Association of dopamine genes and attention-deficit hyperactivity disorder (ADHD)

Julian Buss
ADHD facts

- 5-10% children affected
- 30-50% to adulthood
- Boys 8x likely
- Difficult to characterize
- Difficult to diagnose
- Familial, inheritable (but not consistent with Mendelian patterns)
ADHD characterization

2 Dimensions:
- Inattention
- Hyperactivity/impulsivity

3 subtypes in DSM IV:
- Predominantly inattentive type (ADHD-AD)
- Predominantly hyperactive-impulsive type (ADHD-HI)
- Combined type (ADHD-CT)
Associated but distinct disorders

- Conduct disorders
- Learning disability
- Oppositional defiant disorder
- Tourette’s disorder
- Speech or language disability
- Disorders affecting attention
  - Anxiety disorders
  - Mood disorders
  - Substance abuse
  - Schizophrenia or psychosis
  - Generalized resistance to thyroid hormone
How is ADHD genetically linked?

- Estimated 4-5 genes involved
- 2 genes identified so far (by VNTR)
  - DRD4 (dopamine receptor – 4)
  - DAT1 (dopamine transporter)
- Dopamine pathway likely due to positive treatments of methamphetamine and other dopamine agonists
Genetic linkage - methods overview

- Conduct large-scale assessment of ADHD candidates (1000 or more)
  - DSM III or DSM IV standardized tests
  - Experienced ADHD specialists (doctors, psychologists)
- Identify control and ADHD probands
- Obtain data from family for identifying genetic linkage
- Obtain blood samples from all participants!
Genetic linkage – methods overview

- Exclusions:
  - At least 3 month history of treatment with clinical response to methylphenidate
  - IQ testing (85 min, standardized for children)
  - Absence of comorbid diagnoses
    - Anxiety, mood, conduct, Tourette’s disorder, pervasive developmental disorder, etc.
Genetic linkage – methods overview

- Blood samples:
  - Extract genomic DNA from whole blood by salting out
  - PCR amplification
    - 48-bp region for DRD4
    - 40-bp region for DAT1
  - Separate PCR products via agarose gel electrophoresis
Genetic linkage - statistical analysis

- HRR test (haplotype relative risk)
  - Provides a test for association of an allele with a disorder
  - HRR = \( 4n(w - y)^2 / [(w + y)(4n - w - y)] \)
    - N = total probands
    - W = transmitted allele
    - Y = non-transmitted allele

- TDT analysis (transmission disequilibrium test)
Why DRD4?

- Stimulant medications affect dopamine pathway
- In vitro studies suggest that the receptor encoded by the DRD4 7-repeat allele are sub-sensitive to endogenous dopamine compared to DRD4 2-repeat and DRD4 4-repeat alleles
Why DRD4?

- D4 receptors are present in cortical areas including the anterior cingulate gyrus, a brain region that plays an important role in normal attention and abnormal attention.
- Dopamine genes had been found to be responsible for other neurobehavioral disorders.
Dopamine Receptor D4, DRD4

- Van Tol et al. (1991) cloned D4 receptor
- 11p15.5
- Allelic variants (48-bp sequence in the putative third cytoplasmic loop of exon-III:
  - Direct repeat (D4.2)
  - 4-fold repeat (D4.4)
  - 7-fold repeat (D4.7)
## DRD4 research

- 5 studies to date on DRD4-ADHD relationship

<table>
<thead>
<tr>
<th>Reference</th>
<th>DRD4-ADHD association</th>
<th>N for probands</th>
<th>Statistics used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swanson et al.</td>
<td>+</td>
<td>52</td>
<td>HRR</td>
</tr>
<tr>
<td>Rowe et al.</td>
<td>+</td>
<td>116</td>
<td>HRR</td>
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<tr>
<td>Smalley et al.</td>
<td>+</td>
<td>133</td>
<td>TDT</td>
</tr>
<tr>
<td>LaHoste et al.</td>
<td>+</td>
<td>39</td>
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<tr>
<td>Castellanos et al.</td>
<td>-</td>
<td>41</td>
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</table>
## DRD4 allele proportions

<table>
<thead>
<tr>
<th>Alleles</th>
<th>7 – Repeats</th>
<th>4 - Repeats</th>
<th>2 - Repeats</th>
<th>Other Repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FBA designs</strong></td>
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<tr>
<td>Swanson et al:</td>
<td></td>
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</tr>
<tr>
<td>Parents – 208</td>
<td>0.226</td>
<td>0.663</td>
<td>0.072</td>
<td>0.039</td>
</tr>
<tr>
<td>Probands – 104</td>
<td>0.279</td>
<td>0.635</td>
<td>0.077</td>
<td>0.018</td>
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<tr>
<td>Controls – 104</td>
<td>0.173</td>
<td>0.692</td>
<td>0.069</td>
<td>0.067</td>
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<tr>
<td>Smallet et al:</td>
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<tr>
<td>Parents – 440</td>
<td>0.293</td>
<td>0.439</td>
<td>0.152</td>
<td>0.116</td>
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<tr>
<td>Probands – 220</td>
<td>0.350</td>
<td>0.414</td>
<td>0.141</td>
<td>0.095</td>
</tr>
<tr>
<td>Controls – 220</td>
<td>0.236</td>
<td>0.464</td>
<td>0.164</td>
<td>0.136</td>
</tr>
<tr>
<td><strong>PBA designs</strong></td>
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<td></td>
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</tr>
<tr>
<td>LaHoste et al:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probands – 78</td>
<td>0.282</td>
<td>0.513</td>
<td>0.154</td>
<td>0.051</td>
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<tr>
<td>Controls – 78</td>
<td>0.115</td>
<td>0.756</td>
<td>0.128</td>
<td>0.000</td>
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<tr>
<td>Castellanos et al:</td>
<td></td>
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<tr>
<td>Probands- 82</td>
<td>0.220</td>
<td>0.683</td>
<td>0.073</td>
<td>0.024</td>
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<tr>
<td>Controls – 112</td>
<td>0.205</td>
<td>0.723</td>
<td>0.027</td>
<td>0.045</td>
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<tr>
<td>Rowe et al:</td>
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<tr>
<td>Probands – 214</td>
<td>0.243</td>
<td>0.617</td>
<td>0.061</td>
<td>0.079</td>
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<tr>
<td>Controls - 116</td>
<td>0.129</td>
<td>0.690</td>
<td>0.112</td>
<td>0.112</td>
</tr>
</tbody>
</table>
DRD4 expression

- DRD4 – 7 repeat allele presence:
  - Reduced activity in the mesolimbic dopamine pathway
  - May code for sub-sensitive dopamine receptors in the frontal lobes and produce underactivity in the neural networks involved in executive function
Phillip, the hyper-hypo
DAT1 – another susceptibility gene

- DAT1 acts to take released dopamine back up into presynaptic terminals
- Vandenberghe et al. (1992) cloned cDNA’s
- Mapped to 5p15.3 via in situ hybridization and PCR amplification of rodent/human somatic cell hybrid DNAs
- Knockout mice for the DAT1, showing compromised dopamine transport, exhibit extreme hyperactivity
Dopamine transporter KO mice

- Dopamine persists at least 100 times longer in synaptic space
- Mice show indifference to cocaine and amphetamine
- Mice show spontaneous hyperlocomotion
- Dopamine transporter is the target of cocaine and amphetamine
DAT1 functioning at synapse
## DAT1

- 40-bp repeat sequence
- Common variants include 9 or 10 repeats

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<td>+</td>
<td>57</td>
<td>HRR</td>
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<td>Gill et al.</td>
<td>+</td>
<td>40</td>
<td>HRR</td>
</tr>
<tr>
<td>Waldman et al</td>
<td>+</td>
<td>122</td>
<td>TDT</td>
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</table>
## DAT1 allele proportions:

<table>
<thead>
<tr>
<th>Alleles</th>
<th>10 – Repeat</th>
<th>9 – Repeat</th>
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<tr>
<td>FBA Designs</td>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents – 168</td>
<td>0.768</td>
<td>0.226</td>
<td>0.006</td>
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<tr>
<td>Probands – 84</td>
<td>0.857</td>
<td>0.143</td>
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<tr>
<td>Controls – 84</td>
<td>0.679</td>
<td>0.321</td>
<td>0.000</td>
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<tr>
<td><em>Gill et al:</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Parents – 124</td>
<td>0.710</td>
<td>0.290</td>
<td>0.000</td>
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<tr>
<td>Probands – 62</td>
<td>0.839</td>
<td>0.161</td>
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<tr>
<td>Controls – 62</td>
<td>0.645</td>
<td>0.335</td>
<td>0.000</td>
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<tr>
<td><em>Waldman et al:</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Probands - 234</td>
<td>0.69</td>
<td>0.29</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Treatments

- Stimulant medications
  - Methamphetamines
  - Amphetamines
- Typically 1-2 times a day (oral)
- Until symptoms subside (may take years)
Future research

- Still directed at understanding dopamine pathway, role of dopamine in neurobehavioral disorders
- Possible identification of other target genes involved
- Twin and family based studies
References

References

- http://www.rcsb.org/