Cognitive Endophenotypes: A Tool in Understanding the Genetic Architecture of Neuropsychiatric Disorders

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Department of Psychiatry
Neuroscience and Mental Health
Overview

- ADHD / Neurodevelopmental Disorders
  - Etiological challenges
- Endophenotypes: definition and rationale
- Criteria for validating
- Response Inhibition, example in ADHD
- Application in clinical sample
- Large population sample: Spit for Science
- Cross Disorder application and considerations
ADHD

- Attention Deficit Hyperactivity Disorder
  - Impulsiveness
    - Shout out, disruptive, careless errors
  - Hyperactive
    - Cannot suppress need to move about
  - Inattentive
    - Attention is drawn from one stimulus to another
- Prevalent (4-8%)
- Persistent (50 % +)
- Impairing
  - Associated learning, behavioral, social and emotional problems
  - Employment difficulties, relationship failures, substance use
  - Increase in health care costs
- Acquired causes
  - Brain injury, prematurity, consequences of treatment of leukemia, fetal alcohol exposure, institutional neglect
ADHD Etiology

• Genetic influence
  – Family studies
    – First degree relatives 4 times more likely to have ADHD
  – Adoption studies
    – ADHD > in biological relative than adoptive
  – Twin studies
    – Highly heritable (average of .76 across 30+ studies)
Summary of Genetic Findings

• Candidate gene studies
  – 8 candidates (e.g. DAT1, SNAP25, DRD4)
  – Not always replicated; highest OR 1.5; 3% of variance

• GWAS and linkage
  – No findings of genome-wide significance
  – Recent Meta-analysis combined international samples 4000+, no GWAS hit

• Explanations and remedies
  – Etiologically heterogeneous; phenotypically complex
  – Many genes of small effect
  – Insufficient sample size (e.g., Sz 30,000 cases)
  – Insensitive genetic methods
    • Many candidate genes not well covered in GWAS
    • Population admixture – variations due to ethnicity
    • Rare variants
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Phenotypic Heterogeneity

- Heterogeneity in psychiatric disorders reduces the ability to identify underlying neurobiological factors
- Clinical symptoms are difficult to measure and difficult to collate into consistent clinical groups
- Not clear that the clinical symptoms/diagnosis have a connection with the underlying neurobiology
Model of Neurodevelopmental disorders

Multi-factorial, polygenic, threshold disorders

- Quantitative traits, (attention, repetitive behaviours, communication, social development, hyperactivity)
- Inhibiting, planning, preparing, holding, inhibiting, switching, error detection and adjustment
- Functional connectivity, neurotransmission
- Grey and white matter
- Smoking, drinking, brain trauma, toxins
- Common and rare variants

Genes

Proteins & Structures

Environment and epigenetic

Physiology

Cognition

Behaviour

Inatt Hyp Imp

(Crosbie, Peruse, Barr, Schachar 2008)
Endophenotypes

• Heterogeneity at the behavioural level
  – lead to efforts to dissect the phenotype of ADHD

• “Endo” = within

• Endophenotypes are biological markers that are in the pathway from underlying genetic risk to disease phenotype.
Model of Neurodevelopmental disorders

Multi-factorial, polygenic, threshold disorders

Genotype

Phenotype

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(Crosbie, Peruse, Barr, Schachar 2008)
Endophenotypes

• Markers of pathophysiological processes within the same biological pathways as the genetic disease (Tsuang, Faraone, & Lyons, 1993).

• Endophenotypes are closer to the underlying neuropathology, of a disease than the behavioural manifestation

• Increase power to detect genetics risks

• Increase precision of etiological studies
Validity Criterion Endophenotypes

1. Associated with disorder(s)
   • Meta-analysis (effect sizes-truth is not binary)

2. Heritable
   • Siblings of affected individuals should show deficit
   • Shared genetic variance with disorder

3. State independent (i.e. trait)
   • Stable, evident at every age
   • Persist despite remission

4. Feasible
   • Easy to administer, reliable, quantitative

5. Biologically Plausible/ informative

6. Psychometrically sound
   • High reliability, norms (age, sex, ethnicity, etc)
   • No ceiling or floor effect - behaviour trait

(Crosbie, Peruse, Barr, Schachar 2008)
Response Inhibition
Response Inhibition

• Current theories of psychopathology hold that response inhibition is a viable endophenotype for ADHD (and possibly other neuropsychiatric disorders)
Response inhibition

• Various forms of inhibition –
  – Perceptual inhibition (Flanker, Stroop)
  – Response withholding/restraint (go no-go task)
  – Response cancellation (stop task)

• Stop task
  – An “extreme form” of executive control
  – Analogue of driving and braking suddenly
  – Conditions involving rapid responses and occasional urgent cancellation
Response inhibition
Stop Task

GO Trials

GO Trials

STOP Trials
Stop Signal Task

Diagram:

- 500 ms
- 1000 ms
- 500 ms

- *
- X/O
- X/O
- X/O

*Signal*
| respond |
| inhibit |
stop signal
Validation

Response Inhibition

ADHD
Inhibition Deficit in ADHD

Stop signal reaction time (SSRT)

Schachar & Logan, 1990
Inhibitory control and psychopathology: A meta-analysis of studies using the stop signal task

JONATHAN LIPSZYC,1,2 AND RUSSELL SCHACHT 1,2


- Children, adolescents and adults
- SSRT Not explained by IQ, Go RT, reading
- No difference in GO RT or RT SD
- Deficit not specific to any disorder
Inhibitory control and psychopathology: A meta-analysis of studies using the stop signal task

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Inhibition and Family History of ADHD

Crosbie & Schachar (2001)

- Poor Inhibition: 48.1%
- Good Inhibition: 18.5%
- Normal Control: 7.7%
Inhibition of Motor Responses in Siblings Concordant and Discordant for Attention Deficit Hyperactivity Disorder

Russell J. Schachar, M.D.
Jennifer Crobie, Ph.D.
Cathy L. Barr, Ph.D.
Tisha J. Ornstein, Ph.D.
James Kennedy, M.D.
Molly Malone, Ph.D.
Wendy Roberts, M.D.
Abel Ickowicz, M.D.
Rosemary Tannock, Ph.D.
Shirley Chen, M.D., M.P.H.
Tejaswee Pathare, M.A.
Validation and Extension of the Endophenotype Model in ADHD Patterns of Inheritance in a Family Study of Inhibitory Control

Lisa M. Goos, Ph.D.
Jennifer Crosbie, Ph.D., C.Psych.
Shalaine Payne, B.Sc.(Hons.)
Russell Schachar, M.D., F.R.C.P.(C.)

Objective: Endophenotypes, markers of underlying liability to psychiatric disorders, can improve the power to detect genetic risks relative to a complex clinical endpoint. Motor response inhibition is a prime candidate endophenotype in ADHD. In this study, the authors sought to extend the endophenotype model and further demonstrate its utility by investigating the parental origin of shared genetic influences.

Results: The results confirmed an inhibitory control deficit in children with ADHD as well as in their parents, independent of symptom severity in both generations. Inhibitory control ability in children was significantly predicted by the ability of their parents, particularly their fathers.

Conclusions: These findings indicate that an inhibitory control deficit is a cognitive marker of genetic risk shared by

- SSRT deficit in parents of ADHD children
- Deficit in children predicted by parents performance
Stop Signal and Conners’ Continuous Performance Tasks

Test–Retest Reliability of Two Inhibition Measures in ADHD Children

Noam Soreni
University of Toronto, Ontario and McMaster University, Hamilton, Canada

Jennifer Crobie
Abel Ickowicz
Russell Schachar
University of Toronto

\[ R^2 = .78 \text{ over 3 weeks} \]
The persistence of cognitive deficits in remitted and unremitted ADHD: a case for the state-independence of response inhibition

Tara McAuley, Jennifer Crosbie, Alice Charach, and Russell Schachar

1Department of Psychology, University of Waterloo, Waterloo, ON, Canada; 2Department of Psychiatry Research, Hospital for Sick Children, Toronto, ON, Canada

- Children with ADHD followed from 8 years to 12-14 years
- Persistent SSRT deficit regardless of remission status.
- Spatial or verbal working memory no sig difference from NC at follow-up
Heritability

KIDNET

- 131 twin pairs
- Heritability of SSRT = .5
Response Inhibition and ADHD Traits: Correlates and Heritability in a Community Sample

J. Crosbie · P. Arnold · A. Paterson · J. Swanson · A. Dupuis · X. Li · J. Shan · T. Goodale · C. Tam · L. J. Strug · R. J. Schachar

- N = 16,099
- Ages 6-18
- Heritability of SSRT
- SSRT coheritable with ADHD traits
Endophenotypes

• By conditioning analyses on endophenotypes one can increase the power to identify a subset of the genetic/ neurobiological risks contributing to neurodevelopmental (complex) disorders
Stopping and Risk Alleles

- **SNAP-25**
  - Fusion of synaptic vesicle and plasma membrane
  - Animal model has inattention, learning problems
  - Significant association with ADHD and with SSRT

- **Dopamine receptor D4**
  - 7 repeat VNTR is sub-sensitive to endogenous dopamine
  - Animal model is hyperactive
  - Not significant in ADHD, but significant for SSRT

- **Dopamine transporter (DAT1)**
  - Controls intensity of dopamine signalling
  - Methylphenidate blocks DAT1 and result in a significant increase in extracellular dopamine
  - Evidence of difference in DAT in ADHD is variable
  - Not associated with SSRT

Feng, Crosbie, et al, 2005)
The Problem of Genetics

• Although initial promising results for ADHD...
  – Samples too small
  – Very expensive to genotype
  – Collecting clinical samples – time consuming, expensive
  – Lack of well controlled comparison groups

• Task
  – Large sample, quickly, cost effectively
  – Measurable, reliable, informative trait
  – Control group screened for the trait of interest
  – Analytic approach that maximized power while limiting sample size
Population Design

- Ontario Science Centre
- **17,000** children and youth
- Psychiatric traits
- Cognitive performance
- DNA
- Community Dx and FH
- Largest cognitive genetics sample in the world
Spit for Science: Collection

• Score
  – Standardized quantitative behavioural questionnaires
  – ADHD – SWAN
  – OCD – TPOCS
  – Self and family history regarding other psychiatric conditions

• STOP
  – Stop Signal Task

• Spit
  – DNA collected using Oragene saliva kits
Spit for Science: Sample

Child Diagnostic History
• ADHD  6%
• OCD  0.77%
• Learning problem  10.54%
• Anxiety  3.2%

Family History
• ADHD  5.62%
• OCD   2.25%
• Anxiety  8.19%
Analytical Methods

**Phenotyping**

Sample Collection – Ontario Science Centre
17,263 youth (6-17 years of age)

Samples with complete demographic information, valid SSRT and 4 Caucasian grandparents N = 7,454

Adjust scores of individuals on stimulant medication within 48 h of testing
(SSRT + 0.75 x 154.5)

Create SSRT z-scores controlling for age, age * age and gender

Select one sibling per family

Sample before genotyping N = 5,366

**Genotyping**

Extract & quantify DNA from Saliva
DNA Genotek method & Pico Green
(minimum 50 µg/ml)

Pre-Array Quality Control (QC)
DNA quality using agarose gels

HumanCoreExome array (~500,000 SNPs) &
OMNI1M array (~1,000,000 SNPs)

Standard post-array QC with Principle components analysis (PCA) for population stratification N = 4,687

Imputation using IMPUTE2

Quantitative GWAS (MAF > 1%)
Linear regression with significant principle components as covariates
Sample Characteristics

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<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>N</td>
<td>4683</td>
</tr>
<tr>
<td>Age (mean, SD)</td>
<td>11.0 (2.8)</td>
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<tr>
<td>Gender – Male (Freq, %)</td>
<td>2420 (52%)</td>
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<tr>
<td>On Stimulant Medication within 48 h of testing (Freq, %)</td>
<td>128 (2.7%)</td>
</tr>
<tr>
<td>SSRT (mean, SD)</td>
<td>317.9 (162.1)</td>
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</table>

![Histogram of SSRT z-scores](image)
SSRT GWAS

Crosbie, Burton, Arnold, Schachar, et al 2013
<table>
<thead>
<tr>
<th>Rank</th>
<th>Chr Location</th>
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Crosbie, Burton, Arnold, Schachar, et al 2013
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Crosbie, Burton, Arnold, Schachar, et al 2013
Spit for Science: More to come

- What’s next?
- Confirmation/refinement analyses
- Rare variant
- HD GWAS ***
- Other: OCD, ADHD quantitative traits
Cross disorder collaboration
Background and Rationale

• Neuropsychiatric disorders such as ASD, ADHD, OCD and ID are highly heterogeneous
  – Phenotype
  – Neurobiology
  – Genetic

• Also overlapping
  – Genetic
  – Phenotypic
  – Neurobiological?
The plan: assessment

- Clinical Assessment
  - n=1000
- Genetics
  - n=1000
- Cognitive Phenotyping
  - n=300
- Epigenetics
  - n=300
- Imaging
  - n=150
- Autonomic Nervous System
  - n=140
- Clinical Trial
  - n=60
Model of Neurodevelopmental disorders
Multi-factorial, polygenic, threshold disorders

ADHD, OCD, ASD, ID

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Inhibiting, planning, preparing, holding, inhibiting, switching, error detection and adjustment

Functional connectivity, neurotransmission

Grey and white matter

Smoking, drinking, brain trauma, toxins

Common and rare variants

Adapted from Crosbie et al, 2008
Response Inhibition – ND disorders

• No studies with ADHD, OCD and/or ASD have been compared directly in order to determine whether an inhibition deficit is unique to a single mental illness or common to several.

• No direct comparisons of the structural or functional correlates on response inhibition could determine whether deficient response inhibition in ADHD, OCD and possibly ASD arises from a common neural mechanism.
Aim

- Compare ASD, ADHD and OCD with age, sex and matched controls from the ADHD clinic.

- Hypotheses
  - ADHD, OCD and ASD will exhibit deficient RI compared to age matched controls.
Method

• Cross site, cross disorder analyses involving the clinical assessment/phenotyping and experimental cognitive phenotyping

• All sites; SickKids, McMaster and Holland Bloorview, Lawson.
Sample
# POND Flow of Cases

<table>
<thead>
<tr>
<th>Stage</th>
<th>ASD</th>
<th>ADHD</th>
<th>OCD</th>
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<td>Stage 4</td>
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<td>Valid Stop</td>
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<td>99</td>
<td>51</td>
<td>2 (1)</td>
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Cross Disorder Phenotypes
ADHD Traits - SWAN

SWAN ADHD IN

Symptom #

SWAN ADHD Hyp - Imp

Symptom #
Interpolated Stop Reaction Time (Controlling for age and sex and with medication correction)

Control vs ASD p<0.0001

Control vs OCD p<0.0001

Control vs ADHD p=0.0004
Interpolated Stop Reaction Time (Controlling for age, sex, number of inattentive symptoms and with medication correction)

Control vs ASD $p<0.0002$

Control vs OCD $p<0.0006$

Control vs ADHD $p=0.08$
Go reaction time (Controlling for age and sex)

ADHD vs OCD p=0.05

OCD vs ASD P = 0.05
Post Error Slowling (Controlling for age and sex)

Control vs ADHD $p=0.004$

ADHD vs OCD $p=0.005$
POND Summary

• SSRT – significant deficits across groups
  – Clear evidence of ASD deficit
  – Supported by other resent findings by Ellen Van der Plas in the OSC sample.
  – Clear evidence of OCD deficit – previously not clear in children

• Post Error Slowing – ADHD deficit (further exploration ongoing)

• Reaction time – no significant differences
• Reaction time variability – no significant differences
POND Future Work

- Explore variance accounted for by quantitative phenotypic trait measures
- Neuroimaging (analysis ongoing)
- Genetics
Overview Conclusions

• ADHD / Neurodevelopmental Disorders
  – Etiological challenges
• Endophenotypes: definition and rationale
• Criteria for validating
• Response Inhibition, example in ADHD
• Application in clinical sample
• Large population sample : Spit for Science
• Cross Disorder application and considerations
Investigators and Collaborators

Department of Psychiatry
Russell Schachar
Paul Arnold
Tara Goodale
Rebecca Jantzen
Janet Shan
Annie Dupuis

Genetics & Genomic Biology Sickkids
Stephen Scherer
Christian Marshall
Lisa Strug
Andrew Paterson

Other institutions
Evdokia Anagnostou + POND
Steve Faraone + PGC (Broad Institute)
Gordon Logan (Vanderbilt)