by age. P value for age as a continuous predictor of ASD (P = .55), using the recommended score threshold of 10 or greater on the Autism Diagnostic Observation Schedule, Module 4.
Recurrence Rates and Inherited Transmission in Autism

**MZ concordance:** 90+%  
**DZ concordance:** 20%  
**Non-twin sib recurrence:** 18%  
**General population risk** 1%

“**SPORADIC” (60%)**

**FAMILIAL (40%)**

**De novo (germline)**
- Rare inherited

**Common var. (polygenic)**
- Rare inherited

Swedish Study (2.0M) JAMA. 2014 May 7;311(17):1770-7. The familial risk of autism. Sandin S¹, Lichtenstein P², Kuja-Halkola R², Larsson H², Hultman CM², Reichenberg A³.

Insights into Autism Spectrum Disorder Genomic Architecture and Biology from 71 Risk Loci


Highlights

- De novo copy number variants (dnCNV) are associated with Autism Spectrum Disorder/ASD

- De novo mutations are associated with ASD in individuals with a high IQ

- Small de novo deletions, but not large dnCNVs, contain one high-effect ASD risk gene

- Identifies 6 ASD loci and 65 ASD genes, many of which target chromatin or the synapse
Whole-genome sequencing of quartet families with autism spectrum disorder

Ryan K C Yuen\(^1\), Bhooma Thiruvahindrapuram\(^1\), Daniele Merico\(^1\), Susan Walker\(^1\), Kristiina Tammimies\(^1,2\), Ny Hoang\(^3\), Christina Chrysler\(^4\), Thomas Nalpathamkalam\(^1\), Giovanna Pellecchia\(^1\), Yi Liu\(^1,5\), Matthew J Gazzellone\(^1\), Lia D’Abate\(^1\), Eric Deneault\(^1\), Jennifer L Howe\(^1\), Richard S C Liu\(^1\), Ann Thompson\(^4\), Mehdi Zarrei\(^1\), Mohammed Uddin\(^1\), Christian R Marshall\(^1,6\), Robert H Ring\(^7\), Lonnie Zwaigenbaum\(^8\), Peter N Ray\(^6\), Rosanna Weksberg\(^3,9\), Melissa T Carter\(^3,10\), Bridget A Fernandez\(^11,12\), Wendy Roberts\(^10\), Peter Szatmari\(^10,13,14\) & Stephen W Scherer\(^1,15\)

<table>
<thead>
<tr>
<th>Category</th>
<th>Shared mutations</th>
<th>Non-shared mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sibling 1</td>
<td>Sibling 2</td>
</tr>
<tr>
<td>Comm ((n = 11))</td>
<td>5.00 ± 2.6</td>
<td>5.36 ± 2.0</td>
</tr>
<tr>
<td>Social ((n = 11))</td>
<td>9.36 ± 2.3</td>
<td>9.27 ± 3.2</td>
</tr>
<tr>
<td>Socom ((n = 11))</td>
<td>14.36 ± 4.2</td>
<td>14.64 ± 4.9</td>
</tr>
<tr>
<td>Play ((n = 8))</td>
<td>1.88 ± 1.2</td>
<td>2.63 ± 1.3</td>
</tr>
<tr>
<td>Behav ((n = 11))</td>
<td>4.20 ± 1.5</td>
<td>3.80 ± 2.2</td>
</tr>
<tr>
<td>IQ ((n = 7))</td>
<td>67 ± 24.4</td>
<td>64 ± 29.2</td>
</tr>
</tbody>
</table>

Comm, Communication; social, Reciprocal Social Interaction; socom, Communication and Social Interaction; behav, Stereotyped Behaviors and Restricted Interests. Values are means ± sd (paired, one-sided \(t\)-test).
Autism recurrence in half siblings: strong support for genetic mechanisms of transmission in ASD

Constantino, Todorov, Geschwind et al. (2013)

- Transmission through unaffected parents
  - If additive, accounts for 60% of causal variance.
  - High proportion of population-attributable-risk for autism likely polygenic

<table>
<thead>
<tr>
<th></th>
<th>Full Siblings</th>
<th>Maternal Half Siblings</th>
<th>Paternal Half Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Subjects</td>
<td>4,832</td>
<td>619</td>
<td>55</td>
</tr>
<tr>
<td>Recurrence Rate</td>
<td>.095</td>
<td>.052</td>
<td>(.000)</td>
</tr>
</tbody>
</table>

IAN
ACE
IAN
ACE
IAN
ACE
Most genetic risk for autism resides with common variation

A key component of genetic architecture is the allelic spectrum influencing trait variability. For autism spectrum disorder (herein termed autism), the nature of the allelic spectrum is uncertain. Individual risk-associated genes have been identified from rare variation, especially de novo mutations\textsuperscript{1–8}. From this evidence, one might conclude that rare variation dominates the allelic spectrum in autism, yet recent studies show that common variation, individually of small effect, has substantial impact \textit{en masse}\textsuperscript{9,10}. At issue is how much of an impact relative to rare variation this common variation has. Using a unique epidemiological sample from Sweden, new methods that distinguish total narrow-sense heritability from that due to common variation and synthesis of results from other studies, we reach several conclusions about autism’s genetic architecture: its narrow-sense heritability is \( \sim 52.4\% \), with most due to common variation, and rare de novo mutations contribute substantially to individual liability, yet their contribution to variance in liability, \( 2.6\% \), is modest compared to that for heritable variation.
Excess of rare, inherited truncating mutations in autism

Niklas Krumm1,5, Tychele N Turner1,5, Carl Baker1, Laura Vives1, Kiana Mohajeri1, Kali Witherspoon1, Archana Raja1,2, Bradley P Coe1, Holly A Stessman1, Zong-Xiao He3, Suzanne M Leal3, Raphael Bernier4 & Evan E Eichler1,2

Figure 3 Transmitted mutations and their effect on phenotype. (a) Private, inherited LGD SNVs are enriched in probands with autism or PDD diagnosis but not Asperger’s syndrome (AS) diagnosis. (b) Private, inherited LGD SNVs are primarily enriched in probands with lower IQ than average (<100). (c) We observe transmission disequilibrium of rare, inherited CNVs in SRS-discordant families (proband SRS score > 75, sibling SRS score < 50) but not in families where the SRS score was mild or more balanced between the proband and sibling. (d) Rare, inherited CNVs are enriched in probands (versus their siblings) with IQ <70, but the effect is not significant in probands with IQ >70. All tests and reported P values are paired t tests based on proband-sibling pairs. All analyses were restricted to genes with RVIS values below the 50th percentile. NS, not significant.

differential of 3.7% for private LGD SNVs in conserved genes (RVIS below the 10th percentile) for SRS-discordant quads only (proband, 484/923; siblings, 450/923), whereas SRS-concordant quads had only a 1.6% differential (proband, 419/863; siblings, 405/863).

CNV discovery and validation

Because exome and SNP microarray data provide the opportunity to accurately detect a subset of smaller CNVs within the exonic regions of genes12, we also revisited the burden of both inherited and de novo CNVs with respect to autism. We characterized CNVs from 1,266 quads with available SNP microarray data (validation shown in Supplementary Fig. 4) and tested an additional 50 samples with CNVs of interest identified by array comparative genomic hybridization (aCGH). We focused in particular on validating smaller CNV events that affected genes recurrently affected by de novo SNVs, such as DSCAM, CHD2, ARID1B and TNRC6B (Supplementary Table 5).
Low load for disruptive mutations in autism genes and their biased transmission

Ivan Iossifov\textsuperscript{a,b,1}, Dan Levy\textsuperscript{a}, Jeremy Allen\textsuperscript{a}, Kenny Ye\textsuperscript{c}, Michael Ronemus\textsuperscript{a}, Yoon-ha Lee\textsuperscript{a}, Boris Yamrom\textsuperscript{a}, and Michael Wigler\textsuperscript{a,b,1}

\textsuperscript{a}Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; \textsuperscript{b}New York Genome Center, New York, NY 10013; and \textsuperscript{c}Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY 10461

Contributed by Michael Wigler, August 19, 2015 (sent for review June 12, 2015; reviewed by David B. Boldstein and David H. Skuse)

We previously computed that genes with de novo (DN) likely gene-disruptive (LGD) mutations in children with autism spectrum disorders (ASD) have high vulnerability: Disruptive mutations in many of these genes, the vulnerable autism genes, will have a high likelihood of resulting in ASD. Because individuals with ASD have lower fecundity, such mutations in autism genes would be under strong negative selection pressure. An immediate prediction is that these genes will have a lower LGD load than typical genes in the human gene pool. We confirm this hypothesis in an explicit test by measuring the load of disruptive mutations in whole-exome sequence databases from two cohorts. We use information about mutational load to show that lower and higher intelligence quota (IQ) affected individuals can be distinguished by the mutational load in their respective gene targets, as well as to help prioritize gene targets by their likelihood of being autism genes. Moreover, we demonstrate that transmission of rare disruptions in genes with a lower LGD load occurs more often to affected offspring; we show transmission originates most often from the mother, and transmission of such variants is seen more often in offspring with lower IQ. A surprising proportion of transmission of these rare events comes from genes expressed in the embryonic brain that show sharply reduced expression shortly after birth [Willsey AJ, et al. (2013) Cell 155(5):997–1007].

From recurrence and overlap analysis of DN LGD targets, we estimate ~500 causative ASD target genes in the affected individuals with lower intelligence quota (IQ), and these targets are enriched in certain functional classes (11). Because there are so many autism targets, the penetrance of any given disruptive mutation in a specific target cannot be individually observed at present. However, from the size of the causative target set, DN mutation rate, and ASD incidence rates, we can directly compute what we call “vulnerability” in these genes: the likelihood that a disruptive mutation in the gene results in ASD. We define an “autism gene” as one that, when mutated, may contribute to ASD diagnosis. We computed that roughly half of the time in males, a DN LGD mutation within an autism gene will produce severe ASD (11). Because people with ASD have lower fecundity than the general population, a disruptive mutation in an autism gene will be under strong purifying selection and quickly eliminated from the population (12). A clear prediction is that autism genes will have a smaller load of disruptive mutations than “typical” genes, as we first observed for FMRP-associated genes (5, 13).

Indeed, recent reports indicate that the targets of disruptive DN mutation in affected children do have a lighter load of disruptive mutation in the human population (14, 15). The methods...
Developmental brain dysfunction: revival and expansion of old concepts based on new genetic evidence

Andres Moreno-De-Luca*, Scott M Myers*, Thomas D Challman, Daniel Moreno-De-Luca, David W Evans, David H Ledbetter

The distribution of social and cognitive impairments in a population of children with a specific genetically-defined syndrome is often wide, and for 22q11.2 and 16p11.2 manifest correlations with “genetic background” distributions indexed by variation in the subjects’ parents.

HIGHLY-PENETRANT MUTATIONS RESULT IN PREDICTABLE PATHOLOGICAL SHIFT FROM WHAT WOULD BE “EXPECTED” ON THE BASIS OF AN INDIVIDUAL’S GENETIC BACKGROUND

Figure 1: Cognitive effects of copy number variant syndromes in full-scale intelligence quotient scores (A) The intelligence quotient (IQ) distribution curve in individuals with deletion 22q11.2 (red line) is shifted 2 SD to the left of the IQ distribution in the general population (mean 100; SD: 15; light blue line). # (B) Individuals with deletion 16p11.2 have a mean IQ of 76.1 (dark blue line), which is significantly lower than the mean IQ of their non-carrier first-degree relatives (108.3; green line). The higher IQ of first-degree relatives compared to the general population was previously discussed by Zufferey and colleagues as likely due to ascertainment bias. For both copy number variant syndromes, many deletion carriers have IQ scores within the normal range (>70); this is often referred to as incomplete penetrance when cognitive function is viewed as a qualitative, dichotomous trait (normal intelligence vs intellectual disability) based on a cutoff of 70 points of IQ (dotted line), but may be better interpreted as variable expressivity of a continuous, quantitative trait.
The Social Responsiveness Scale

Quantifies presence and severity of social impairment within the autism spectrum and differentiates it from that which occurs in other disorders

Ages:
2.5 years through adulthood

Administration Time:
15 to 20 minutes

Format:
Parent and/or teacher and/or other adult informant rating scale

Norms:
Total Score;
Scores for DSM-5 Subscales
Life Course, 4-20 yrs

ASD probands
Unaff. sibs of ASD probands

Constantino et al., manuscript in preparation
Small shifts in diagnostic threshold can result in large changes in prevalence
ANOVA (brothers) $F=16.2; \text{df}=2,188; p<.000001$

Constantino et al. (2006)
*Am J Psychiatry* 163(2):294-6
Evidence That Autistic Traits Show the Same Etiology in the General Population and at the Quantitative Extremes (5%, 2.5%, and 1%)

Elise B. Robinson, ScD, MPH; Karestan C. Koenen, PhD; Marie C. McCormick, MD, ScD; Kerim Munir, MD, ScD; Victoria Hallett, PhD; Francesca Happé, PhD; Robert Plomin, PhD; Angelica Ronald, PhD

Arch Gen Psychiatry. 2011;68(11):1113-1121

... support for a continuous risk hypothesis, which argues that inherited genetic risk sets are associated with both subthreshold autistic traits and the clinical ASD phenotype. Furthermore, continuous genetic liability implies that clinical thresholds are etiologically arbitrary because clinical disorders exist as the quantitative extreme of a continuum.

Table 2. Cross-twin Cross-Affectation-Level Correlations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tetrachoric Correlation (95% CI)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>≥99% T2</td>
</tr>
<tr>
<td>MZ twins</td>
<td></td>
</tr>
<tr>
<td>≥99% T1 (n=22)</td>
<td>0.89 (0.81-0.98)</td>
</tr>
<tr>
<td>≥95% T1 (n=86)</td>
<td>0.79 (0.68-0.90)</td>
</tr>
<tr>
<td>≥90% T1 (n=225)</td>
<td>0.63 (0.49-0.77)</td>
</tr>
<tr>
<td>DZ twins</td>
<td></td>
</tr>
<tr>
<td>≥99% T1 (n=36)</td>
<td>0.51 (0.31-0.71)</td>
</tr>
<tr>
<td>≥95% T1 (n=230)</td>
<td>0.25 (0.07-0.43)</td>
</tr>
<tr>
<td>≥90% T1 (n=568)</td>
<td>0.18 (0.02-0.34)</td>
</tr>
</tbody>
</table>

Abbreviations: DZ, dizygotic; MZ, monozygotic; NE, not estimated; T1, twin 1; T2, twin 2.

*One tetrachoric correlation could not be estimated because only 1 T2 scored below the 90th percentile (high scores too strongly associated; odds ratio > 200).
Social Responsiveness, an Autism Endophenotype: Genomewide Significant Linkage to Two Regions on Chromosome 8

LOD 4.03

Chromosome 8 SRS Linkage, AGRE, n=800

Lowe et al., Am J Psychiatry in press
16p11.2
Moreno-DeLuca, Ledbetter et al., in press

(A) - 1.7 SD (ICC = 0.42)
(B) + 2.2 SD (ICC = 0.52)
(C) - 1.3 SD (ICC = 0.21)
(D) + 1.0 SD (ICC = 0.40)
Parent-child correlation = 0.30

Upper quintile mating doubles RR for clinical ASD in offspring

Mother-father correlation = 0.30 (mate selection)

Nurses Health II Study  N=756 cases, 3,000 controls
Sisters of ASD probands fall at the 1 percentile extreme for girls 8 times more commonly than in the gen. pop.

...but they are only half as likely to be diagnosed as brothers at the 1 percentile extreme for boys

<table>
<thead>
<tr>
<th>Proportion affected, defined by...</th>
<th>Male Siblings (n=831)</th>
<th>Female siblings (n=854)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current sx: Categorical ASD Diagnosis</td>
<td>0.12</td>
<td>0.04</td>
<td>3:1</td>
</tr>
<tr>
<td>Current sx: 1&lt;sup&gt;st&lt;/sup&gt; %ile of pop. dist. for gender</td>
<td>0.12</td>
<td>0.08</td>
<td>3:2</td>
</tr>
</tbody>
</table>

Am J Psychiatry 2010
In multiplex ASD families (in which unaffected parents transmit risk to multiple offspring), a polygenic model would predict quantitative trait distributions that are pathologically shifted.
A Higher Mutational Burden in Females Supports a “Female Protective Model” in Neurodevelopmental Disorders

Sébastien Jacquemont, Bradley P. Coe, Micha Hersch, Michael H. Duyzend, Niklas Krumm, Sven Bergmann, Jacques S. Beckmann, Jill A. Rosenfeld, and Evan E. Eichler

Figure 3. Excess of Maternally Inherited Deleterious Autosomal CNVs
(A) Data on inheritance (maternal, paternal, or de novo) were available for 1,826 and 1,735 CNVs from Signature Genomics and the BCA, respectively. These CNVs were selected by cytogeneticists, and inheritance was tested on the basis of the likelihood of their association with the proband’s neurodevelopmental phenotype. Maternal ratio in percentage, associated 95% confidence interval, and p values represent the enrichment of maternally versus paternally inherited CNVs. The p values were computed with a binomial test, and the null hypothesis was a balanced 50/50 inheritance. The CNVs were stratified on the basis of size (400 kb and 1 Mb as cutoffs). An additional and previously published filter was applied on the basis of the presence of an ND gene (see Material and Methods). Compared to small CNVs, large CNVs showed increased maternal inheritance.

(B) Data on inheritance were available for all CNVs identified in 762 SSC probands ascertained for ASD. The ratio of maternally inherited CNVs is represented with the 95% confidence interval and associated p value. The CNVs were stratified on the basis of size and the disruption of an ND gene. Large CNVs disrupting ND genes were preferentially maternally inherited.

N.B. Quantitative severity scores showed no association with CNV or SNV burden.
4:1 M:F ratio in ASD
likely a manifestation of markedly reduced penetrance in females, across numerous / diverse mechanisms of transmission

Most (but not all) females appear to be protected from most (but not all) forms of autism susceptibility

MONOGENIC SYNDROMES

POLYGENIC RISK: Very High Levels of Quantitative Autistic Traits in Unaffected Sisters
Recurrent Transmission through unaffected mothers (maternal half sibs)
(Constantino et al., Mol Psychiatry 2013; Gronborg et al., JAMA Pediatrics 2013)
The female protective effect in autism spectrum disorder is not mediated by a single genetic locus

Jake Gockley¹, A Jeremy Willsey¹,², Shan Dong¹,³, Joseph D Dougherty⁴,⁵, John N Constantino⁴*, and Stephan J Sanders¹,²*

A Tier 1: Unique to chromosome X and escapes X inactivation

B Tier 2: All of chromosome X

C Tier 3: Genome-wide
**Trans-generational Sampling Strategy for Comprehensive Examination of FPE**

**Behavioral phenotyping for quantitative traits**

Population: Sisters of ASD Probands

*N = 90*

**Aim 1: Estimation of Offspring Risk**

**Aim 2: Identification of neural signature of FPE**

*Compensatory neural signature*

*N = 12*

**Aim 3: Explore cellular signature of FPE**

*Patient biomaterials → ipso-derived neuron → cellular signature*

*RELATE NEURAL SIGNALS TO CELLULAR SIGNATURE*

*RELATE MATERNAL BEHAVIORAL TRAITS TO OFFSPRING PHENOTYPE*

*Affected and unaffected male offspring*
Proof of Principle

iPSC APPROACH
Review

Etiologies underlying sex differences in Autism Spectrum Disorders

Sara M. Schaafsma *, Donald W. Pfaff

Laboratory of Neurobiology and Behavior, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

ABSTRACT

The male predominance of Autism Spectrum Disorders (ASD) is one of the best-known, and at the same time, one of the least understood characteristics of these disorders. In this paper we review genetic, epigenetic, hormonal, and environmental mechanisms underlying this male preponderance. Sex-specific effects of Y-linked genes (including SRY expression leading to testicular development), balanced and skewed X-inactivation, genes that escape X-inactivation, parent-of-origin allelic imprinting, and the hypothetical heterochromatin sink are reviewed. These mechanisms likely contribute to etiology, instead of being simply causative to ASD. Environments, both internal and external, also play important roles in ASD's etiology. Early exposure to androgenic hormones and early maternal immune activation comprise environmental factors affecting sex-specific susceptibility to ASD. The gene–environment interactions underlying ASD, suggested here, implicate early prenatal stress as being especially detrimental to boys with a vulnerable genotype.
ASD occurs in Turner Syndrome

Four-core genotype mouse model:
- XX
- XY
- XY lacking SRY
- XY with SRY on autosome

Y genes expressed in brain

Few if any Y mutations implicated in ASD
(B) Number of X chromosomes / X-inactivation

Females
X-linked genes can express from both Xm and Xp

XmXp
Dosage compensation by X-inactivation: X-linked genes express either from Xm or Xp

Balanced X-inactivation

No dosage compensation
(i.e., genes escaping X-inactivation or before X-inactivation): X-linked genes express from both Xm and Xp

Skewed X-inactivation

Males
X-linked genes can express only from Xm

XmY

No skewing of X inactivation in ASD

For dosage compensation, see Gockley et al *in press*
(C) Parent-of-origin allelic imprinting of specific genes

Imprinting of a gene on \( X_m \) leads to expression of that gene in females only in some, but not all, nerve cells of the female's brain.

Imprinting of a gene on \( X_p \) leads to expression of that gene in both sexes, but only in males is the gene expressed in all nerve cells.

- Females: \( X_mX_p \)
- Males: \( X_mY \)

- Females: \( X_mX_p \)
- Males: \( X_mY \)

\( \text{\_\_} = \text{DNA methylation} \)

Inconclusive findings from Turner Syndrome (sample sizes not large enough)

Epigenetic marks complex, change over development and by tissue type.
(D) Heterochromatin sink

Hypothetically limited supply of epigenetic factors in a nerve cell makes them less available to autosomal chromosomes

**Males**

Many epigenetic factors are available to the autosomes

![Diagram](image1)

*No X-inactivation*

**Females**

Limited supply of epigenetic factors are available to the autosomes due to X inactivation

![Diagram](image2)

*Inactivation of Xm*

or

*Inactivation of Xp*

**Fig. 1 (continued)**

Observed in *Drosophila*, but evidence in mammals is limited
## Convergent Cellular Phenotypes?

Prilutsky et al., 2014

<table>
<thead>
<tr>
<th>Disease</th>
<th>Disease-related phenotype in iPSC-derived neurons</th>
<th>Methods to characterize disease-related phenotype</th>
<th>Time from harvest of fibroblasts to phenotype of neurons</th>
<th>Refs</th>
</tr>
</thead>
</table>
| **Rett syndrome**            | Reduced spine density and number of synapses, smaller soma size, altered calcium signaling, and electrophysiological defects | Immunocytochemistry and neuronal morphology quantification (cell soma size, neuronal dendrites and spines, synapse quantification); Cell cycle analysis (at level of neuroprogenitors); Calcium imaging; Electrophysiology | Total: ~8–11 weeks  
Differentiation: 4–5 weeks | [13] |
| **Reduced nuclear size**     | Neuronal nuclei measurement                                                                                        |                                                                                                              | Total: ~7–10 weeks  
Differentiation: 30 days | [14] |
| **Reduced neuronal soma size** | Morphology (soma size)                                                                                            |                                                                                                              | Total: ~12–15 weeks  
Differentiation: ~8–9 weeks | [15] |
| **Defects in neuronal maturation:**  
Reduced expression of cellular markers at mature neuronal stage | Immunocytochemistry; Gene expression analysis: quantitative RT-PCR; Determination of apoptotic cells |                                                                                                              | Total: ~7–10 weeks  
Differentiation: 25 days | [16] |
| **Fragile X syndrome**       | Fewer and shorter neurites                                                                                         | Immunocytochemistry; Morphology                                                                               | Total: ~6–9 weeks  
Differentiation: (from progenitors to neurons) 19 days | [20] |
|                              | Synaptic proteins: decreased PSD95 expression; Synaptic density: decreased PSD95 puncta density; Neurite outgrowth: decreased neurite length; Calcium imaging: functionally abnormal neurons, increased amplitude/frequency, and altered response to glutamate uptake | Immunocytochemistry; Synaptic protein expression: immunoblot; Calcium imaging; Neurite analysis (neurite outgrowth, number of roots, and number of branch points); Synaptic density | Total: ~7–12 weeks  
Differentiation: ~4–6 weeks | [21] |
ORIGINAL ARTICLE

Modeling non-syndromic autism and the impact of TRPC6 disruption in human neurons


Figure 1. Mapping the breakpoints in the autism spectrum disorder (ASD) individual with the 46, XY, t(3;11)(p21;q22) karyotype. (a) The allele frequency distribution plot for chromosomes 3 and 11 generated by single-nucleotide polymorphism (SNP) array genotyping showed no gain or loss of genetic material on these chromosomes. (b) The schematic view of the bacterial artificial chromosome (BAC) probes used and the surrounding breakpoint area on chromosome 3. RP11 probes marked in red span the breakpoint, whereas the black ones do not. The shared region between probes RP11-780020 and RP11-109N8 narrows the breakpoint area to a region inside the VPRBP gene. The blue arrows indicate open reading frames. (c) Fluorescent in situ hybridization (FISH) imaging showing that RP11-780020 probe (red signal) binds to normal and derivative chromosome 3 and to derivative chromosome 11, indicating that the probe spans the breakpoint (arrows). (d) A schematic view of the BAC probes used and the surrounding areas on chromosome 11. A shared region between probes RP11-153K15 and RP11-141E21 places the breakpoint in TRPC6. (e) FISH image showing the BAC probe RP11-153K15 (green signal) bound to normal chromosome 11 and both derivatives chromosomes 3 and 11 (arrows).
Figure 3. Derivation of neural progenitor cells (NPCs) and neurons from induced pluripotent stem cells (iPSCs). (a) Representative images depicting morphological changes during neuronal differentiation from control and TRPC6-mut iPSCs. Bar = 100 μm. (b) NPCs are positive for the neural precursor markers Musashi-1 and Nestin. Bar = 50 μm. (c) Representative images of cells after neuronal differentiation. iPS-derived neurons express neuronal markers such as γ-aminobutyric acid (GABA), microtubule-associated protein 2 (MAP2) and synapsin I. (d) Examples of distinct cortical neuronal subtypes present differentiating cultures after 3 weeks. Bar = 30 μm. (e) We obtained 30% neurons in our cultures with this protocol, as measured by MAP2 staining and infection with the syn::enhanced green fluorescent protein (EGFP) lentiviral vector. Most MAP2-positive cells expressed vesicular glutamate transporter-1 (VGLUT1), in contrast with 12% of neurons expressing GABA. Ctip2-positive neurons were more abundant (16%), whereas Tbr1-positive neurons were present in a small percentage in the population (6%) at the end of the differentiation protocol. (f) Morphology of neurons patched for electrophysiological recording. (g) Representative recordings of evoked action potentials in iPSC-derived neurons in response to current steps under current patch clamps. (h) Representative Na⁺ and K⁺ currents in iPSC-derived neurons. The error bars in all panels show the s.e.m.
induced pluripotent stem cell (iPSC)-derived neuronal cells and mouse models, we demonstrate that TRPC6 reduction or haploinsufficiency leads to altered neuronal development, morphology and function. The observed neuronal phenotypes could then be rescued by TRPC6 complementation and by treatment with insulin-like growth factor-1 or hyperforin, a TRPC6-specific agonist, suggesting that ASD individuals with alterations in this pathway may benefit from these drugs. We also demonstrate that
Sexually dimorphic RB inactivation underlies mesenchymal glioblastoma prevalence in males

Tao Sun,1 Nicole M. Warrington,1 Jingqin Luo,2 Michael D. Brooks,1 Sonika Dahiya,3 Steven C. Snyder,1 Rajarshi Sengupta,1 and Joshua B. Rubin1,4

Figure 6. Sex differences in RB inactivation in Nfr1–/– DNp53 astrocytes. (A) Western blot analysis for RB and phospho-RB (p-RB) in protein lysates from cultures of male and female Nfr1–/– DNp53 astrocytes serum starved for 48 hours (t = 0) and after addition of serum for the indicated times. Actin served as loading control. Shown are representative blots from 1 of 3 independent experiments. (B) Quantification of Western blot analysis of RB phosphorylation. Shown is the ratio of p-RB/RB as a function of time in serum (***P = 0.0001, ANOVA). (C) RB inactivation was measured with an E2F-Luc reporter in 4 independent cultures of male and female Nfr1–/– DNp53 astrocytes. For each measurement, bioluminescence was normalized to EGFP fluorescence, which was linearly related to cell number in both male and female astrocytes (inset). Male values were normalized to female values within each experiment (*P < 0.05, t test). (D) G1 fraction obtained from cell cycle analysis of male and female Nfr1–/– DNp53 astrocytes cultured in serum or serum starved for 48 hours. Quantitation of 4 independent experiments is shown (*P = 0.028, t test).
Stable X Chromosome Reactivation in Female Human Induced Pluripotent Stem Cells

Tahsin Stefan Barakat,1,5,6 Mehrnaz Ghazvini,1,2,5 Bas de Hoon,1,4 Tracy Li,1,2 Bert Eussen,3 Hannie Douben,3 Reinier van der Linden,2 Nathalie van der Stap,1,2 Marjan Boter,3 Joop S. Laven,4 Robert-Jan Galjaard,3 J. Anton Grootegoed,1 Annelies de Klein,5 and Joost Gribnau1,*

This erosion of XCI is detrimental for studies involving cell types generated from female hiPSCs, as it can be expected that many of these cell types will be prone to gene dosage inequalities. Therefore, the availability of such hiPSC lines with stable XCR, having two active X chromosomes as in mESCs, would greatly advance research on modeling of X-linked human diseases and studies on regulatory mechanisms of human XCI.

The varying results regarding XCR and XCI obtained for hiPSCs may be explained by different reprogramming techniques and the growth conditions in which hiPSCs are generated and maintained.
Figure 1. If the iPSC has undergone reactivation and therefore has two active X chromosomes then when it differentiates its progeny will undergo random X-inactivation and the resultant population will have an approximately evenly distributed ratio of inactivated X chromosomes. If the original iPSC has an inactivated X chromosome then all of its progeny will maintain that identical X-inactivation status.
Medication for Attention Deficit–Hyperactivity Disorder and Criminality

Paul Lichtenstein, Ph.D., Linda Halldner, M.D., Ph.D., Johan Zetterqvist, M.Ed., Arvid Sjölander, Ph.D., Eva Serlachius, M.D., Ph.D., Seena Fazel, M.B., Ch.B., M.D., Niklas Långström, M.D., Ph.D., and Henrik Larsson, M.D., Ph.D.

METHODS
Using Swedish national registers, we gathered information on 25,656 patients with a diagnosis of ADHD, their pharmacologic treatment, and subsequent criminal convictions in Sweden from 2006 through 2009. We used stratified Cox regression analyses to compare the rate of criminality while the patients were receiving ADHD medication, as compared with the rate for the same patients while not receiving medication.

Modeling resilience (compensatory function) across diverse causes of ASD

Source 1: NIH Autism Center of Excellence (MH 100027, J.N. Constantino Co-P.I., n=1000 families)
Source 2: W.U. Longitudinal Family Study (HD 042541, n=300 families)
Source 3: IAN National Volunteer Register (Carrier females identified in Aim 1 of this protocol)
Source 3: AGRE Multiplex Autism Collection (n=1500 families)
Biological correlates of genetic background in early development

Figure 3. Salience maps for typically-developing 2-year-olds (top) and for 2-year-olds with autism (bottom). Images at right show color data scaled from black to transparent and overlaid on the still image from the video scene.

Courtesy, Ami Klin and Warren Jones, Emory University
Attention to eyes is present but in decline in 2–6-month-old infants later diagnosed with autism

Warren Jones¹,²,³ & Ami Klin¹,²,³

Figure 2 | Growth charts of social visual engagement for typically developing children and children diagnosed with ASD. a, b, Fixation to eyes, mouth, body and objects from 2 until 24 months in TD (a) and ASD (b) children. c, d, Contrary to a congenital reduction in preferential attention to eyes in ASD, children with ASD exhibit mean decline in eye fixation. e–h, Longitudinal change in fixation to eyes (e), mouth (f), body (g), and object (h) regions; between-group comparisons by functional ANOVA. Thick lines...
Conclusions

- Additive genetic risk is a major factor in the causation and transmission of ASD. Its phenotypic manifestations exhibit...
Conclusions

• Additive genetic risk is a major factor in the causation and transmission of ASD. Its phenotypic manifestations exhibit:
  – A unitary factor structure
  – Continuity and causal overlap with social variation in the entire human population
  – A range of expression in familial ASD that is continuously distributed in males, bimodally distributed in females
  – Major effects on mate selection
  – Possible adaptation at lower levels and impairment at higher levels
Conclusions

• Identification of convergent mechanisms of causation across the diversity of additive and high-penetrance genetic influences on ASD will present important opportunities for novel treatments that would benefit significant subsets of the ASD population (as occurs for the effect of stimulant medication on a majority of cases of ADHD).
  – Heritable developmental phenotypes conferring risk for ASD
  – Compensatory factors conferring resilience
  – Biological mechanisms underlying highly deleterious (rare) mutations
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