From Genes to Pathology:

The Path Forward in Genetically Complex Neurodevelopment Disorders

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Overview

Gene discovery in early-onset disorders, including so-called idiopathic ASD, and Tourette disorder (TD) has had a strikingly different result than for “adult-onset” disorders. Rare de novo loss of function/likely gene disrupting mutations of large effect. This accounts for a minority of clinical cases but offers distinct avenues to elaborate biology. Critical issue is differentiating biology from pathophysiology. Consider several avenues to the development of novel “precision” therapies.
Natural selection has limited window to act, so large effects mutations are not yet weeded out.

Het, likely gene disrupting (LGD) variants in the coding portion of the genome are more common in affected individuals.

But there is no group difference in silent mutations or the number of affected individuals with multiple de novo mutations compared to their siblings.
Werling et al Nat Genet 2018
An et al Science 2018
Total Population
Genetic Risk

Unaffected
Clinic/Affected

Large effect de novo mutation

SCZ, ID, Epilepsy, SLI

ASD

Large effect de novo mutation
Tourette Disorder

Vocal and motor tics: very high rates of co-morbidity

No common variants from GWAS to date

Rare mutations: Histaminergic neurotransmission; neurite outgrowth (Gulhan Sencicek et al *NEJM* 2010; Abelson et al *Science* 2005)

Tic Genetics: Multi-Site international collaboration

Exome sequencing of trios
De Novo Coding Variants Are Strongly Associated with Tourette Disorder

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Shari Duga,1,7,12 Kevin E. Samocha,1,7,12 Tourette International Collaborative Genetics (TIC Genetics)

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Matthew W. State,1,7,12,13,14 and Gary A. Heiman1,7,12

A Maximum likelihood estimate of number of TD risk genes

B Recurrently Mutated Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>LGD</th>
<th>Mis3</th>
<th>p value</th>
<th>q value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWC1</td>
<td>1</td>
<td>1</td>
<td>7.3 x 10^-9</td>
<td>0.096</td>
</tr>
<tr>
<td>CELSR3</td>
<td>1</td>
<td>1</td>
<td>1.7 x 10^-8</td>
<td>0.14</td>
</tr>
<tr>
<td>NIPBL</td>
<td>0</td>
<td>2</td>
<td>5.4 x 10^-5</td>
<td>0.22</td>
</tr>
<tr>
<td>FN1</td>
<td>0</td>
<td>2</td>
<td>5.9 x 10^-5</td>
<td>0.26</td>
</tr>
</tbody>
</table>

p and q values estimated with TADA
De Novo Sequence and Copy Number Variants Are Strongly Associated with Tourette Disorder and Implicate Cell Polarity in Pathogenesis

Table 4. TD Risk Genes Identified in this Study

<table>
<thead>
<tr>
<th>Gene</th>
<th>LGD</th>
<th>Miss</th>
<th>p Value</th>
<th>q Value</th>
<th>q Value in Phase 1</th>
<th>Risk Status in Phase 1</th>
<th>Intolerant</th>
<th>pLJ</th>
<th>Missense Z Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWCT</td>
<td>1</td>
<td>1</td>
<td>1.93 × 10^-6</td>
<td>0.069</td>
<td>0.096</td>
<td>hTD</td>
<td>no</td>
<td>0.02</td>
<td>1.27</td>
</tr>
<tr>
<td>CEL5R3</td>
<td>1</td>
<td>3</td>
<td>2.23 × 10^-6</td>
<td>0.073</td>
<td>0.14</td>
<td>pTD</td>
<td>yes (LGD and Miss)</td>
<td>1.00</td>
<td>6.17</td>
</tr>
<tr>
<td>CPA1</td>
<td>0</td>
<td>2</td>
<td>6.70 × 10^-6</td>
<td>0.11</td>
<td>0.72</td>
<td>NA</td>
<td>yes (LGD)</td>
<td>0.99</td>
<td>1.83</td>
</tr>
<tr>
<td>NRNP2</td>
<td>0</td>
<td>2</td>
<td>1.13 × 10^-6</td>
<td>0.16</td>
<td>0.22</td>
<td>pTD</td>
<td>yes (LGD and Miss)</td>
<td>1.00</td>
<td>5.04</td>
</tr>
<tr>
<td>FN1</td>
<td>0</td>
<td>2</td>
<td>1.22 × 10^-6</td>
<td>0.19</td>
<td>0.26</td>
<td>pTD</td>
<td>no</td>
<td>0.06</td>
<td>1.39</td>
</tr>
<tr>
<td>FBN2</td>
<td>0</td>
<td>2</td>
<td>1.29 × 10^-6</td>
<td>0.22</td>
<td>0.98</td>
<td>NA</td>
<td>yes (LGD3)</td>
<td>1.00</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Five genes with recurrent de novo variants meet our thresholds for association: two of these are high-confidence TD (hTD) risk genes (CELSR3 and WWCT1; FDR ≤ 0.1), and four of these are probable TD (pTD) risk genes (CPA1, NRNP2, FN1, and FBN2; FDR ≤ 0.3). We excluded genes with only one de novo variant from this table (3 pTD genes; see Table S7). See also Figure S6 and Tables S6 and S7.

*Wilsey et al. (2017).

*Probability of being loss-of-function (LoF) intolerant, from Exome Aggregation Consortium (ExAC). pLJ ≥ 0.9 is considered intolerant.

*Z score for missense mutations, from ExAC. Miss z ≥ 3.891 is considered intolerant.

*We previously identified WWCT1 as an hTD gene and CELSR3, NRNP2, and FN1 as pTD genes (Wilsey et al., 2017). Four of these six TD risk genes are considered intolerant to variation: determined based on pLJ and missense Z score.
Biology vs. pathology
ASD genes point to the synapse, chromatin modification and FMRP

Figures by Montana Morris and Sarah Pyle
Temporal-spatial transcriptional profiling mid-fetal human cortical development

Modified from Willsey et al Cell 2013
Willsey et al Cell 2013

13 hcASD genes (FDR < 0.02)
269 pASD genes (FDR < 0.45)
Excludes all phase 1 genes

33 hcASD genes (FDR < 0.02)
146 pASD genes (FDR < 0.3)
Includes all phase 1 & 2 genes

Ben David et al Mol Psych 2012
Parikshak et al Cell 2013
Xu et al J Neuroscience 2014
Uddin et al Nat Genet 2014
Genes as targets (CRISPR/ASO)
Illuminate molecules and neural cells as targets (eCog/DBS)
Circuits as targets (eCog/DBS)
Target Protein levels

Pluses:

› haplo-insufficiency is a shared mechanism (despite individual rare variants)
› We know the phenotype (protein levels)
› Increasing expression may not need to get to 50%
› Multiple ways to get here with functional genomic and/or pharmacology
› Top mutations/genes, while rare, carry very large risks
› There may be a window to wait until first symptoms
› AAV and ASOs in spinal muscular atrophy points (broadly) to plausibility
› UBE3A: Huang et al Nature 2011; Lee et al Mol Aut 2018
Target Protein levels

Minuses:

› Top genes, while rare, carry very substantial risks (but for what?)
› May (or may not) be a window to wait until first symptoms and genetic diagnosis;
› Going earlier (e.g. fetal delivery) seems unlikely initially given the phenotype/cost benefit analysis
› Challenge of identifying individuals carrying rare mutations
› Targeting deep layer glutamatergic or striatal neurons
› CNS penetration and off target effects
Summary:

De novo mutations in sequence and structure of DNA carry large risks for ASD and TS (and Epilepsy, OCD, ADHD)
Genes are not fate. The risk is not specific: mutations may also confer risk for epilepsy, intellectual disability, schizophrenia, specific language impairment...
Genic large-effect mutations provide relatively direct avenues to translation
Biology is not synonymous with pathophysiology
Convergence approaches versus sticking close to the gene
Perspective on how far the field has developed in the last half decade
Sanders & State:
- Joon An
- Donna Werling
- Shan Dong
- Michael Gilson
- Claudia Dastmalchi
- Lindsay Liang
- Eirenne Markenscoff-Papadimitriou

Talkowski:
- Harrison Brand
- Ryan Collins
- Joseph Glessner
- Matthew Stone

Marth & Quinlan:
- Andrew Farrell
- Dillon Lee
- Ryan Layer

Roeder & Devlin:
- Lingxue Zhu
- Kevin Lin
Connecticut
  Thomas Fernandez
  Robert King

California
  Young Shin
  Kim
  Bennett
  Leventhal
  Matthew
  State
  Jeremy
  Willsey

Iowa
  Samuel Kuperman

New Jersey
  Gary Heiman
  Jay Tischfield

Florida
  Barbara Coffey

Ohio
  Donald Gilbert

Pennsylvania
  Lawrence Brown

Washington State
  Samuel Zinner

USA

Denmark
  Kerstin von Plessen

Germany
  Kirsten Mueller-Vahl
  Alexander Münchau
  Veit Roessner

Netherlands
  Andrea Dietrich
  Pieter Hoekstra
  Chaim Huyser
  Athanasios Maras

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  Astrid Morer

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  Kyungun Jhung
  Yun-Joo Koh
  So-dahm Kook
  Dong-Ho Song
  Jungeun Song