Attention Deficit Hyperactivity Disorder (ADHD) as an Example for the Genetics of Complex Traits

Max Muenke, M.D.
Chief, Medical Genetics Branch
Director, Medical Genetics and Genomic Medicine Training
National Human Genome Research Institute
National Institutes of Health
No Conflict
Holoprosencephaly

Muenke Syndrome
Holoprosencephaly

ADHD

Muenke Syndrome

Fatty Liver
Outline

• Introduction
• Genetics of ADHD
• Genetic Approaches to Understanding ADHD
• ADHD Susceptibility Genes: *LPHN3* & *NCAM1*
• Pharmacogenetic Studies in ADHD
• LPHN3 in Animal Studies
• Conclusion
Attention Deficit Hyperactivity Disorder (ADHD)

- Excessive inattention, hyperactivity and impulsivity for a given developmental level
  - Multi-point DSM-IV criteria for each category

Most common behavioral disorder of childhood
  - Prevalence: 3.0-7.5% of children ages 6-12

- Associated DSM-IV disorders
  - ODD - Oppositional Defiant Disorder
  - CD - Conduct Disorder
  - SUD – Substance Use Disorder

- Evidence for genetic basis of ADHD
  - Familial ADHD
  - Twin and adoption studies
ADHD: Inattention

- careless errors, inattentive to detail
- sustains attention poorly
- *appears* to not be listening
- follows through poorly on obligations
- disorganized
- avoids or dislikes sustained mental effort
- loses needed objects
- easily distracted
- forgetful
ADHD: Hyperactivity/Impulsivity

Often

- fidgets or squirms
- can’t stay seated
- restless (subjective in adolescents)
- loud, noisy
- always “on the go”
- talks excessively
- blurts out
- impatient
- intrusive
DSM IV Diagnosis of ADHD

- 6/9 symptoms required for each subtype
- Clear impairment (social, academic, or occupational)
- Some symptoms cause impairment by age 7
- Impairment present in more than one setting
- Not accounted by another condition (e.g. autism, psychosis, depression, ...)
Bradley (1937) — original conceptualization of ADHD involved testing of response to stimulant.
History

• Hippocrates: 493 BCE “patients with quickened responses to sensory experience, but also less tenaciousness because the soul moves on quickly to the next impression. Proposed etiology “overbalance of fire over water”. Treatment included: “barley rather than wheat bread, fish rather than meat, water drinks and many natural and diverse physical activities”.

• In 1845 Dr. Heinrich Hoffmann a German Pediatrician wrote a book for children with illustrations, about children and inappropriate behaviors.
Slovenly Peter
or Cheerful Stories and Funny Pictures for Good Little Folks

Dr. Heinrich Hoffmann
translated into English jingles by

Mark Twain
The Story of Fidgety Philip

"Let me see if Philip can Be a little gentleman; Let me see if he is able To sit still for once at table." Thus spoke, in earnest tone, The father to his son; And the mother looked very grave To see Philip so misbehave.
Seht, ihr lieben Kinder, seht,
wie's dem Philipp weiter geht!
Oben steht es auf dem Bild.
Seht! er schaukelt gar zu wild,
bis der Stuhl nach hinten fällt.
Da ist nichts mehr, was ihn hält.
Nach dem Tischtuch greift er, schreit.
Doch was hilft's? Zu gleicher Zeit
fallen Teller, Flasch und Brot.
Vater ist in großer Not,
und die Mutter blicket stumm
auf dem ganzen Tisch herum.

Nun ist Philipp ganz versteckt,
und der Tisch ist abgedeckt.
Was der Vater essen will,
unten auf der Erde rollt.
Suppe, Brot und alle Bissen,
alles ist herabgerissen.
Suppenschüssel ist entzwei,
und die Eltern stehen dabei.
Beide sind gar zornig sehr,
haben nichts zu essen mehr.
As he trudged along to school, 
It was always Johnny’s rule 
To be looking at the sky 
And the clouds that floated by; 
But what just before him lay, 
In his way, 
Johnny never thought about; 
So that every one cried out - 
“Look at little Johnny there, 
Little Johnny Head-In-Air!”
Einst ging er an Ufers Rand mit der Mappe in der Hand.
Nach dem blauen Himmel hoch sah er, wo die Schwalbe flieg,
also daß er kerzengrad immer mehr zum Flusse trat.
Und die Fischlein in der Reih sind erstoben sehr, alle drei.

Doch zum Glück die kommen zwei Männer aus der Nähe herbei,
und die haben ihn mit Stangen aus dem Wasser aufgelangen.

Seht nur steht er triefend naß!
Es, das ist ein schlechter Spaß!
Wasser läuft dem armen Wicht aus den Haaren ins Gesicht,
aus den Kleidern, von den Armen,
und es friert ihn zum Erbarmen.

Doch die Fischlein alle drei,
swimmen hurtig gleich herbei:
streckens Köpfe aus der Flut,
lachen, daß man's hören tut,
lachen fort noch lange Zeit.
Und die Mappe schwimmt schon weit.

Noch ein Schritt und plumps! der Hanns stürzt hinab kopfüber ganz! —
Die drei Fischlein, sehr erschreckt, haben sich sogleich versteckt.
Is ADHD Genetic?
What causes ADHD?

Environment

Genetics

ADHD
Adding or removing one of these factors doesn’t really tip the scale; that particular factor isn’t the cause of the disease.
Understanding Genetic and Environmental Influences Using Twin Studies

*Monozygotic (MZ) Twins*
- 100% genes
- 100% home environment

*Dizygotic (DZ) Twins*
- 50% genes
- 100% home environment

*We are a combination of our genes and environment.*
Genetics of ADHD

Twin concordance studies in ADHD

Heritability >70%
(regardless of criteria and country studied)
Studies Show ADHD Is a Genetic Disorder

Average Genetic Contribution of ADHD Based on Twin Studies

Hudziak, 2000
Nadder, 1998
Levy, 1997
Sherman, 1997
Silberg, 1996
Gjone, 1996
Thapar, 1995
Schmitz, 1995
Edelbrock, 1992
Gillis, 1992
Goodman, 1989
Willerman, 1973

Breast cancer
Asthma
Schizophrenia
Height

<table>
<thead>
<tr>
<th>Name</th>
<th>Life prevalence</th>
<th>Heritability</th>
<th>Essential characteristics</th>
<th>Notable feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>0.132</td>
<td>0.58</td>
<td>Dementia, defining neuropathology</td>
<td>Of the top ten causes of death in the United States, Alzheimer’s disease alone has increasing mortality</td>
</tr>
<tr>
<td>Attention-deficit hyperactivity disorder (ADHD)</td>
<td>0.053</td>
<td>0.75</td>
<td>Persistent inattention, hyperactivity, impulsivity</td>
<td>Costs estimated at ~US$100 \times 10^9 per year</td>
</tr>
<tr>
<td>Alcohol dependence (ALC)</td>
<td>0.178</td>
<td>0.57</td>
<td>Persistent ethanol use despite tolerance, withdrawal, dysfunction</td>
<td>Most expensive psychiatric disorder (total costs exceed US$225 \times 10^9 per year)</td>
</tr>
<tr>
<td>Anorexia nervosa</td>
<td>0.006</td>
<td>0.56</td>
<td>Dangerously low weight from self-starvation</td>
<td>Notably high standardized mortality ratio</td>
</tr>
<tr>
<td>Autism spectrum disorder (ASD)</td>
<td>0.001</td>
<td>0.80</td>
<td>Markedly abnormal social interaction and communication beginning before age 3</td>
<td>Huge range of function, from people requiring complete daily care to exceptional occupational achievement</td>
</tr>
<tr>
<td>Bipolar disorder (BIP)</td>
<td>0.007</td>
<td>0.75</td>
<td>Manic-depressive illness, episodes of mania, usually with major depressive disorder</td>
<td>As a group, nearly as disabling as schizophrenia</td>
</tr>
<tr>
<td>Major depressive disorder (MDD)</td>
<td>0.130</td>
<td>0.37</td>
<td>Unipolar depression, marked and persistent dysphoria with physical and cognitive symptoms</td>
<td>Ranks number one in the burden of disease in the world</td>
</tr>
<tr>
<td>Nicotine dependence (NIC)</td>
<td>0.240</td>
<td>0.67</td>
<td>Persistent nicotine use with physical dependence (usually cigarettes)</td>
<td>Major preventable risk factor for many diseases</td>
</tr>
<tr>
<td>Schizophrenia (SCZ)</td>
<td>0.004</td>
<td>0.81</td>
<td>Long-standing delusions and hallucinations</td>
<td>Life expectancy decreased by 12–15 years</td>
</tr>
</tbody>
</table>

*Most of these definitions are made more restrictive by requiring persistence over time (for example, the criteria for SCZ require \( \geq 6 \) months of symptoms), substantial impairment and presence across multiple different contexts. See Supplementary information S1 (table) for more detail. Additional sources are REFS 1, 2, 181–183.*
Clinical Approaches to ADHD

Determination of phenotypic severity

ADHD subtypes

- Inattention
- Hyperactivity
- Impulsivity

Comorbidities

- ODD, CD
- Alcohol dependence
- Nicotine dependence
- Depression
- Anxiety, Phobias

Severity

ADHD Comorbid Clusters
Populations Segregate into Phenotypic Clusters

Multi-point DSM-V criteria for specific disorders

Comorbidities

Inattention
Hyperactivity
Impulsivity
ODD
CD
Nicotine
Alcohol
Research Designs for Human Genetic Studies

• Possible designs:
  – Determine heritability estimates
  – Candidate gene studies
  – Genome wide scans

• Types of studies:
  – Sib pairs
  – Family studies (TDT, linkage, etc)
  – Association studies (case/control)
Genetic Approaches to ADHD

Advantages:
- Power
- Homogeneity

Disadvantages:
- Heterogeneity
- Large samples

Identifying ADHD Genes

Small pedigrees

Disadvantages:
- Genetic isolate
- Bi-lineality

Large pedigrees

Genetic isolate
- Bi-lineality
Genetics of Population Isolates: Paisas in Antioquia
Genetics of Population Isolates: Paisas in Antioquia

Co-morbidities

- ADHD
- CD (Conduct disorder)
- Alcohol dependence
- Nicotine dependence
- ODD (Oppositional defiant disorder)

Possibly affected
Genome-Wide Scan for ADHD Linkage

- 8p23.1
- 17p11
- 11q22
- 4q13.2
- 5q33.3
- 17p11
- 8p23.1
- 11q22

Chromosome 4
Latrophilin 3 is a member of the LPHN subfamily of G-protein coupled receptors (GPCRs).
Haplotypes were determined at markers in the minimal critical region: rs7678046, rs1901223, rs6813183 and rs1355368.

Haplotype **A-G-C-G** is associated with increased risk of ADHD, and is termed the “susceptibility” haplotype.

Haplotype **G-G-G-A** is associated with the absence of ADHD, and is termed a “protective” haplotype.

### LPHN3 Haplotype Variants: Susceptibility and Protection

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>ADHD cases</th>
<th>Controls</th>
<th>ADHD freq</th>
<th>Control freq</th>
<th>RR</th>
<th>95% Low</th>
<th>95% High</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-A-C-G</td>
<td>88</td>
<td>91</td>
<td>0.287</td>
<td>0.362</td>
<td>1.7</td>
<td>0.63</td>
<td>4.789</td>
<td>0.051</td>
<td>0.8208</td>
</tr>
<tr>
<td>A-A-G-A</td>
<td>53</td>
<td>61</td>
<td>0.139</td>
<td>0.209</td>
<td>1.5</td>
<td>0.555</td>
<td>3.837</td>
<td>1.795</td>
<td>0.1803</td>
</tr>
<tr>
<td>A-G-C-G</td>
<td>76</td>
<td>48</td>
<td>0.417</td>
<td>0.213</td>
<td>4.3</td>
<td>1.413</td>
<td>12.96</td>
<td>12.33</td>
<td>0.00044</td>
</tr>
<tr>
<td>G-G-C-G</td>
<td>45</td>
<td>49</td>
<td>0.133</td>
<td>0.162</td>
<td>1.8</td>
<td>0.596</td>
<td>5.411</td>
<td>0.015</td>
<td>0.902</td>
</tr>
<tr>
<td>G-G-G-A</td>
<td>22</td>
<td>35</td>
<td>0.025</td>
<td>0.054</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4.547</td>
<td>0.03298</td>
</tr>
</tbody>
</table>
Metabolic Brain Analysis Using Proton Magnetic Resonance Spectroscopy (1H-MRS)

- 1H-MRS provides an index of neuronal number, metabolism or viability.
- N-acetylaspartate/creatine ratio is decreased in ADHD individuals.
- We screened in the Paisas:
  - 15 individuals with at least one copy of the susceptibility haplotype
  - 10 individuals with at least one copy of the protective haplotype
  - 8 individuals with other, different haplotype variants
Metabolic Brain Analysis: N-acetylaspartate to Creatine ratios (NAA/Cr)

**Right medial thalamus**

**Right lateral thalamus**

The NAA/Cr ratio varies inversely with the number of ADHD susceptibility alleles.
Prevalence of Diagnosis & Medication Treatment for ADHD Among Children Aged 4-17 Years, US, 2007

Source: CDC. MMWR 2010;59(44).
The susceptibility allele is associated with response to stimulant medication.

Arcos-Burgos et al. 2010
Latrophilin 3 Confers Susceptibility to ADHD

- LPHN3 identified by linkage analysis
- Replication of association between LPHN3 and ADHD in worldwide samples (n=6360)
- LPHN3 is expressed in brain areas related to attention
- LPHN3 susceptibility variants alter neural circuits implicated in ADHD
- LPHN3 variants associated with response to stimulant medication

Arcos-Burgos et al. 2010
Latrophilin 3 and ADHD

Contribution of LPHN3 to the genetic susceptibility to ADHD in adulthood: a replication study

M. Ribasés*,†,‡, J. A. Ramos-Quiroga†,§, C. Sánchez-Mora†,‡, R. Bosch†, V. Richarte†, G. Palomar†, X. Gastaminza†, A. Bielsa†, M. Arcos-Burgos‖, M. Muenke‖, F. X. Castellanos***,†, B. Cormand†,§,‖, M. Bayés*** and M. Casas†,§

Keywords: Adult ADHD, attention-deficit/hyperactivity disorder, case–control association study, LPHN3

Received 22 March 2010, revised 2 July 2010 and 24 August 2010, accepted for publication 31 August 2010
Behavioral Genetics in Zebrafish

1. Inject morpholino against gene of interest into fertilized egg

2. Check splice blocking with RTPCR

3. Raise to 5 days post fertilization when larvae are freely swimming
During phototaxis, larvae rapidly approach a target light cue.

Phototaxis requires selectively increasing the initiation frequency of forward movements in larvae facing the target.
LPHN3 and ADHD

ORIGINAL ARTICLE
The ADHD-susceptibility gene lphpn3.1 modulates dopaminergic neuron formation and locomotor activity during zebrafish development

M Lange¹, W Norton¹, M Coolen¹, M Chaminade¹, S Merker², F Proft², A Schmitt², P Vernier³, K-P Lesch² and L Bally-Cuif¹
Deeann Wallis⁴°, Denise S. Hill⁵, Ian A. Mendez⁴°, Louise C. Abbott⁴, Richard H. Finnek⁴, Paul J. Wellman⁶, Barry Setlow⁷

April 17, 2012
Genetics of Attention Deficit Hyperactivity Disorder

To correlate molecular and clinical findings to identify predictors of long-term prognosis in ADHD and related disorders

- Ongoing Studies / Future Plans:
- Predictors of treatment response
- Predictors of long-term outcome including:
  - Severity of core symptoms
  - Association with comorbid disorders (ODD, CD, SUD, and others)
- Personalized diagnosis and treatment
Thank You
Attention Deficit Hyperactivity Disorder (ADHD) as an Example for the Genetics of Complex Traits

Max Muenke, M.D.
Chief, Medical Genetics Branch
Director, Medical Genetics and Genomic Medicine Training
National Human Genome Research Institute
National Institutes of Health