Attention-Deficit/Hyperactivity Disorder Endophenotypes

Alysa E. Doyle, Erik G. Willcutt, Larry J. Seidman, Joseph Biederman, Virginie-Anne Chouinard, Julie Silva, and Stephen V. Faraone

Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable disorder with a multifactorial pattern of inheritance. For complex conditions such as this, biologically based phenotypes that lie in the pathway from genes to behavior may provide a more powerful target for molecular genetic studies than the disorder as a whole. Although their use in ADHD is relatively new, such “endophenotypes” have aided the clarification of the etiology and pathophysiology of several other conditions in medicine and psychiatry. In this article, we review existing data on potential endophenotypes for ADHD, emphasizing neuropsychological deficits because assessment tools are cost effective and relatively easy to implement. Neuropsychological impairments, as well as measures from neuroimaging and electrophysiological paradigms, show correlations with ADHD and evidence of heritability, but the familial or genetic overlap between these constructs and ADHD remains unclear. We conclude that these endophenotypes will not be a quick fix for the field but offer potential if careful consideration is given to issues of heterogeneity, measurement and statistical power.

Key Words: ADHD, endophenotype, genetic, neuropsychology, executive functions

Behavioral genetic studies leave no doubt that genes play a significant role in the development of attention-deficit/hyperactivity disorder (ADHD). Heritability estimates from twin studies are consistently high, ranging from .6 to .9 (e.g., Hudziak et al 1998; Rhee et al 1999; Sherman et al 1997). Yet, molecular genetic studies of ADHD have yielded conflicting results. Candidate gene studies show an inconsistent pattern of replication (Faraone et al 2005), and the three research groups that have conducted genome scans of ADHD thus far have identified largely nonoverlapping chromosomal regions as potentially harboring susceptibility genes (Arcos-Burgos et al 2004; Bakker et al 2003; Fisher et al 2002; Ogdie et al 2002). Such inconsistencies, although often found in complex phenotypes in which multiple genetic and nongenetic factors are acting in concert, present challenges to understanding the genetic architecture of ADHD.

Two reasons behind inconsistencies in molecular genetic studies of complex conditions are low statistical power to detect genes of small effect and heterogeneity (Faraone et al 1999) and research suggests that these characteristics are true of ADHD. In a recent meta-analysis, candidate genes from the catecholamine and serotonin systems that were significantly associated with ADHD showed pooled odds ratios ranging from 1.2 to 1.5 (Faraone et al 2005). Suarez et al (1994) have also shown low power to find genes of small magnitude could lead to an inconsistent pattern of replication across genome scans. Both twin and family studies raise the further possibility of genetic heterogeneity in ADHD (Faraone, unpublished data; Rasmussen et al 2002; Todd et al 2001). Although subgroups have not been definitively parsed, promising delineations might occur via comorbidity (e.g., with conduct and bipolar disorders [Doyle and Faraone 2002; Faraone et al 1998]), persistence of ADHD into adolescence (Faraone et al 2000), empirically derived latent classes (Todd 2000), and, in population but not clinical samples, DSM-IV subtypes (Faraone 2002). Molecular genetic studies have begun to explore sources of heterogeneity (McCraken et al 2000; Rowe et al 1998; Waldman et al 1998), but results have not been definitive because large samples are needed for subgroup analyses.

To address these challenges, there is growing interest in using endophenotypes in molecular genetic studies. The term “endophenotype” has been used in various ways. Most definitions refer to a phenotype more proximal to the biological etiology of a clinical disorder than its signs and symptoms and influenced by one or more of the same susceptibility genes as the condition (e.g., Almasy and Blangero 2001; Gottesman and Gould 2003; Skuse 2001). The power of these biologically based phenotypes is based on several assumptions, most important of which is that the endophenotype is less genetically complex than the disorder it underlies. This reduced complexity is due both to the endophenotype’s relative proximity to gene products in the chain of events leading from gene to behavior and to its potential to target one of likely several pathophysiological deficits that combine to create the overall condition. Because the endophenotype is influenced by fewer genetic (and environmental) risk factors than the disorder as a whole, its use would result, theoretically, in greater statistical power to detect the effects of the individual genes. Additionally, endophenotypes can also be used to help elaborate on or revise the suspected pathophysiological basis of the condition (Freedman et al 1999; Gottesman and Gould 2003), including heterogenous processes, via subsequent expression studies.

Although there is no definitive pathophysiological model of ADHD, evidence for frontostriatal impairment in ADHD comes from the success of stimulant medications as well as animal models of hyperactivity that implicate dopamine pathways consistent with these regions (e.g., Gainetdinov et al 1999; Rubinstein et al 1997). Additionally, behavioral similarities exist between adult patients with frontal lesions and children with ADHD (Mates 1980). Dysfunction in frontostriatal pathways has also been demonstrated by neuroimaging studies (e.g., Seidman et al 2005), electrophysiological studies (Chabot and Serfontein...
Proposed criteria for useful endophenotypes in psychiatry (e.g., Almasy and Blangero 2001; Gottesman and Gould 2003; Leboyer et al 1998; Skuse 2001) vary somewhat but share several key elements. Specifically, researchers suggest that useful endophenotypes should 1) co-occur with the condition of interest; however, because an endophenotype may be useful for understanding heterogenous conditions, it need not be universal within the disorder; 2) be measured by tools with good psychometric properties, including reliability; 3) show evidence of heritability; and 4) show familial–genetic overlap with the disorder in question. The issue of familial overlap is important because, without such evidence, we could find genes for a biologically based phenotype, but they may not be genes for the disorder of interest. Because an endophenotype is conceptualized as an expression of the genetic liability for a disorder, it should appear in individuals who carry genes for a condition but do not express the disorder itself, that is, the unaffected relatives of diagnosed individuals. Deficits found in affected but not unaffected relatives raises the possibility that impairments are a result of the disorder itself or of unique environmental factors. The presence of an endophenotype in unaffected relatives may further augment the statistical power of molecular genetic studies because of their increased prevalence in families compared with the disease entity.

In this article, we focus on association with ADHD, heritability, and familial overlap of candidate deficits from neuropsychological measures of inhibition to assess their suitability as ADHD endophenotypes. We then briefly summarize these criteria as they relate to neuromaging and psychophysiological measures. We address measurement issues in our discussion of strategies to move the field forward. For more in-depth discussion of measurement issues related to neuropsychological endophenotypes for ADHD, including sensitivity, construct and discriminant validity, and developmental factors, we refer the reader to Doyle et al (in press-b).

Endophenotypes: Criteria

Association with ADHD—Executive Functions

Association with ADHD—Other Neuropsychological Constructs

Other neuropsychological mechanisms such as impairments in state regulation and delay aversion are interesting candidate endophenotypes to consider in conjunction with EF deficits because their association with ADHD is supported empirically and because they may relate to the neuropsychological heterogeneity within ADHD samples. Because of space constraints, we refer the readers to recent reviews of theoretical models that encompass these constructs (Sergeant 2005; Sonuga-Barke 2005) for more detailed explications. Briefly, one of the main contributions of Sergeant and colleagues' cognitive energetic model of ADHD (Sergeant 2000) is their hypothesis that impairments on tasks requiring effortful control of attention and executive processes could be due, at least in part, to deficiencies in activation, arousal, and effort that control the allocation of cognitive resources rather than impaired cognitive resources per se. One potential index of such state regulation difficulties is variability of reaction time (RT), a measure of the consistency of a response after presentation of a stimulus. As reviewed by Castellanos and Tannock (2002), RT variability is one of the most replicated deficits in ADHD. Yet like EF deficits, RT variability does not appear to be universal within ADHD samples (Nigg et al 2005).

Delay aversion is a construct grounded in an animal model of altered reinforcement and extinction processes. Such processes are hypothesized to relate to dysfunction in the meso-limbic-cortical branch of the dopamine system (Johansen et al 2002; Sagvolden and colleagues posited that goal-directed behavior in ADHD youth requires frequent, potent reinforcers proximal to the
behavior being reinforced. If such reinforcers are lacking or distal, inattention and motor impulsivity occur. Consistent with this model, Sonuga-Barke and colleagues have shown that children with ADHD exhibit aversion to delay. That is, they show preferences for immediate but smaller rewards compared with delayed larger rewards, particularly when the immediate reward reduces the length of a task (e.g., (Sonuga-Barke et al 1996). Solanto et al (2001) found that measures of inhibitory control and delay aversion were not highly correlated in ADHD subjects; however, the two measures together identified the majority of ADHD cases in a discriminant function analysis. Based on these data, which were replicated in preschoolers (Sonuga-Barke et al 2003), Sonuga-Barke (2003) has proposed a dual-pathway model of ADHD involving both EF and delay aversion. Although further data are needed to determine whether the predictions of this model are borne out, this theory marks an important contribution to the field as the first formal model of neuropsychological heterogeneity in ADHD.

Association with ADHD—Summary

Although no single neuropsychological deficit has emerged as a necessary and sufficient cause of ADHD, those related to EFs, state regulation (especially RT variability), and delay aversion show replicated association with ADHD and thus fulfill criterion 1 for an ADHD endophenotype. Although we have discussed these three neuropsychological constructs separately in line with their individual theoretical literatures, some researchers may argue that state regulation and the ability to withstand delay are aspects of executive processes. Further work is needed to map the relationship between these constructs and the heterogeneity of these impairments within ADHD samples.

Heritability

For neuropsychological measures to be useful endophenotypes for ADHD, they should show evidence of heritability. A significant literature suggests that general cognitive functioning (IQ) is highly heritable (Plomin 1999); however, data on the heritability of specific neuropsychological functions are limited. Table 1 shows twin studies that have examined constructs that are relevant to ADHD. For ease of explication, the table is divided according to measures of attention, EFs, and other functions; however, it should be noted that many of these measures are multifactorial in nature and are thus supported by multiple cognitive functions. In these samples, heritabilities range from zero to 88%, with the majority of studies showing at least some genetic influence. These data provide preliminary evidence that measures of attention and EF show genetic influence. Yet many studies are characterized by small sample sizes, and only a limited number of measures have been examined.

Although larger samples are needed to estimate more accurately the heritability of relevant neuropsychological measures, current data suggest that these measures may have lower heritability than ADHD. These lower heritability estimates may partially reflect measurement issues. For example, one possibility is that these lower estimates are due to error variance or low reliability. Furthermore, measures that are not normally distributed may not be amenable to quantitative genetic analyses, even after data transformation procedures. Yet even if such measures are less heritable than ADHD, they may be more useful for finding genes than the disorder itself if a smaller number of genes contribute to individual differences on the EF measure than contribute to the overall ADHD diagnosis (because the magnitude of effect for a single gene depends on the number of genes involved; Faraoane et al 2000; Risch 1990a).

To date, few studies have investigated the relationship of individual genes with performance on neuropsychological measures. Studies have suggested an association between the catechol-O-methyltransferase (COMT) val allele and perseverative errors (Egan et al 2001; Joober et al 2002; Malhotra et al 2002), and other studies suggest a role in aspects of attention of the dopamine 4 receptor gene (DRD4; Auerbach et al 2001; Fossella et al 2002) and a region of the monoamine oxidase A gene related to transcription induction (MAOA-LPR; Fossella et al 2002). Thus far, however, none of these studies has documented a gene contributing more than 5% of the variance to test performance. Additionally, studies (Auerbach et al 2001; Fossella et al 2002) also provide evidence that multiple genes are likely to be contributing to these measures. Thus, these data raise the possibility that at least some neuropsychological measures may themselves be complex phenotypes.

Overlap with ADHD

Family Studies. Table 2 illustrates family studies that have assessed neuropsychological deficits in relatives of ADHD youth. Two studies failed to find such deficits in parents of ADHD probands (Asarnow et al 2002; Murphy and Barkley 1996). Studies that have distinguished between affected and unaffected relatives of ADHD probands suggest subtle deficits in unaffected relatives and greater deficits in relatives who themselves have ADHD (Doyle et al, in press-a; Nigg et al 2004; Seidman et al 2000; Slaats-Willemse et al 2003), with a lack of consistency in the specific deficits found to be impaired in unaffected relatives. Nonetheless, although findings are not definitive, the fact that several studies find some evidence of deficits in unaffected relatives provides support for partial familial overlap of ADHD and neuropsychological weaknesses, for example, on measures of inhibition and processing speed.

Adoption Studies. Two adoption studies of ADHD have examined neuropsychological performance. In one, biological parents of ADHD children performed more poorly on measures of visual attention and reaction time than did adoptive relatives of ADHD children (Alberts-Corush et al 1986), but no differences between biological and adoptive parents were found on an impulsivity measure. In the second (Nigg et al 1997), biological parents of ADHD boys showed hemispheric asymmetry on a visuospatial orienting task compared with the adoptive parents of ADHD boys and parents of control boys. Together these studies suggest impairments on measures of visual attention may be part of the genetic susceptibility to ADHD.

Twin Studies. Twin designs can provide estimates of bivariate heritability (h^2g), a statistic ranging from 0 to 1.0 that indicates the extent to which variability in one trait is attributable to the same genetic influences that impact another trait. To date, two twin studies have used objective neuropsychological measures to assess bivariate heritability with ADHD. Kuntsi et al (2001) examined the bivariate heritability of extreme hyperactivity and measures of working memory, delay aversion, and reaction time. Bivariate heritability was not estimated for a measure of response inhibition because it did not differ between the hyperactive group and controls in initial phenotypic analyses. A composite measure of tasks that best discriminated groups with and without hyperactivity was also examined; the composite included measures of reaction time, omission errors, delay aversion task, and verbal IQ. Results showed statistically significant genetic overlap between extreme hyperactivity and RT variability (h^2g = .64) and...
<table>
<thead>
<tr>
<th>Study</th>
<th>N (Pairs: MZ/DZ)</th>
<th>Measure</th>
<th>Function</th>
<th>Twin Intraclass Correlations</th>
<th>Heritability (h²)</th>
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<td></td>
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<td>rMZ</td>
<td>rDZ</td>
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<td></td>
<td>Heritability not calculated</td>
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<td>Goodman and Stevenson 1989</td>
<td>102/111</td>
<td>E scan</td>
<td>Visual attention/scanning</td>
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<td>.33</td>
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<td>Bartfai et al 1991</td>
<td>10 (MZA)/10</td>
<td>SPAN</td>
<td>Selective attention</td>
<td>.53</td>
<td>-.06</td>
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<td>Myles-Worsley and Coon 1997</td>
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<td>SPAN accuracy</td>
<td>Selective attention</td>
<td>.19</td>
<td>.31</td>
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<td></td>
<td></td>
<td>SSAT baseline accuracy</td>
<td>Average identification accuracy</td>
<td>.44</td>
<td>.01</td>
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<td>Selective attention</td>
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<td>.20</td>
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<td></td>
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<td>DS-CPT d'</td>
<td>Target discrimination/vigilance</td>
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<td>.08</td>
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<td>Fan et al 2001</td>
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<td>DS-CPT beta</td>
<td>Decision criteria</td>
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<td>-.14</td>
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<td></td>
<td>ANT Alerting</td>
<td>Maintenance of alert state (vigilance)</td>
<td>.47</td>
<td>.38</td>
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<td></td>
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<td>ANT Orienting</td>
<td>Visual orienting (selective attention)</td>
<td>.10</td>
<td>.40</td>
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<td>20 / 20</td>
<td>CPT-IP matches</td>
<td>Vigilance</td>
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<td>.53</td>
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<td>.79</td>
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<td>.73</td>
<td>.28</td>
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<td></td>
<td></td>
<td>WCST perseverative errors</td>
<td>Perseveration</td>
<td>.49</td>
<td>.21</td>
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<td>Pennington et al 1995</td>
<td>20/30</td>
<td>WCST total errors</td>
<td>Impulsivity/abstract problem solving</td>
<td>.60</td>
<td>.16</td>
</tr>
<tr>
<td>Fan et al 2001</td>
<td>26/26</td>
<td>ANT Conflict</td>
<td>Executive control of attention</td>
<td>.73</td>
<td>.28</td>
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<td>Holmes et al 2002</td>
<td>20/20</td>
<td>MFFT number correct</td>
<td>Impulse control (includes aspects of attention)</td>
<td>.79</td>
<td>-.42</td>
</tr>
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<td>Campana et al 1996</td>
<td>15/9</td>
<td>MFFT number incorrect</td>
<td>Impulse control</td>
<td>.73</td>
<td>-.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CPT-IP false alarms</td>
<td>Impulse control</td>
<td>-.10</td>
<td>.38</td>
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<td>WCST Categories</td>
<td>Abstract problem-solving/set-shifting</td>
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<td>-.06</td>
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<td>Perseveration</td>
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<td>-.01</td>
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<td></td>
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<td>WCST Total Errors</td>
<td>Impulsivity/abstract problem solving/set-shifting</td>
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<td>-.03</td>
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<td>Ando et al 2001</td>
<td>143/93</td>
<td>Revision of spatial and verbal working memory span tasks and developed by Shah and Miyake (1996)</td>
<td>Verbal working memory</td>
<td>.44</td>
<td>.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spatial working memory</td>
<td>Spatial working memory</td>
<td>.50</td>
<td>.22</td>
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the composite score \((h^2_g = .80)\). Bivariate heritability estimates were relatively high for commission errors \((h^2_g = .60)\); however, these were not statistically significant due to the small sample size. Delay aversion did not show any evidence of genetic overlap with hyperactivity \((h^2_g = -.06)\).

The second study examined a larger sample of twins selected for DSM-IV ADHD (Chhabildas et al, unpublished data). The battery included measures of response inhibition, working memory, vigilance, set shifting, and processing speed. Estimates of bivariate heritability were somewhat lower than those obtained by Kuntsi and Stevenson \((h^2_g = 20–38)\) but were significant for all variables with the exception of set shifting. Higher bivariate heritabilities were obtained for inattentive symptoms than for hyperactive–impulsive symptoms. Similar to Kuntsi et al, the highest bivariate heritability was obtained for a composite score that included measures of processing speed, vigilance, working memory, and response inhibition.

### Candidate Gene Studies of Neuropsychological Deficits in ADHD Samples

The small number of molecular genetic studies of ADHD and neuropsychological measures are generally inconclusive. Although three studies find an association between DRD4 and test performance, only one study (Langley et al 2004) found this association to be with the 7-repeat allele that is typically associated with increased risk for ADHD. The other two studies (Manor et al 2002; Swanson et al 2000) suggest that the short alleles of DRD4 were associated with slow, variable, and impulsive responses on computerized attention tests, raising the possibility that both high and low levels of synaptic dopamine could be associated with neurocognitive deficits (Fossella et al 2002). Although one study found an association between the dopamine 5 receptor gene \((GRIN2A)\) and inhibitory control and verbal short term and working memory (Adams et al 2004), however, the association between these latter genes and the ADHD diagnosis overall remains unclear.

### Summary of Neuropsychological Literature

Considered together, family, twin, and adoption studies suggest that impairments on neuropsychological measures related to EF as well as processing speed, visual attention, and response variability may be associated with the genetic liability to ADHD. Family and twin studies also suggest that familial–genetic overlap is most robust for scores based on multiple neurocognitive measures. Yet the low magnitude of bivariate heritability and the relatively small effect size of deficits in unaffected relatives also indicate that either a significant proportion of the genetic influences on ADHD differ from the genetic influences on these measures or else that some factor is limiting the detection of the extent of the shared genetic influences. Such a factor could be measurement issues, underlying neurocognitive heterogeneity of ADHD, or a combination of these. We return to these latter issues in the context of recommendations for future studies.

### Neuroimaging and Electrophysiological Endophenotypes for ADHD

#### Neuroimaging

**Association with ADHD.** Both structural and functional neuroimaging studies have documented abnormalities in frontal-subcortical circuits that regulate attention, inhibition, and intentional motor behavior in ADHD samples (Seidman et al 2005); however, the majority of studies with implications for endophenotype research involve structural neuroimaging. Volumetric differences have been found repeatedly in the dorsolateral prefrontal cortex, the dorsal anterior cingulate cortex, the cau-
<table>
<thead>
<tr>
<th>Study</th>
<th>Diagnostic System</th>
<th>Relatives</th>
<th>N</th>
<th>Measures</th>
<th>Measures Impaired in Relatives</th>
<th>Neuropsychologic Function(s) Implicated by Impairments in Unaffected Relatives (or Correction by Relative ADHD Status)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murphy and Barkley 1996</td>
<td>DSM-IIIR</td>
<td>Parents</td>
<td>25; probands w/ severe ADHD = 25; probands w/ mild ADHD = 25; control subjects = 25</td>
<td>WCST, CPT, Verbal Selective Reminding Test, Digit Span</td>
<td>None (did not distinguish between affected and unaffected)</td>
<td>N/A</td>
</tr>
<tr>
<td>Asarnow et al 2002</td>
<td>DSM-IIIR</td>
<td>Parents</td>
<td>190; control probands = 115</td>
<td>DS-CPT, Trails B, SPAN</td>
<td>None (did not distinguish between affected and unaffected)</td>
<td>N/A</td>
</tr>
<tr>
<td>Seidman et al 2000</td>
<td>DSM-IIIR</td>
<td>Siblings of boys</td>
<td>40; unaffected sibs of ADHD probands = 40; unaffected sibs of ADHD probands = 116; Control subjects = 118</td>
<td>Stroop, WCST, ROCF WRAML, CVLT, Auditory CPT, LC</td>
<td>Affected relatives: Overall battery, Stroop Word, Color and Color–Word subtests, WCST Perseverative Errors, WRAML list learning (overall battery fell short of significance, ( p = .06 ))</td>
<td>Verbal learning (general EFs, based on near-significance of overall battery)</td>
</tr>
<tr>
<td>Slaats-Willemse et al 2003</td>
<td>DSM-IV</td>
<td>Siblings from multiplex families</td>
<td>25; unaffected sibs of ADHD probands = 25; Control subjects = 48</td>
<td>Stroop Go–NoGo and SAT from Amsterdam Neuropsychologic Battery</td>
<td>Affected relatives: Go–NoGo, SAT accidental responses, Stroop Interference Unaffected relatives: No significant differences vs. control subjects, but linear effect across affected, unaffected, and control groups on Impulsivity/response inhibition all tests</td>
<td></td>
</tr>
<tr>
<td>Nigg et al 2004</td>
<td>DSM-IV</td>
<td>Parents and siblings</td>
<td>165; ADHD—combined type = 165; ADHD—inattentive type = 80; control subjects = 141</td>
<td>SS Task, Trails B, Tower of London, Stroop</td>
<td>Before correction for relative ADHD: SSRT in mothers of female probands, Trails B in relatives of children with ADHD-C, variability of basic RT in mothers; After correction for relative ADHD: SSRT in mothers of female probands, Trails B in relatives of children with ADHD-C,</td>
<td>Response inhibition and set shifting/ processing speed in specific subgroups</td>
</tr>
<tr>
<td>Doyle et al, in press</td>
<td>DSM-IV (and DSM-IIIR)</td>
<td>Parents and siblings of girls</td>
<td>106; unaffected relatives of ADHD probands = 106; control subjects = 243</td>
<td>Wechsler Digit Span, Oral Arithmetic, Digit Symbol/Coding; Stroop, WCST, ROCF WRAML, CVLT, Auditory CPT, WRAT-R Reading and Math</td>
<td>Affected relatives: Overall battery, Stroop Word, Color, WRAT-R Reading and Math Unaffected relatives: Overall battery, Stroop Color Word, Stroop Interference, WRAT-R Math</td>
<td>Interference control (and/or processing speed/naming), mathematics skills; other aspects of EF (e.g., working memory)</td>
</tr>
</tbody>
</table>

CPT, Continuous Performance Test; CVLT, California Verbal Learning Test; DS, Degraded Stimulus; EF, executive functions; LC, Letter Cancellation; SPAN, Span of Apprehension test; ROCF, Rey–Osterreith Complex Figure; WCST, Wisconsin Card Sorting Test; WRAML, Wide Range Assessment of Memory and Learning. SAT, Sustained.

\(^a\)Significant for DSM-IIIR diagnoses as well.
date nucleus, the putamen, and the globus pallidus (Castellanos and Tannock 2002; Ernst et al 1994; Fararone and Biederman 1998; Giedd et al 2001; Seidman and Valera 2002; Zanetkin et al 1990). Yet recent studies suggest more widespread abnormalities that include cortical regions and the cerebellum (Berguin et al 1998; Castellanos et al 2002; Mostofsky et al 1998, 2002). In one large study (Castellanos and Tannock 2002), after controlling for total cerebral volume, only the difference for cerebellar volume remained significant. Similar to neuropsychological studies, imaging studies of ADHD also show significant variability (Seidman et al 2005). One possibility is that this variability is due to low statistical power because the majority of structural neuroimaging studies have included groups smaller than 20 individuals. Given their sample sizes, most studies have not had the ability to address issues related to heterogeneous subgroups within ADHD.

**Heritability.** Although few in number, twin studies using structural neuroimaging paradigms suggest that the volume of relevant brain regions is under significant genetic control. As in neuropsychological studies, however, specific estimates of heritability should be interpreted cautiously because of limited sample sizes. In a small sample of twins, Pennington and colleagues (2000) found heritabilities of .56–.97 for subcortical and cortical volumes, left and right neocortex, and total cerebral volume. Regions of particular interest to ADHD researchers have also shown high levels of heritability. For example, two studies reported heritabilities of .5–.9 for frontal regions (Carmelli et al 2002; Thompson et al 2001). In a multifactor twin design, Poshuntma et al (2000) found that 88% of the variation of cerebellar volume was found to be due to genetic factors after the effects of age, gender, height, and intracranial space were parsed out.

**Family–Genetic Overlap with ADHD.** To date, only one study has assessed the familial overlap between ADHD and structural magnetic resonance imaging (MRI) measurements. Durston et al (2004) examined 30 ADHD youth, their 30 unaffected siblings, and 30 matched control subjects. Results showed reduced volumes in both the ADHD group and their unaffected siblings in right prefrontal gray matter and left occipital gray and white matter. Intracranial volume was reduced in ADHD youth, with a statistical trend toward reductions in unaffected siblings. Reductions in other cortical areas were observed; however, these did not reach statistical significance. Of interest was the fact that right cerebellar volume was reduced almost 5% in ADHD subjects versus control subjects, with no statistical difference between unaffected relatives and control subjects for this region. This finding was interpreted as suggesting that cerebellar reductions were associated with disease status rather than with the familial vulnerability to ADHD. Yet the effect size between unaffected relatives and control subjects was .26, suggesting that with a larger sample size, reduced volume of this region may also be implicated in the familial liability to ADHD.

**Electrophysiology**

**Association with ADHD.** A substantial literature has documented an association between ADHD and electrophysiological measures of brain functions. Event-related potentials (ERPs) measure changes in brain electrical activity in response to specific stimuli. Barry, Johnston, and Clarke’s (2003b) review of this literature suggests ADHD versus control differences across a variety of ERP paradigms. Particularly robust is the finding in children aged 12 years and younger of reduced amplitude of the posterior P3 wave, thought to peak between 300 and 500 msec after presentation of a stimulus, in response to an auditory oddball task that taps aspects of attention and working memory. Reduced P3 amplitude is also evident in the visual attention mode and with regard to anomalous processing (a reduced frontal N2 peak) after stimuli that evoke inhibitory processes.

Variability of electrophysiological findings is also evident across ADHD samples. Although more work is needed to explore whether such differences can be accounted for by small sample sizes and methodological differences across laboratories, reviews highlight different possibilities for delineating true heterogeneity (e.g., with regard to DSM-IV subtypes [Barry et al 2003a] or across subtypes based on groupings from cluster analyses involving different patterns of hypo- and hyperarousal [Clarke et al 2002]).

**Heritability.** A conceptual and meta-analytic review of twin studies of electrophysiological measures indicates that genetic factors contribute significantly to both EEG and ERP measures (van Beijsterveldt and van Baal 2002), with heritability of EEG alpha power and alpha peak frequency estimated to be .8 and ERP P3 amplitude and latency.6 and .5, respectively. Large interstudy discrepancies were also noted and attributed to small sample sizes and methodological differences. Other EEG and ERP parameters show evidence of genetic influence but have only been examined in a limited number of studies.

**Family–Genetic Overlap with ADHD.** To date, no studies have addressed the familial overlap of ADHD and electrophysiological measures. Meta-analytic findings, however, have documented reduced P3 amplitude in men with a family history of alcoholism compared with control subjects, particularly on visual tasks (Polich et al 1994), and Carlson et al (2002) found that in twins discordant for alcoholism, both affected and unaffected co-twins had reduced P3 amplitudes compared with nonalcoholic control twins. Iacono and colleagues (2002) have shown that this familial relationship is not specific to alcoholism. In their study, paternal antisocial personality disorder, in addition to alcohol abuse and dependence, was associated with reduced P3 amplitudes on a visual oddball test, and a similar but nonsignificant effect size for reduced P3 amplitude was found for sons of fathers with other substance abuse and dependence. These and other data (for a review, see Iacono et al 2003), such as the link between P3 amplitude reductions and a range of externalizing disorders including ADHD, the frequent comorbidity of externalizing and alcohol/substance use disorders, and evidence for shared genetic liability between externalizing disorders themselves have led Iacono, Malone, and McGue (2003) to hypothesize a genetically influenced latent trait of behavioral disinhibition that underlies a range of externalizing disorders and that can be indexed by reduced P3 amplitude. This hypothesis is interesting in light of family studies of ADHD suggesting cosegregation of ADHD and antisocial and bipolar disorders as well as higher relative risks associated with these comorbidities in ADHD families. Therefore, although research is needed in ADHD samples, these results highlight the potential utility of reduced P3 amplitude as an endophenotype for ADHD.
Summary: Neuroimaging and Electrophysiology Measures

Data from neuroimaging and electrophysiological paradigms suggest anomalies in individuals with ADHD when compared with control subjects. Patterns of MZ versus DZ correlations suggest that significant portions of variation in volumetric measures of the brain as well as in both EEG and ERP measures are due to genetic factors, suggesting that these measures meet one important criterion for a potential ADHD endophenotype. Yet further data are needed to determine the familial genetic overlap of measures from these paradigms with ADHD. Additionally, given the phenotypic, genetic, and likely neuropsychological heterogeneity in ADHD, the heterogeneity that has been observed in the neuroimaging and electrophysiological literatures should be explored further rather than simply attributing these inconsistencies to limited statistical power in studies with small sample sizes.

Discussion and Recommendations for Future Studies

The convergence of data from neuropsychological, neuroimaging, and electrophysiological studies suggest that neural mechanisms are disrupted in the ADHD brain and that these disruptions offer a potential window into the inherited diathesis of ADHD. If a single deficit or characteristic pattern of findings on these measures were identified in ADHD samples, particularly one that was reliable, heritable, and cofamilial, such a deficit would be an obvious candidate for use in molecular genetic studies. However, the literature does not yet yield definitive evidence for a specific endophenotype for ADHD.

Neuropsychological impairments in the construct of EFs broadly conceived, as well as impairments in state regulation and delay aversion, are associated with the ADHD. Such measures also show preliminary evidence of heritability and at least some familial–genetic overlap with ADHD diagnosis. Thus, they offer clear potential for molecular genetic studies. Of particular interest is evidence from family and twin studies suggesting familial–genetic overlap of ADHD and weaknesses in response inhibition, interference control, and processing speed. Yet the variability of deficits across studies, the partial rather than substantial overlap between these and other measures and ADHD in family and twin studies, the generally lower levels of heritability of neuropsychological measures compared with ADHD, and the lack of evidence for individual genes accounting for more than 5% of the variance in different tests all suggest that there is more work to be done to capitalize on the potential advantages of neuropsychological measures as ADHD endophenotypes.

The potential utility of neuroimaging and electrophysiological measures as endophenotypes may be constrained by their expense and the specialized equipment and training required for their implementation. Nonetheless, because such paradigms show association with ADHD and evidence of heritability, and also offer a window into neural pathways, these measures may still prove to be useful targets in molecular genetic studies of ADHD, particularly if they were to show robust familial or genetic overlap with the disorder. Given that only one structural neuroimaging study and no electrophysiological studies to date have examined unaffected ADHD relatives, a comparison of the relative advantage of candidate neuroimaging and electrophysiological versus neuropsychological endophenotypes is premature. Family and twin studies that assess overlap of these measures with ADHD are of interest to the field, particularly those large enough to address questions about heterogeneity.

In the remainder of the paper, we recommend additional strategies to move research in ADHD endophenotypes forward. We emphasize neuropsychological measures due to their ease of administration to large samples relative to neuroimaging and electrophysiological studies and to the fact that many research groups may already have neuropsychological data available.

Further Exploration of Neuropsychological Heterogeneity in ADHD

Examination of neuropsychological heterogeneity may allow targeting of more homogenous endophenotypes, which, in turn, would increase their utility for finding genes. Previously, we have argued for attention to family history, comorbidity, and DSM-IV subtypes and symptom dimensions to assist with endophenotype selection by determining whether qualitative or quantitative differences on these dimensions exist within ADHD samples (Doyle et al, in press-b). The benefits of this further cognitive analysis of ADHD would be most easily seen if distinct subtypes exist within ADHD that reflect unique pathophysiological deficits (e.g., as hypothesized by Sonuga-Barke’s [2002] dual pathway model). However, such research may not yield definitive answers for endophenotype studies if heterogeneity at the neurocognitive level does not reflect genetic heterogeneity (Faraone and Tsuang 2003; Tsuang and Faraone 1995). For example, it is possible that ADHD could arise from a single, fixed pool of genetic influences, yet appear to be neuropsychologically heterogeneous, because of the influence of measurement issues, development, co-occurring conditions, and the other genetic or environmental influences that affect a specific individual. In an equally plausible model, ADHD cases may arise from various combinations of risk factors from a much larger pool, such that risk factors are potentially but not necessarily overlapping (Faraone and Tsuang 2003; Tsuang and Faraone 1995). Additionally, the same neurobiological deficits could be associated with different genes in different samples (e.g., deficits in different subcortical regions that project to the frontal cortex may produce similar “executive” impairments; Pennington and Ozonoff 1996). This possibility is reasonable because the prefrontal cortex is one of the most widely interconnected regions in the brain (Goldberg and Seidman 1991). Thus, attention to heterogeneity offers important potential for identifying subtypes that would be more strongly associated with candidate endophenotypic constructs; however, further cognitive analysis alone may not clarify the optimal neuropsychological endophenotypes for ADHD.

Direct Assessment of Statistical Power

Empirical strategies, focused on maximizing the power of potential endophenotypes in individual samples, offer further potential either in conjunction with or as an alternative to analysis of neuropsychological heterogeneity. Risch (1990b) has demonstrated that the statistical power of a linkage study increases with the magnitude of risk ratios (i.e., λs), which are computed by dividing the affection rate among each relative type by the rate of affection in the population. Low λ values may be due to a variety of factors, such as oligogenic transmission, genetic heterogeneity, phenocopies, and low penetrance. Risch (1990b) has suggested that defining disease status in a manner that increases λ would increase the power of linkage studies. Faraone et al (1995) reviewed λ values from schizophrenia studies and showed their utility in discriminating endophenotypes based on their potential for increasing the statistical power of linkage studies. Egan et al (2001) used this strategy in families of patients with schizophrenia and found relative risk to be
increased for neuropsychological measures of processing speed, set shifting and verbal learning compared with the relative risk for the diagnosis. For ADHD, the potential value of endophenotypes is seen in the fact that λ values for the transmission of the ADHD diagnosis in family studies are consistently low, ranging from 2 to 3 for the risk to siblings and 2 to 8 for the risk to parents (Faraone et al 2000).

Increasing Statistical Power Through Reduction of Error Variance

An additional way to maximize power is to reduce error variance. Individual studies have demonstrated evidence of test–retest, inter-rater, or internal consistency reliability for neuropsychological (e.g., Kuntsi et al 2001), neuroimaging (e.g., Seidman et al 1999), and electrophysiological measures (e.g., Iacono et al 2002). Yet further attention to the psychometric properties of measurement tools in these domains is needed. For example, with regard to neuropsychological measures, reliability may differ across levels of ability (Pennington et al 1996) or when different algorithms are applied to a given experimental measure (Kuntsi et al 2001; Logan et al 1997). Our recent review (Doyle et al, in press-b) highlights other issues that could contribute error to measures of EF, including the limited sensitivity and the multifactorial nature of clinical neuropsychological measures, developmental changes, and state factors, such as sleep disturbances. Experts have advocated greater attention to the psychometric properties of EF measures (e.g., Denckla 1996; Pennington et al 1996), and such investigations are essential for determining whether heterogeneity is real. Measures that show good psychometric properties across the full range of performance, including better than normal, may also be more useful for genetic studies than those with a truncated distribution in which most individuals score perfectly (e.g., commission errors on a continuous performance test). This would allow for quantitative trait analyses and could also capitalize on statistically powerful designs using discordant relative pairs in which one is high and the other low on a quantitative trait (e.g., Dolan and Boomsma 1998; Eaves and Meyer 1994; Risch and Zhang 1995).

Computerized experimental measures borrowed from cognitive neuroscience may offer greater precision of measurement than clinical neuropsychological tasks (Nigg 2001) via assessment of reaction time rather than correct versus incorrect responses or the use of control tasks or constraints on alternative problem solving strategies (MacDonald and Carter 2002). Yet such measures should be used cautiously as they may have limited standardization across labs and minimal normative data. Other methods of reducing error variance in endophenotype studies include aggregating data from multiple sources. Rice and Todorov (1994) recommend the use of longitudinal or repeated measures designs for diagnostic assessment to reduce measurement errors in genetic studies. This strategy could be applied to neuropsychological data. Factor scores or conceptually derived scales that aggregate information from more than one neuropsychological measure of a construct could also reduce error associated with any one test. The literature reviewed here underscores the utility of this strategy because aggregate measures in twin and family studies have shown greater familial overlap with ADHD than individual measures in several studies. Yet the use of aggregated measures should also be undertaken with care, given that one reason to use endophenotypes is to simplify complex phenotypes into component parts (BF Pennington, personal communication). Therefore, measures should ideally be aggregated to provide better measures of specific constructs rather than a summary score for a broad construct. Factor analysis may also provide a means of capturing multiple neurocognitive deficits were they to exist in ADHD, and such a strategy has been useful in the assessment of familial neurocognitive deficits in schizophrenia (Krabbendam et al 2001).

Selection of Measures Showing High Heritability or for Which a Given Gene Contributes a Significant Amount of Variance

Because the literature on the heritability of neuropsychological measures is sparse, twin studies with large samples are needed to better document which measures are most heritable. Such studies may benefit from collaborations across research groups to achieve adequate sample sizes. In the absence of twin data, family studies can be used to test whether a putative endophenotype is familial and to calculate upper limits of heritability. Additionally, studies should further assess the complexity of neuropsychological measures themselves because identification of measures for which specific genes contribute a large amount of variance would assist in the selection of measures for further study.

Statistical Methods for Phenotype Selection

Finally, recent advances in statistical methods may offer empirical strategies for selecting endophenotypes for analyses in molecular genetic studies when a priori specification of specific measures is premature. For example, Lange and colleagues (2003) have developed a strategy to test the association of multiple quantitative phenotypes with a given marker that eliminates the need to adjust for multiple comparisons in a subsequent family-based association test. In the first stage, the association phenotypes and the marker locus is tested using a population-based statistic grounded in generalized estimating equations that model the quantitative phenotypes as a function of genotypes of interest using noninformative families. The phenotype with the strongest genetic component can then be tested for association with the marker. For linkage analyses, Hauser and colleagues (2004) have developed a strategy to identify subsets of families, based on their score on a covariate, that provide the greatest evidence for linkage. Such strategies provide useful methods for endophenotype selection in light of the fact that the multifactorial nature of ADHD may not yield definitive evidence for choosing between multiple candidate measures.

Conclusions

Although evidence for genetic influences on ADHD has been accumulating since the 1960s (Lopez 1965), a great deal of work still lies ahead to understand the mechanisms linking genes to brain dysfunction and the expression of ADHD symptoms. Specification of genetic and environmental risk factors and their associated pathophysiological risk mechanisms will help characterize early predictors of persistence and morbidity that, in turn, will pave the way for more refined treatment and primary prevention strategies for ADHD. Although their use in ADHD is relatively new, endophenotypes have aided the clarification of the etiology and pathophysiology of several other conditions in medicine and psychiatry (e.g., Borecki et al 1990; Freedman et al 2001). The data reviewed here suggest that neuropsychological, neuroimaging, and electrophysiological endophenotypes for ADHD offer potential to move molecular genetics research forward. Such studies will, however, require careful consideration of heterogeneity and measurement to reduce the complexity of the endophenotypes themselves and take advantage of
their potential to target a more homogenous piece of the etiological puzzle of ADHD.

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