Cognitive functioning in affected sibling pairs with ADHD: familial clustering and dopamine genes

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Background: This paper examines familiality and candidate gene associations of cognitive measures as potential endophenotypes in attention-deficit/hyperactivity disorder (ADHD). Methods: The sample consists of 540 participants, aged 6 to 18, who were diagnosed with ADHD from 251 families recruited for a larger genetic study of ADHD. All members of the family underwent psychiatric interviews and children were administered a large battery of cognitive tasks. Subjects were genotyped for several dopaminergic candidate genes (DAT1, DRD4, and DRD5). Results: Performance on measures of intelligence, working memory, and set-shifting had the highest sibling correlations and exhibited significant familial clustering. The 7-repeat allele of the dopamine receptor D4 (DRD4) gene was associated with poor performance on measures of intelligence, color naming, interference control, and working memory. There were no significant associations with DAT1 and DRD5. Conclusions: Sibling correlations, familial clustering and candidate gene associations provide strong support for verbal working memory as a candidate endophenotype for ADHD. More complex models of, and larger sample sizes for, genetic association with cognitive functions are encouraged for future study. Keywords: Endophenotype, working memory, DRD4, genetics, executive function, neuropsychology, parent psychopathology.

Attention-deficit/hyperactivity disorder (ADHD; American Psychiatric Association (APA), 1994) is a prevalent childhood psychiatric disorder that often continues into adulthood. The etiology is unknown; however, a large body of literature strongly supports the influence of genes in conferring risk for the disorder. Family and twin studies have demonstrated that ADHD is a highly heritable disorder, with ~76% of the variance attributable to genetic influences (Faraone et al., 2005). Among the four genome scans conducted to date, the results are largely mixed; however, three have identified overlapping regions of linkage (of varying magnitude) on chromosomes 5p and 17p (Arcos-Burgos et al., 2004; Bakker et al., 2003; Hebebrand et al., 2006; Ogdie et al., 2003). Association studies have focused on the dopaminergic genes due to the hypothesized role of dopamine in the pathophysiology of ADHD (Swanson et al., 2007) as well as the efficacy of psychostimulants in treating ADHD. Recent meta-analyses suggest statistically significant yet small effect sizes for several genes and ADHD, including the dopamine transporter (DAT1), dopamine receptors D4 (DRD4) and D5 (DRD5) (1.13, 1.45 and 1.24 respectively; Faraone et al., 2005). Current evidence suggests that ADHD is a polygenic disorder, probably the result of several genes of small to moderate effect.

Taken together, these results support genetic influences, complex inheritance, and likely genetic heterogeneity across ADHD populations. Investigations of intermediate phenotypes or endophenotypes in ADHD may help reduce sources of etiological heterogeneity and increase linkage or association signals. Endophenotypes have been increasingly used in psychiatric genetics as they are thought to be closer to gene action than diagnoses (Gottesman & Gould, 2003). Endophenotypes for ADHD such as inhibition, working memory, delay aversion, and temporal processing have been suggested based on the extant neuroscience literature (Castellanos & Tannock, 2002).

A step in this direction has been to test the association between dopaminergic candidate genes and cognitive processes implicated in ADHD, of which there have been a handful of studies. Of these, DRD4 has been most frequently studied; however, the results are mixed. Two groups have found that the DRD4-7-repeat allele of the 48-bp VNTR, the variant that has been shown to be a risk factor for ADHD, is associated with better cognitive functioning when compared to children who do not have the 7-repeat allele (Manor et al., 2002; Swanson et al., 2000). Specifically, the 7-absent group had a longer reaction time, more commission errors, and a more variable reaction time than children carrying the 7-repeat allele. In contrast, other studies (Kieling, Roman, Doyle, Hutz, & Rohde, 2006; Langley et al., 2004) have found that ADHD children who had at least one copy of the 7-repeat allele exhibited a fast and impulsive response style and higher rates of commission errors than 7-absent and normal control children. One longitudinal study (Barkley, Smith, Fischer, & Navia, 2003) as well as the efficacy of psychostimulants in treating ADHD. Recent meta-analyses suggest statistically significant yet small effect sizes for several genes and ADHD, including the dopamine transporter (DAT1), dopamine receptors D4 (DRD4) and D5 (DRD5) (1.13, 1.45 and 1.24 respectively; Faraone et al., 2005). Current evidence suggests that ADHD is a polygenic disorder, probably the result of several genes of small to moderate effect.

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Conflict of interest statement: No conflicts declared.
2006) found no differences in cognitive functioning or adult adjustment according to DRD4 genotype.

Similarly, tests of association between the DAT1 risk genotype, homozygous for the 10-repeat allele of the 480-bp VNTR, have been mixed. Initial studies report that ADHD children who were homozygous for the 10-repeat allele exhibited increased response variability and deficits in sustained attention and response inhibition when compared to children who had one or more copies of the 9-repeat allele (Cornish et al., 2005; Loo et al., 2003). There have been two recent studies that have found that the children who were heterozygous for the 9-repeat and 10-repeat alleles exhibited higher error rates and poorer adult outcomes than those homozygous for the 10-repeat allele (Barkley et al., 2006; Kim, Kim, & Cho, 2006). The one study of the DRD5-148bp allele reported findings in the expected direction, with the DRD5 148bp allele being associated with increased commission errors, longer reaction time, and more variable reaction time on the TOVA (Manor et al., 2004).

There are several possible reasons why such conflicting results have been found; the most notable possibility is small samples sizes resulting in spurious results. The sample sizes for nearly all of the studies have generally been small and often not larger than 50 subjects within the group of the less prevalent genotype tested. Given that ADHD is such a heterogeneous and polygenic disorder, sampling differences, ascertainment and other methodological differences across studies are likely to affect the distribution and resultant patterns of deficits according to genotype. In addition, none of the studies listed above have included multiple genes and their additive effects which may have influenced the findings and subsequent interpretations.

In the current study, we examined the genetic effects on cognitive performance in a large multiplex ADHD sample in several different ways. First, we examined the familiality of cognitive task performance to identify putative endophenotypes based on two factors: shared variance in affected sibling pairs with ADHD and cognitive task performance in offspring as a function of parental ADHD. We hypothesized that children with ADHD who have at least one parent affected with ADHD would have a higher genetic loading for ADHD and salient endophenotypes would be reflected in worse performance on the cognitive measures. Finally, we examined the association between three dopaminergic candidate genes in ADHD (DRD4, DRD5 and DAT1) and performance on the cognitive measures.

Methods

Participants
The sample consists of 540 participants, aged 6 to 18, from 251 families recruited for a larger genetic study of ADHD. Families were ascertained from a variety of sources, including community, clinical and pediatric referrals (see Smalley et al., 2000, for details). After receiving verbal and written explanations of study requirements, all participants provided written informed consent and assent approved by the UCLA Institutional Review Board.

The affected sibling pair (ASP) members did not differ in IQ, gender, ADHD subtype, or rates of co-morbidity. Briefly, the sample of 237 older ASP members were an average age of 12.3 years (SD = 2.7), were 72% male (N = 170), had a mean IQ of 107 (SD = 13.7), were 48% inattentive subtype, and had 60% Oppositional Defiant or Conduct Disorder and 16% reading disability. The younger ASP member had an average age of 9.7 years (SD = 2.8), were 74% male, had a mean IQ of 104 (SD = 14.7), were 40% inattentive subtype, 59% Oppositional Defiant or Conduct Disorder, and 21% reading disability. The families were 82% Caucasian, 11% Hispanic, 1% African-American, and 9% Other or Mixed Ethnicity.

Procedure
Families were scheduled for evaluation if at least two children between the ages of 6 and 18 had either been diagnosed with ADHD or exceeded the cutoff on a telephone screen using the SNAP-IV (Swanson, 1992) behavior rating scale. Subjects needed to be English speaking and have both biological parents available to participate. Other eligibility criteria for the current study included: full scale IQ of 70 or above, absence of a known genetic condition associated with ADHD (e.g., tuberous sclerosis, fragile X), and absence of schizophrenia and autism. At least one sibling in each ASP met full DSM-IV criteria for ADHD, while the other sibling could be diagnosed as definite or probable ADHD (i.e., one symptom short of diagnostic criteria but impairment was present). For a complete description of the assessment protocol see Smalley et al. (2000).

Diagnostic procedures. Parents and children were interviewed directly using the semi-structured interviews, the Schedule of Affective Disorders and Schizophrenia for School Age Children (K-SADS-PL; Kaufman et al., 1997) for ages 5–11, and the Schedule of Affective Disorders and Schizophrenia (SADS-LAR; Fyer, Endicott, Mannuzza, & Klein, 1995) for parents. The K-SADS-PL was administered to the mother followed by a direct interview with the child if age 8 years or older. Parental diagnoses were based on self-report on the SADS, which was supplemented by the KSADS Behavioral Disorders section to query directly for ADHD symptomatology. All interviews were conducted by clinical psychologists or highly trained interviewers with extensive experience in psychiatric diagnoses. ‘Best estimate’ diagnoses were determined after individual review of diagnoses, symptoms, and impairment level by senior clinicians (JJM, JTM). Inter-rater reliabilities were computed with a mean weighted kappa of .84 across all diagnoses with a greater than 5% occurrence in the sample. For complete diagnostic procedures and inter-rater reliabilities, see Smalley et al. (2000).
Cognitive measures

The following cognitive measures were given in the same order for all subjects. All subjects were off stimulant medication during cognitive testing. Some tasks were added to the study after data collection began, resulting in differing sample sizes for various tasks. The total sample size for each task is presented in Table 1 along with the sibling correlations. The Stop Signal Task (Logan, Schachar, & Tannock, 1997) was used as a measure of response inhibition during which participants were told to withhold a prepotent response when a tone (the stop signal) sounds during the task. The version of the Stop Task used in the present experiment used a tracking algorithm to identify the Stop Signal Delay (SSD) at which the probability of a correct inhibition was 50%. Three dependent variables are generated from this task: the Stop Signal Reaction Time (SSRT), the standard deviation of the go-reaction time (SDRT), and the reaction time on go trials (Go-RT), which reflects processing speed. The Stroop Color-Word Test (Golden, 1978) was used as a measure of interference control in response to incongruent stimuli. Participants were first asked to read as many words (red, blue, green) in a minute (Word condition), then asked to say the color of the ink (red, blue, green) (Color condition) and finally, to say the color of the ink, even though it was not congruent with the printed word (Color-Word condition). The dependent variable for each of the conditions was the number of items correctly identified in one minute. The Stroop interference score was also calculated as suggested in Golden (1978). The Trail Making Test, parts A & B (Reitan, 1979), is a paper and pencil test of complex visual search, mental flexibility, set-shifting, and motor function while connecting letters (Part A) or letters and numbers (Part B) in consecutive order. The dependent variables for Trails part A and part B are the amount of time (in seconds) to connect the stimuli, which were log transformed due to the non-normal distribution of these scores. Finally, the Wechsler Intelligence Scales for Children, Third edition (WISC-3; Wechsler, 1991) was used as a measure of general intellectual functioning (Full Scale, Verbal and Performance IQ). In addition, the Freedom from Distractibility Index (FFD) was used to measure working memory and is derived from the Arithmetic and Digit Span subtests.

Genotyping

Blood samples were collected from each family member and DNA was isolated using the Puregene Kit following the manufacturer’s recommendations (Gentra Systems, Minneapolis, MN, USA). Both DRD4 polymorphisms (48-bp VNTR and 120-bp repeat) and the DRD5 and DAT1 polymorphisms were genotyped according to standard procedures and have been reported elsewhere (Kustanovich et al., 2004). Differences in sample sizes for genotyping are due to several reasons such as: insufficient DNA for genotyping after inclusion in other genetic investigations and/or genotyping failure (<1%).

Statistical analyses

Statistical analyses were carried out using the Statistical Package for the Social Sciences 12.1 and SAS v. 9.1. To correct for multiple comparisons, we employed a false discovery rate (FDR; Benjamini & Hochberg, 1995) to maintain the family-wise experimental error at $p < .05$. Proc Mult-test was used to generate FDR-adjusted $p$-values, which are presented in addition to raw $p$-values; the threshold for statistical significance was set at $p \leq .05$.

Sibling correlations. To give an estimate of familiality, two-tailed Pearson product moment correlations were run. In families who had more than two affected probands, only the eldest and second eldest children were used in this analysis. Because age is significantly correlated to performance on many tasks, we ran partial correlations adjusting for differences in age to account for age effects between the two siblings. The upper limit of heritability is estimated by doubling the sibling correlation.

Effect of parental ADHD status. Parents were classified as ADHD if they had a definite lifetime diagnosis of ADHD. Given the recent findings of gender differences in endophenotypic expression (Nigg, Blaskey, Stawicki, & Sachek, 2004), we ran separate analyses for mothers and fathers. To account for the non-independence of the data due to the inclusion of multiple siblings from one family, a linear mixed model ANOVA was used specifying family as the cluster variable. This adjusts for the sibling correlation within the data which may lead to an over-estimate of the $F$ and $p$ values. Age and intelligence were used as covariates in all analyses.

Association analyses. The association of candidate genes to cognitive task performance was also examined using a mixed model ANOVA for all ADHD subjects including probands and siblings. We tested for association between hypothesized risk genotypes in two

Table 1 Sibling correlations for cognitive measures

<table>
<thead>
<tr>
<th>Cognitive Measure</th>
<th>$r$</th>
<th>$p$-value</th>
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<tr>
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<tr>
<td>Verbal IQ</td>
<td>.47</td>
<td>&lt;.001</td>
<td>229</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>.39</td>
<td>&lt;.001</td>
<td>229</td>
</tr>
<tr>
<td>Full Scale IQ</td>
<td>.48</td>
<td>&lt;.001</td>
<td>234</td>
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<tr>
<td>FFD</td>
<td>.32</td>
<td>&lt;.001</td>
<td>165</td>
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<tr>
<td>Coding</td>
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</tr>
<tr>
<td>SDRT</td>
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<td>.09</td>
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</tr>
<tr>
<td>SSRT</td>
<td>.17</td>
<td>.16</td>
<td>70</td>
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<tr>
<td>Trail Making Test</td>
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</tr>
<tr>
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<tr>
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<tr>
<td>Stroop Interference</td>
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<td>.39</td>
<td>89</td>
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</table>

Note. WISC-III = Wechsler Intelligence Scale for Children, 3rd edn, IQ = intelligence quotient, FFD = freedom from distractibility index, RT = reaction time, SDRT = standard deviation of reaction time, SSRT = stop signal reaction time.
variants of the DRD4 gene on chromosome 11p: 1) the 7-repeat polymorphism of the 48-base-pair (bp) variable number of tandem repeats (VNTR) in exon 3 and 2) the 120-bp duplication in the 5’ untranslated region. In addition, we tested the 148-bp allele of the DRD5 dinucleotide repeat and the 480-bp polymorphism of a 40-bp repeat VNTR in the 3’ region of the dopamine transporter gene (DAT1; also known as SLC6A3). To control for effects associated with age and intelligence, these were used as covariates for all analyses. Next, we tested the haplotype of the two DRD4 variants, which has been previously associated with ADHD (McCracken et al., 2000). Lastly, we examined interactions between the dopaminergic candidate genes and with parental ADHD for additive polygene effects on cognitive functioning using mixed model ANOVA.

**Results**

**Familial clustering of cognitive functioning.** Correlational analyses revealed significant sibling similarity on several cognitive tasks as shown in Table 1. Performance on measures of intelligence (Verbal IQ: \( r = .47 \), Performance IQ: \( r = .39 \), Full Scale IQ: \( r = .48 \), working memory (FFD: \( r = .32 \)) and set-shifting (Trails B: \( r = .41 \)) had the highest sibling correlations. The inhibition measures (Stop signal: \( r = .17 \), Stroop Color-Word: \( r = .16 \)) exhibited more modest sibling correlations and were not statistically significant within this sample.

Within our sample, 11% of children had both parents affected with ADHD, 24% had a father with ADHD, 24% with maternal ADHD, and 41% had neither parent affected with ADHD. Using parental ADHD status as a proxy for higher genetic load for ADHD, analyses indicated significant effects of maternal ADHD on certain cognitive tasks. Specifically, performance on measures of working memory (FFD: \( F(1,185.7) = 6.10, p = .05 \)), set-shifting (Trails B: \( F(1,116.7) = 10.13, p = .02 \)), conflict resolution (Stroop Color-Word: \( F(1,109.7) = 7.56, p = .05 \)), and speeded naming (Stroop Word: \( F(1,120.2) = 6.40, p = .05 \)) were worse among probands that had a mother with ADHD when compared to children without maternal ADHD (see Table 2). The results did not change when age, intelligence, and ADHD subtype were used as covariates. Paternal ADHD was not associated with differential performance on any of the cognitive tasks. These findings suggest that children who have a mother with ADHD (an index of a higher load of ADHD genes) do worse on certain types of cognitive functioning measures.

**Association between candidate genes and cognition.** Presented in Table 3 are the results of the candidate gene analyses for DRD4, DRD5 and DAT1. After FDR-adjustment, there were no significant associations between the DAT1 or DRD5 and any of the cognitive tasks tested. Overall, children who had the ‘risk’ allele for DRD4, the presence of the 7-repeat genotype, had lower scores on intelligence functioning (Verbal IQ: \( F(1,1417.2) = 7.56, p = .03 \); Performance IQ: \( F(1,1406.6) = 8.69, p = .02 \); Full Scale IQ: \( F(1,1422.4) = 9.83, p = .02 \)), speeded naming (Stroop color: \( F(1,172.9) = 6.36, p = .04 \)), interference control (Stroop Color-Word: \( F(1,162.5) = 7.17, p = .03 \)) and working memory (FFD: \( F(1,302.96) = 6.08, p = .03 \)) than those who had the DRD4 7-absent genotype. Those who were homozygous for the DRD4 240-bp allele exhibited significantly slower reaction time (Go-RT: \( F(1,128.5) = 6.83, p = .05 \)) on the Stop Signal Task than those who had at least one copy of the 120-bp allele.

**Gene × gene interactions and cognitive functions.** We then conducted exploratory tests of the association of cognitive task performance with the DRD4 haplotype, gene–gene interactions, and additive polygene effects. For the DRD4 haplotype, there were several significant associations, with the 240-bp allele/7-present group generally having the worst scores. The haplotype results were driven primarily by the DRD4 7-present group, which, regardless of

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**Table 2** Significant effects of maternal ADHD status on child cognitive performance

<table>
<thead>
<tr>
<th></th>
<th>No maternal ADHD</th>
<th>Maternal ADHD</th>
<th>F</th>
<th>Raw p-value</th>
<th>FDR-adjusted p-value</th>
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<td>SD</td>
<td>N</td>
<td>Mean</td>
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*Note. ADHD = attention-deficit hyperactivity disorder, FDR = false discovery rate, IQ = intelligence quotient, FFD = freedom from distractibility index, RT = reaction time, SDRT = standard deviation of reaction time.*

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the DRD4-120/240 status, had the same pattern of lower scores on the intelligence, speeded naming and conflict resolution tasks. The tests of gene × gene interactions and additive gene effects among the dopaminergic genes were uniformly statistically non-significant.

Gene × environment (parent ADHD status) and cognitive functioning. Lastly, we tested the effects of maternal ADHD status and candidate genes in association with cognitive processes. Children were grouped according whether there was maternal ADHD (Yes/no) and dopamine genotype (e.g., DRD4-7 absent or DRD4-7-present) to create four groups. Results can be summarized as falling into one of two patterns: one pattern where maternal ADHD appears to have an additive effect by affecting the strength of the association between the candidate gene genotype and cognitive functioning. Thus, if children had the ‘risk genotype’ and a mother with ADHD, they had the worse performance when compared to those children who did not have the risk genotype or a mother with ADHD. This occurred with the DRD4-7 genotype on WISC FFD ($F(3,252.9) = 3.82$, raw $p = .01$, FDR adjusted $p = .05$) and Stroop Color ($F(3,139.6) = 5.0$, raw $p = .002$, FDR adjusted $p = .03$) with a trend toward significance on Stroop Color-Word ($F(3,128.6) = 3.12$, raw $p = .03$, FDR adjusted $p = .06$). Maternal ADHD also strengthened the association between the DRD4-240-bp genotype resulting in longer go reaction times on the Stop Signal task (Go-RT: $F(3,106.8) = 3.73$, raw $p = .01$, FDR adjusted $p = .18$) when compared to children with no maternal ADHD and the DRD4 120-bp genotype; however, this result became non-significant with adjustment for multiple corrections (see Figure 1).

The second pattern that emerged was that maternal ADHD mediated the relationship between candidate gene genotype resulting in opposite

<table>
<thead>
<tr>
<th>DRD4 haplotype</th>
<th>120/7-absent (1)</th>
<th>240/7-absent (2)</th>
<th>120/7-present (3)</th>
<th>240/7-present (4)</th>
<th>post-hoc</th>
<th>$F$</th>
<th>$p$</th>
<th>FDR adj $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal IQ</td>
<td>97</td>
<td>109.04</td>
<td>15.12</td>
<td>127</td>
<td>108.39</td>
<td>15.01</td>
<td>44</td>
<td>103.43</td>
</tr>
<tr>
<td>Perform IQ</td>
<td>97</td>
<td>106.85</td>
<td>16.02</td>
<td>127</td>
<td>105.44</td>
<td>14.7</td>
<td>44</td>
<td>100.66</td>
</tr>
<tr>
<td>Full Scale IQ</td>
<td>100</td>
<td>108.47</td>
<td>14.88</td>
<td>127</td>
<td>107.4</td>
<td>14.06</td>
<td>44</td>
<td>102.27</td>
</tr>
<tr>
<td>Stop Signal Go-RT</td>
<td>38</td>
<td>663.55</td>
<td>131.77</td>
<td>42</td>
<td>709.29</td>
<td>93.25</td>
<td>15</td>
<td>630.67</td>
</tr>
<tr>
<td>Stroop Color</td>
<td>38</td>
<td>44.79</td>
<td>7.51</td>
<td>44</td>
<td>45.59</td>
<td>8.4</td>
<td>15</td>
<td>44.2</td>
</tr>
<tr>
<td>Stroop Color Word</td>
<td>38</td>
<td>46.63</td>
<td>8.98</td>
<td>44</td>
<td>46.05</td>
<td>9.45</td>
<td>15</td>
<td>44.6</td>
</tr>
</tbody>
</table>

Note. IQ = intelligence quotient, FFD = freedom from distractibility index, RT = reaction time, DRD4 = dopamine receptor D4, FDR adj $p$ = false discovery rate adjusted $p$-value

The summary of significant candidate gene associations with cognitive task performance is shown in Table 3.

**Table 3 Summary of significant candidate gene associations with cognitive task performance**

<table>
<thead>
<tr>
<th>Gene</th>
<th>$N$</th>
<th>Mean</th>
<th>SD</th>
<th>$N$</th>
<th>Mean</th>
<th>SD</th>
<th>$F$</th>
<th>$p$</th>
<th>FDR adj $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD4_7</td>
<td>7-Absent</td>
<td>270</td>
<td>108.23</td>
<td>14.96</td>
<td>153</td>
<td>102.75</td>
<td>13.26</td>
<td>7.56</td>
<td>.008</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>270</td>
<td>106.1</td>
<td>15.02</td>
<td>153</td>
<td>100.3</td>
<td>12.91</td>
<td>8.69</td>
<td>.003</td>
<td>.02</td>
</tr>
<tr>
<td>Full Scale IQ</td>
<td>270</td>
<td>107.64</td>
<td>14.25</td>
<td>153</td>
<td>101.67</td>
<td>12.12</td>
<td>9.83</td>
<td>.002</td>
<td>.02</td>
</tr>
<tr>
<td>FFD</td>
<td>202</td>
<td>20.87</td>
<td>5.89</td>
<td>107</td>
<td>18.94</td>
<td>4.5</td>
<td>6.08</td>
<td>.01</td>
<td>.03</td>
</tr>
<tr>
<td>Stroop Color</td>
<td>109</td>
<td>44.68</td>
<td>7.52</td>
<td>71</td>
<td>41.69</td>
<td>8.6</td>
<td>6.36</td>
<td>.01</td>
<td>.04</td>
</tr>
<tr>
<td>Stroop Color Word</td>
<td>109</td>
<td>46.14</td>
<td>8.69</td>
<td>71</td>
<td>42.55</td>
<td>8.14</td>
<td>7.17</td>
<td>.01</td>
<td>.03</td>
</tr>
<tr>
<td>DRD4_240</td>
<td>120/120 or 120/240</td>
<td>202</td>
<td>20.87</td>
<td>5.89</td>
<td>107</td>
<td>18.94</td>
<td>4.5</td>
<td>6.08</td>
<td>.01</td>
</tr>
<tr>
<td>Stop Signal Go-RT</td>
<td>53</td>
<td>654.25</td>
<td>119.12</td>
<td>85</td>
<td>709.93</td>
<td>103.76</td>
<td>6.83</td>
<td>.01</td>
<td>.05</td>
</tr>
</tbody>
</table>

The second pattern that emerged was that maternal ADHD mediated the relationship between candidate gene genotype resulting in opposite
analyses. of maternal ADHD and DRD5 emerged from these $p = .05$). No significant additive or interacting effects results according to genotype status. For example, the DAT-9 genotype was associated with worse performance on the Trails B test in the presence of maternal ADHD status than the DAT-10 when maternal ADHD was not present ($F(3,143.9) = 3.85$, raw $p = .01$, FDR adjusted $p = .10$) whereas the overall main effect of DAT-10 was not statistically significant (see Figure 2). When adjusted for multiple comparisons, this interaction between maternal ADHD and DAT-10 became a statistical trend. A similar effect was also apparent on the Trails B test with the DRD4-7-absent genotype and maternal ADHD associated with significantly worse performance on the Trails B task when compared to the effect of DRD4-7-present genotype and no maternal ADHD ($F(3,120.1) = 3.67$, raw $p = .01$, FDR adjusted $p = .05$). No significant additive or interacting effects of maternal ADHD and DRD5 emerged from these analyses.

Discussion

In this study, we examined familiality and candidate gene associations of cognitive measures among the largest sample to date of affected sibling pairs with ADHD. Our data suggest that there are several cognitive functions that exhibit significant familiality, particularly measures of set-shifting (Trails B), working memory (Freedom from Distractibility), and speeded color naming (Stroop Color). These measures were significantly correlated among ADHD ASPs and differed according to maternal ADHD status, suggesting that a higher genetic load for ADHD genes manifests in poorer cognitive performance. While sibling correlations among ASPs can be elevated due to shared environment as well as genetic similarity or by virtue of their association with ADHD, we look to parental ADHD as an additional indication of familiality of cognitive measures. That these differences were apparent according to maternal ADHD status and not paternal ADHD status is consistent with a hypothesis that females with ADHD may require a higher loading of ADHD genes to express the disorder (i.e., a sex-specific threshold of ADHD (Rhee, Waldman, Hay, & Levy, 1999). These mothers might then have offspring with a higher genetic loading of ADHD genes and, consequently, greater cognitive deficits on working memory, conflict resolution, and speeded naming tasks. These findings are similar to a previous family study in ADHD (Nigg et al., 2004) where the Trails B also received support as a potential cognitive endophenotype for ADHD. The Trails B is thought to be a measure of set-shifting, sequencing, and motor speed, but also appears to tap a working memory component as well (Loo et al., 2007). Significant familiality in the present sample coupled with moderate to large effect sizes between ADHD and control samples on the Trails B and other working memory measures such as FFD (Loo et al., 2007) suggest that working memory is a strong candidate as an endophenotype for ADHD.

The most striking findings of these association analyses between candidate genes and cognition are the association between DRD4-48bp allele 7-repeat genotype and performance deficits on measures of intelligence, working memory, speeded color naming, and interference control. The strength of the DRD4-7 repeat allele drove most of the findings for the DRD4 haplotype as well. We did not find any significant associations between cognitive measures and the DAT1 or DRD5. This finding conflicts with the previous literature, which has generally found small effects of cognitive functioning for both the DRD5 and DAT1, although the putative risk allele has not been consistent across studies. While these conflicting findings may indicate methodologic issues as well as genetic heterogeneity, we also suggest that the divergent results may be due to other factors that are not systematically controlled for as detailed in the paragraph below.

Our most interesting findings involve the interaction between maternal ADHD status and risk genotypes for DRD4 and DAT1. We found that in families with maternal ADHD, there were differential effects of risk alleles at DRD4 and DAT1. This suggests that families with multiple ADHD cases (i.e., familial
ADHD) may differ from sporadic cases. Specifically, in the familial form, we speculate there may be genes of more moderate effect that may interact or overshadow the genes of small effect such as DRD4 and DAT1. In the non-familial form of ADHD, where less moderate genes are present, the effects of many small genes may depend more on environmental factors such as prenatal exposure to alcohol and smoking, family environment and stressors or other medical and psychiatric comorbidities. Interestingly, the interaction between the ‘risk’ genotype and maternal ADHD status changed the direction of the association, making the ‘protective’ genotype (i.e., DAT1-9 and DRD4-7 absent) associated with worse cognitive performance compared to the ‘risk’ genotypes. As maternal ADHD status is not commonly accounted for in genetic analyses, differing rates of maternal ADHD may account for some of the divergent findings in the candidate gene literature to date.

There are several issues that should be considered that may limit the generalizability of the results. The sample is comprised of predominantly Caucasian children from multiplex families with ADHD (i.e., have more than one child with ADHD), in which there may be an increased genetic loading for ADHD. Further work to test the generalizability of these findings to children from singleton families (only one child with ADHD) from other ethnic backgrounds is needed. In addition, we have performed a large number of statistical tests, which may increase the possibility of Type 1 (false positive) error. In order to address this possibility, we have calculated and reported p-values adjusted for the false discovery rate to control the family-wise experimental error rate.

In conclusion, within our sample of ASPs with ADHD, working memory has the most robust support as a candidate endophenotype. Cognitive measures that involve working memory (FFD and Trails B) exhibit the highest sibling correlation and differ in the presence of a higher genetic load for ADHD. Clinically, this suggests that ADHD children who have a mother with ADHD may have more executive functioning difficulties. The DRD4-7 repeat allele is significantly associated with poorer cognitive functioning in a number of areas, including working memory, conflict resolution, and speeded naming. Maternal ADHD status modifies candidate gene effects on some cognitive measures such as working memory and set-shifting. More complex models including parental ADHD status as a putative risk variable in examining genetic association with cognitive functions are encouraged for future study.

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References


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