Understanding Neurodevelopment - Challenges and Aspirations in Child and Adolescent Mental Health

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Prof Louise Gallagher
TCD
Why do we need to understand typical and atypical neurodevelopment?

Will understanding neurobiology lead to improvements in diagnosis, treatment, prevention, quality of life?
Neurodevelopmental disorders

- Communication disorders
- Learning disorders
- Autism
- Chronic tic disorders
- ADHD
- Schizophrenia
- Psychosis

Deficits - language, social cognition, attention, sensory-motor functioning
What have we learned about atypical neurodevelopment?

• Example from Rett Syndrome
• Autism Spectrum Disorders
• Converging neurobiology of neurodevelopmental disorders
• Challenges to integrating understanding neurodevelopment
• Aspirations for the future of neurodevelopmental research
Rett Syndrome

- Early onset neurodevelopmental disorder
- Similarities to ASD
- Predominantly girls
- Well defined syndrome – 4 clear stages of progression:
  1. Stagnation
  2. Regression (6-18 months)
  3. Seizure development
  4. Late motor deterioration
Rett Syndrome – A Rare Neurodevelopmental Syndrome with a Genetic Aetiology

• Physical phenotype
  – Small hands and feet
  – Deceleration of head growth (→ microcephaly)

• Behavioural phenotype
  – social withdrawal,
  – repetitive hand movements,
  – avoidance of eye contact
  – lack of social/emotional reciprocity
  – Impaired nonverbal behaviors
  – Breathholding

• Physical phenotype
  – GI disturbances
  – Seizures
  – Sensory disturbances

Andreas Rett, 1966
Discovery of a gene for Rett

- MECP2 (xq28) – de novo or germline mutations
- CDKL5, FOXG1 ~ 10% cases
- Duplication of MECP2 in males
- MECP2 – Methyl-CpG binding protein – 3 binding domains

Zoghbi et al, 1999
Rett syndrome – phenotypic differences

| Characteristic       | Classic RTT
d | Mild RTT Variants | Severe RTT Variants |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Age at onset</td>
<td>Onset between 6–18 mo</td>
<td>Later onset</td>
<td>Congenital onset</td>
</tr>
<tr>
<td>Head/body size</td>
<td>Small head, body</td>
<td>May have small head, body</td>
<td>Small head, body</td>
</tr>
<tr>
<td>Seizures</td>
<td>Seizures</td>
<td>Speech is preserved</td>
<td>Early seizure onset</td>
</tr>
<tr>
<td>Speech</td>
<td>Loss of speech</td>
<td>Usually ambulatory</td>
<td>Hypotonia, motor deficiencies</td>
</tr>
<tr>
<td>Motor function</td>
<td>Motor deficiencies</td>
<td>Retain hand use</td>
<td></td>
</tr>
<tr>
<td>Hand use</td>
<td>Stereotypical hand motions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social Interactions</td>
<td>Autistic features</td>
<td>Mild or no mental retardation</td>
<td>Mental retardation</td>
</tr>
<tr>
<td>Intelligence</td>
<td>Mental retardation</td>
<td></td>
<td>Scoliosis and/or kyphosis</td>
</tr>
<tr>
<td>Spinal curvature</td>
<td>Scoliosis and/or kyphosis</td>
<td></td>
<td>Breathing dysfunction</td>
</tr>
<tr>
<td>Respiration</td>
<td>Breathing dysfunction</td>
<td></td>
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</tbody>
</table>

* Null alleles or severe inactivating mutations, balanced XCI.

* Hypomorphic alleles (late truncations) with balanced XCI or null alleles with favorably skewed XCI. (Very late truncations and some missense mutations, such as A140V and Q406X, result in no phenotype in females even when XCI is balanced. These same mutations do, however, produce phenotypes in males.)

* Null alleles or severe inactivating mutations, possibly due to unfavorably skewed XCI.

*From Shahbazian and Zoghbi, 2002*
Rett syndrome - Neurochemistry

- **Pontine noradrenergic deficits**
  - ↓ NA functioning, NA excitability in locus coeruleus
  - ↓ TH mRNA expression in mouse models (-/- male and -/+ female)
  - ↓ TH expressing neurons and density of dendritic arborizations – immature neurons
  - Implicated in both respiratory and cognitive dysfunctions

- **Midbrain dopaminergic disturbances**
  - Nigrostriatal dopaminergic deficits – hypothesised to underpin motor abnormalities
  - ↓ TH immunoreactivity in caudate-putamen om -/- mouse model
  - ↓ DA – midbrain and striatal regions – PM animal studies -/- model
  - Changes remain static but behavioural phenotype and deficits progress
  - Oral L-DOPA administered to mouse model ---- amelioration in some motor deficits
Rett – brain structure

• Global brain atrophy – frontal, occipital and dorsal parietal

• Structural neuroimaging - $\downarrow$ FA in left peripheral white matter areas - middle temporal, middle occipital, pre-cuneus and post-central white matter

Oishi et al, 2013
Resting neuron

HDAC

mSin3a

MeCP2

Rat BDNF exon II
Mouse BDNF exon IV

Depolarisation

Rat BDNF exon III

HDAC

mSin3a

MeCP2

PO₃

MeCP2 is phosphorylated and released from BDNF promoter
Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice

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Contributed by Rudolf Jaenisch, December 11, 2008 (sent for review November 9, 2008)
Rate of progress towards potential treatments for Rett Syndrome

- Clinical Description 1966
- Expanding phenotype descriptions
- Identification of MECP2 1999
- MECP2 structure and function
  - MECP2 animal models
- PM studies, neurophysiology
- Downstream molecules - Animal trials, e.g. BDNF
- Human Clinical Trials of IGF-1
Autistic Spectrum Disorders

- Autistic Disorder
- Asperger’s Disorder
- Childhood Disintegrative Disorder
- Rett’s Disorder
- Pervasive Developmental Disorder Not Otherwise Specified

Cognitive impairment
Language impairment
Social impairment
Other behaviours
Autism Genetics

Higher rates of autism in MZ twins compared with DZ twins

Higher rates of autism in first-degree family members

Chromosomal anomalies known to cause autism
Genetic risk factors

Changes in coding sequence

- **Common** – Greater frequency in population with a trait compared with controls, mild increase in the odds of having a trait

- **Rare** – Very low frequency – Significantly increase the odds of having a trait

Changes in chromosome structure

- Amplification Copy Number 6
- 1 Copy Duplication Copy Number 3
- Normal Copy Number 2
- 1 Copy Deletion Copy Number 1
- 2 Copy Deletion Copy Number 0

**SNP**

- Controls
- Cases
Identifying the genetic risk factors in ASD

• Given a heritable component, how have we attempted to discover the genetic risk loci underpinning the family and heritability data?

1. Gross chromosomal anomalies (Syndromal autism)
2. Linkage
3. Association
4. Structural variation
5. Sequence variation
Cytogenetically-visible breakpoints in autism

Source: http://projects.tcag.ca/autism/

BREAKPOINTS
- Translocation
- Deletion
- Inversion
- Duplication
## Genome-wide Association Studies: Major Findings

<table>
<thead>
<tr>
<th>Manuscript</th>
<th>Sample Size</th>
<th>Array Size</th>
<th>Major Findings</th>
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<tbody>
<tr>
<td>Wang et al., 2009</td>
<td>&gt;900</td>
<td>500K</td>
<td>CDH9/CDH10</td>
</tr>
<tr>
<td>Weiss et al., 2009</td>
<td>&gt;800</td>
<td>500K</td>
<td>TAS2R1/SEMA5A</td>
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<tr>
<td>Anney et al., 2010</td>
<td>&gt;1300</td>
<td>1M</td>
<td>MACROD2</td>
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<tr>
<td>Devlin, Anney., in prep</td>
<td>+1000</td>
<td>1M</td>
<td></td>
</tr>
<tr>
<td>PGC., in prep</td>
<td>&gt;5000</td>
<td>&gt;1M</td>
<td></td>
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</tbody>
</table>
Functional Impact of Global Rare Copy Number Variation in Autism Spectrum Disorder

![Bar chart showing percentage of samples with rare CNVs.](chart.png)

- **Deletions** and **Duplications**
  - Cases: 10, Controls: 6
  - Expression data: ASD implicated, ID candidates, and ASD implicated + ID
  - NS: 0.0054, P: 0.0012

![Diagram illustrating cell biology pathways.](diagram.png)

- **Membrane raft**
  - **Organ morphogenesis**
  - **Cell proliferation**
  - **Positive regulation of cell proliferation**
  - **Cell motility**
  - **Cell projection + cell motility**
  - **Cell projection organization**
  - **Cytoskeleton organization**

- **GTPase/Ras signalling**
  - **RhoGAP domain**
  - **GTPase activator**
  - **Regulation of catalytic activity**
  - **Kinase activity/ regulation**

Published in final edited form as:
Implications for diagnosis

- Specific genetic aetiology in up to 15%
- Single gene disorders - Fragile X syndrome, Rett syndrome, TS, PTEN mutations
- Microscopic – Trisomy 15q, sex chromosome aneuploidy, Ig deletions, duplications
- Submicroscopic \(\rightarrow\) CNV at 16p11.2, 15q11-13, 22q11.2, Rare ‘de novo’ CNV
- ‘ASD plus’ \(\rightarrow\) syndromic or ‘complex’
- Non-syndromic \(\rightarrow\) non-syndromic, healthy, normal growth, no major congenital abnormalities

Carter and Scherer, 2013
Rare sequence variants

- Rare functional mutation

2
Family-based re-sequencing of NLGN3 and NLGN4

- Jamain et al., 2003, Nature Genetics
Family-based re-sequencing of SHANK3

- Durand et al., 2007 Nature Genetics
Family-based re-sequencing of SHANK3

- Durand et al., 2007 Nature Genetics
Functional implications of Mutation

Impact on Synapse validation
Chih et al., Hum. Mol. Genetics, 2004

Impact on Neuronal wiring?
Durand et al. Nature Genetics 2007

Courtesy of Thomas Bourgeron. INSERM, Paris
Emerging models of synaptic proteins

Cellular localization, within a representative neuron, of proteins implicated in recent whole exome studies and accompanying follow-up sequencing. Implicated functions include chromatin remodeling/transcriptional regulation (CHD8), DNA binding/mitotic activity (POGZ), nuclear kinase activity (DYRK1A), microtubule binding/severing (KATNAL2), glutamate receptor (GRIN2B), and voltage-gated sodium channel (SCN2A) [EntrezGene].

www.scienceDirect.com

Current Opinion in Genetics & Development 2013, 23:310-315
Animal models of neurodevelopmental disorders and therapeutics
Challenges to integrating what we have learned

- Genetics - Replication and further discovery will require 10s of 1000s of samples
- Imaging the brain provides correlational findings that are difficult to integrate with genetic data
- Overlapping neurobiology between disorders – is there any specificity to symptoms?
- Brain is an inaccessible organ. Inadequate model systems – neuroblastoma cells/ mouse models
• Large clinical databases
• International samples
• Reduction in phenotype definition – increase phenocopies
Evidence for altered brain connectivity in autism

- Long range hypoconnectivity
- Short range hyperconnectivity
- Resting state - hypoconnectivity
Resting state hypoconnectivity

- ↓ cortico-cortical iFC – esp paralimbic and unimodal association areas (FG, STG)
- ↓ lobar connectivity – esp temporal
- ↑ Connectivity sub-cortical regions – primary parietal sensorimotor regions
- Alterations in interhemispheric connectivity
- Suggestive of maturational abnormalities in ASD

Di Martino et al, 2013
Normal brain development

**FIGURE 2** | Normal developmental trajectory of neurogenesis, neuronal migration, and myelination in the human. Neurogenesis and the subsequent migration of neurons to the cortex begin within a few weeks of gestation in the human (Zecevic et al., 2011). Pyramidal neurogenesis and migration of these cells to the cortex occurs by radial migration and is completed by mid-gestation (Naderajsh and Parnavelas, 2002), while genesis and migration of GABAergic interneurons continues into early post-natal life, with emerging evidence based on the presence of molecular markers of immature neurons suggesting that this process continues into adulthood in primates (Gould et al., 1999, 2001; Benier et al., 2002; Fung et al., 2011a; Wang et al., 2011). Prefrontal myelination occurs predominantly in early post-natal life, still increasing through adolescence before reaching adult levels (Kang et al., 2011).
Induced pluripotent stem cells (iPS cells)

'genetic reprogramming'
= add certain genes to the cell

adult cell → induced pluripotent stem (iPS) cell behaves like an embryonic stem cell

culture iPS cells in the lab → differentiation → all possible types of specialized cells

Advantage: no need for embryos!
Future directions - Induced pluripotent stem cells

- OCT4, SOX2, KLF4, c-MYC
- Gene expression data
- CNV data
- Sequence data
- Animal models
- Epigenetics
- Phenotype data
- Behavioural data
- Environmental data
- Physical/medical data
- Neuroimaging
- Electrophysiology
- Longitudinal trajectory
Disruption of Neurexin 1 gene in Irish Families
Stem cells – Neurexin 1 deletion

**Indepth phenotyping patients:**
- Neurocognitive
- Neuroimaging
- Physical
- Behavioural

**Cell line phenotyping:**
- Electrophysiology
- Molecular – test molecular compounds
- Morphology
Summary

• Genetics research has not been in vain! – Emerging neurobiology of neurodevelopmental disorders
• Genetic diagnosis is a reality for a small number of individuals with ASD
• Convergence between the emerging neurobiology and changes observed in functional and structural neuroimaging
• Potential new technologies to interrogate brain function and possibly aid the development of new therapies
• Caution! – we still need to understand the role of development and the relationship between perturbations in the neurobiology and symptoms
• Dimensional approach to symptoms – may aid our understanding of the co-occurrence of symptoms between disorders but we need to figure out why the symptoms are different too!
“Genes and family may determine the foundation of the house but time and place determine its form”

(Jerome Kagan)
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