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ARCHIVAL REPORT

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Background: Reading disabilities (RD) and attention-deficit hyperactivity/ disorder (ADHD) are two common childhood disorders that co-occur by chance more often than expected. Twin studies and overlapping genetic linkage findings indicate that shared genetic factors partially contribute to this comorbidity. Linkage of ADHD to 6p, an identified RD candidate locus, has previously been reported, suggesting the possibility of a pleiotropic gene at this locus. RD has been previously associated with five genes in the region, particularly DCDC2 and KIAA0319.

Methods: To test whether these genes also contribute to ADHD, we investigated markers previously associated with RD for association with ADHD and ADHD symptoms in a sample of families with ADHD (n = 264). Markers were located in two subregions, VMP/DCDC2 and KIAA0319/TTRAP.

Results: Across all analyses conducted, strong evidence for association was observed in the VMP/DCDC2 region. Association was equally strong with symptoms of both inattention and hyperactivity/impulsivity, suggesting that this locus contributes to both symptom dimensions. Markers were also tested for association with measures of reading skills (word identification, decoding); however, there was virtually no overlap in the markers associated with ADHD and those associated with reading skills in this sample.

Conclusions: Overall this study supports a previous linkage study of ADHD indicating a risk gene for ADHD on 6p and points to VMP or DCDC2 as the most likely candidates.

Key Words: ADHD, genetic association, pleiotropic locus, RD

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood disorder that occurs in approximately 5% of the population and is characterized by developmentally disproportionate levels of inattention, impulsivity, and hyperactivity (1). It often co-occurs with reading disabilities (RD), another common disorder of childhood in both clinical (2,3) and population-based (4,5) samples. RD is a specific learning disability that occurs in 3%–6% of the population and is characterized by difficulties with accurate and/ or fluent word recognition and by poor spelling and decoding abilities. These difficulties occur despite adequate cognitive abilities and effective educational opportunities (International Dyslexia Association, 2002; http://www.interdys.org/). In samples selected for ADHD, the rate of comorbid RD is between 25% and 40% (3), whereas in samples selected for RD, the rate of comorbid ADHD ranges from 15% to 26% (6).

Four competing hypotheses have been proposed to explain the comorbidity of RD and ADHD. The first proposed cross- assortative mating between individuals with either disorder (7).

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Four competing hypotheses have been proposed to explain the comorbidity of RD and ADHD. The first proposed cross-assortative mating between individuals with either disorder (7).

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Figure 1. Location of markers investigated in the reading disability (RD) candidate region on 6p. Schematic showing the ~589-kb region on chromosome 6p, with the five candidate genes, VMP, DCDC2, KIAA0319, TTRAP, and THEM2, drawn as thick horizontal arrows at the bottom of the figure. Portions of the 3' region of VMP and DCDC2, 5' region of KIAA0319, and the 3' region of TTRAP have been expanded to show the location of markers genotyped for this study (vertical arrows). The two markers in VMP are 900 bp apart. The marker Rs 3178 in VMP is 35.9 kb away from Rs 793,662 in DCDC2. The region between Rs 793,662 and Rs 807,701 across intron 8 in DCDC2 is 66.6 kb in length. The region across KIAA0319 and TTRAP is 70.2 kb in length. The marker D6S105 is approximately 3.1-Mb away from Rs 2,143,340, toward the centromere of chromosome 6. Untranslated regions (UTR) of these genes are drawn as shorter, clear boxes, and coding exons are depicted as longer, filled boxes.

the DUX1C1 (EKN1) locus in the RD linkage region on 15q (27,28) and DRD4 on 11p (29,30).

The most studied chromosomal region in conjunction with RD has been 6p21.22, where multiple studies have found linkage (16,18,31–37). Willcutt and colleagues (38) investigated linkage of the ADHD phenotype to the RD locus on chromosome 6p in a sample of sibling pairs ascertained for RD. Results from their study showed linkage to ADHD between markers D6S276 and D6S105 (38) (Figure 1). These results remained significant for both markers when the reading phenotypes were regressed and linkage of the residual scores was analyzed, suggesting that linkage to ADHD was not a consequence of reading problems (38). In addition, evidence for significant bidirectional linkage to D6S105 was found, suggesting that the 6p locus was pleiotropic for both RD and ADHD (38). The linkage results of Willcutt and colleagues for ADHD, and the previous linkage studies of RD implicated broad regions on 6p containing multiple genes; thus, it was not clear which of the genes in the region would contribute to RD or ADHD, and further fine mapping was required.

The first study to fine map the 6p region for RD identified associated markers in five candidate genes: vesicular membrane protein, p24 (VMP); recently named Neurexin 1, NRN1, doublecortin domain containing-2 (DCDC2), KIAA0319, TTRAP and TNF associated protein (TTRAP) and a member of the thioesterase superfamily of genes (THEM2) (34) (Figure 1). Additional follow-up studies have since been conducted, and results have converged on KIAA0319 (36,39–41) and DCDC2 (42,43) as the two most likely RD candidates at this locus. We have previously investigated association of markers in these five genes with RD. Results from our study also support a role for KIAA0319; however, some evidence for association was also found with single markers in VMP, the distal neighbor of DCDC2 (42,43) (44). No evidence for association with DCDC2 was identified.

The most significant markers from the bivariate linkage study of Willcutt et al. (38), D6S276 and D6S105, flank the current RD candidate region on 6p, with D6S276 lying proximal to VMP and within DCDC2 (Figure 1). The other RD candidate, KIAA0319, is located less than 5 Mbp centromeric to DCDC2. On the basis of the genetic overlap between these two disorders and the convergence of bivariate linkage to a region surrounding these RD candidate genes on 6p (38), we hypothesized that these specific genes might also be associated with ADHD, particularly the LA symptoms. Therefore, this investigation focused on markers in two regions, VMP/DCDC2 and KIAA0319/TTRAP, previously reported to be associated with RD either individually or as haplotypes. Specifically, we investigated the markers and the haplotypes of Rs 793,662–Rs 807,701 in DCDC2 (42,43), Rs 4,054,469–Rs 2,038,137–Rs 2,143,340 in KIAA0319 and TTRAP (36,40,41), and Rs 4,054,469–Rs 6,935,076 in KIAA0319 (39–41). The two markers in DCDC2 were strongly associated with RD in two independent samples from Germany (42,43). The three markers that were used to derive the second set of haplotypes in KIAA0319 and TTRAP were first associated with RD in samples from the United Kingdom and the United States (36,40,41).

Association of this set of haplotypes with RD was also identified in two studies using population-based samples (41,45). The next set of haplotypes comprising two markers in KIAA0319 were first associated with RD in an additional sample from the United Kingdom (39–41). An additional two markers in VMP, Rs 3178 and Rs 3,829,810, and two in KIAA0319, Rs 12,194,507 and Rs 13,213,672, were also investigated. The first was associated with RD in our previous study (44) and, along with the second, marked tagged variation greater than or equal to 10% within a haplotype block in VMP (http://www.hapmap.org; Rel2/phase2 April 2007) (46). The latter two along with the four previously associated markers in KIAA0319 and TTRAP cover a region of high linkage disequilibrium (LD) across these two genes in which 11 single nucleotide polymorphisms (SNPs) associated with RD in previous studies are located (36,39–41,44).

The association of ADHD and ADHD symptoms was investigated using a sample of families comprising probands meeting DSM-IV criteria for one of the three DSM-IV subtypes of ADHD (28,47,48).

Methods and Materials

Study Sample

The sample consisted of 264 nuclear families from the Toronto area, which includes 264 probands and 55 siblings between the ages of 7 and 16 selected on the basis of an ADHD diagnosis (described subsequently). Of these, 192 were two-parent families, 157 with one child, 55 with two children, and two with three children. There were also 72 single-parent families, 55 with one child, 16 with two children, and one with three children. Nineteen percent of the participants described their ethnicity as "European," and the other 10% were of "other" or "mixed" backgrounds. These included Chinese, African, Indian, and Native American (47). Although this sample is not entirely ethnically homogenous, the association design employed here is robust to population stratification because it evaluates transmission of marker alleles, while the untransmitted alleles within each family are used as internal controls (49).

Diagnostic Assessment

Subjects were recruited following referral to the Child Development and Neuropsychiatry Clinics at the Hospital for Sick Children in Toronto. Children were included in the study if they met DSM-IV criteria for one of the three DSM-IV ADHD subtypes...
(IA, HI, and combined). The diagnosis of ADHD was based on semi-structured interviews with parents (Parent Interview for Child Symptoms [PICS-IV]) (50) and teachers (Teacher Telephone Interviews [TTI-IV]) (51), with additional information on behavior and academic skills collected from standardized questionnaires and assessments: Conners Parent and Teacher Rating Scales—Revised (52); Ontario Child Health Survey Scales—Revised (53); Wide Range Achievement Test—3rd revision (WRAT) (54); Woodcock Reading Mastery Tests—Revised (55); WISC-R (55); Clinical Evaluation of Language Fundamentals, 3rd ed. (56); Children's Depression Inventory (57); and Children's Manic Anxiety Scale (58). All children were free of medication for 24 hours before assessment. The distribution of DSM-IV ADHD subtypes was as follows: 14% were predominantly HI, 27% were IA, and 59% were combined. Information on ADHD symptom dimensions from the PIICS-IV and TTI-IV were used in the quantitative analyses. In this sample, only 31 individuals met criteria for RD on the basis of criteria used in a study of RD that identified individuals who fell, on average, in the lower 5% of the population in reading skills (27). Therefore, a categorical analysis of RD was not conducted in this sample. Measures ascertainment from the WRAT 3 Reading, WRAT Word ID, and WRAT Word Attack subtests were used in the quantitative analyses of reading. Descriptive statistics for this sample can be found in Table 1.

Subjects were excluded if they showed evidence of neurologic or chronic medical illness, bipolar affective disorder, psychotic symptoms, Tourette syndrome, or chronic multiple tics. Children were also excluded if they scored below 80 on both the Performance and Verbal Scales of the Wechsler Intelligence Scale for Children III (59).

Isolation of DNA and Marker Genotyping

DNA was extracted directly from blood lymphocytes using a high-salt extraction method (60). We investigated single nucleotide polymorphism (SNP) markers in the genes for VMP, DCDC2, and in the 5' regions of the genes for KIAA0319 and TTRAP (Figure 1). The specific markers were selected from prior published studies and association findings for RD as well as measures of reading in our previous studies of RD (44). The assays employed here were either predesigned (Assay-on-Demand by Applied Biosystems, Foster City, California; see Table S1A in Supplement 1) or designed from flanking sequence (UCSC database builds 33–35). Assay-by-Design, Table S1B in Supplement 1). Genotyping was conducted with the ABI 7900-HS Sequence Detection System (Applied Biosystems) using the TaqMan 5' nuclease assay for allelic discrimination and the end point analysis mode of SDS software package version 2.0 (Appendix B).

Table 1. Descriptive Statistics for the Attention-Deficit/Hyperactivity Disorder Sample (n = 319)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean</th>
<th>SD</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent inattention (PIICS-IV)</td>
<td>5.20</td>
<td>2.44</td>
<td>-0.49</td>
<td>2.39</td>
</tr>
<tr>
<td>Parent hyperactive/impulsive (PIICS-IV)</td>
<td>4.86</td>
<td>2.74</td>
<td>-0.22</td>
<td>1.95</td>
</tr>
<tr>
<td>Teacher inattention (TTI)</td>
<td>4.63</td>
<td>2.53</td>
<td>-0.42</td>
<td>2.18</td>
</tr>
<tr>
<td>Teacher hyperactive/impulsive (TTI)</td>
<td>3.51</td>
<td>2.70</td>
<td>0.24</td>
<td>1.80</td>
</tr>
<tr>
<td>WRAT 3 Read</td>
<td>97.23</td>
<td>14.31</td>
<td>-0.25</td>
<td>3.42</td>
</tr>
<tr>
<td>WRAT Word ID</td>
<td>95.58</td>
<td>14.43</td>
<td>-0.67</td>
<td>3.84</td>
</tr>
<tr>
<td>WRAT Word Attack</td>
<td>94.15</td>
<td>13.44</td>
<td>-0.59</td>
<td>3.18</td>
</tr>
</tbody>
</table>

PIICS, Parent Interview for Child Symptoms; TTI, Teacher Telephone Interviews; WRAT, Wide Range Achievement Test—3rd revision; WRATM, Woodcock Reading Mastery Tests.

slated Biosystems). The G/T polymorphism Rs 2,038,137 was genotyped using a standard polymerase chain reaction (PCR; annealing temp 58°C) followed by an analysis with the restriction enzyme BstUI (New England Biolabs, Beverly, Massachusetts). PCR primer sequences were as follows: KIAA0319: 8137 F: GTTGAGGAAAAAGACCTCAAA and KIAA0319: 8137 R: GAGGACGAGGAGACAGCATT. The more frequent allele, G, was cut by the enzyme while the other allele, T, was not cut.

Statistical Analysis

Following the genotyping, all markers were tested for Mendelian errors, crossovers, and Hardy-Weinberg equilibrium using Merlin (61). For all 10 SNP assays, genotyping success rates ranged from 99% to 100%. For the single marker categorical analysis, the TDT statistic was calculated using the extended TDT (ETDT) program (62). p values from the TDT analysis of all 10 SNP were corrected using a permutation test, running 1000 permutations in UNPHASED (63). For each permutation, transmission status of the parental haplotypes was randomized and the minimum p value was compared with the minimum p value of all markers from the original analyses. This provides a correction over all markers in the analyses (63). Haplotype transmission for ADH defined as a categorical trait was analyzed using the TRANSMIT program (64). For these analyses, the p values are only reported for haplotypes with frequencies greater than 10%, and those with a frequency of less than 10% were included in the global chi-square test. Analysis of the quantitative measures of IA and HI as reported by both parents and teachers was carried out using the Family Based Association Test (FBAT) program and the HBAT component for the quantitative analysis of haplotypes (65). The null hypothesis (H0) for the FBAT analysis was no linkage and no association. The following offsets based on means from a healthy screened control sample were used to mean center traits (66): Parent IA = 1.45, Parent HI = 1.05, Teacher IA = 1.1, Teacher HI = .74.

Results

Ten markers in VMP, DCDC2, KIAA0319, and TTRAP were tested for association with ADHD using a single-marker categorical analysis (n = 264 affected children). Both markers in the gene for DCDC2 were significantly associated with ADHD. These were Rs 793,862 (χ2 = 7.049, p = 0.008) and Rs 807,701 (χ2 = 16.990, p = 0.0004; Table 2). In the gene for VMP, significant evidence for association of ADHD was observed with Rs 3,829,810 (χ2 = 5.896, p = 0.015) and Rs 3,178 (χ2 = 5.586; p = 0.018; Table 2). Single markers in the genes for KIAA0319 and TTRAP were not associated with ADHD (Table 2). The p values for all 10 markers were corrected for multiple testing using 1000 permutations (63). The corrected global p value was 0.002.

Two studies of ADHD symptom dimensions have identified shared and independent genetic factors contributing to both the IA and the HI dimensions, indicating that risk genes may contribute to both or either of the dimensions (57). On this basis, we also analyzed two symptom dimensions separately as quantitative measures. Following the prediction from twin studies of a stronger genetic relationship between IA and RD (12), we hypothesized that genes contributing to RD were more likely to be associated with the IA symptoms. Because these were secondary analyses based on our initial findings from the TDT that were corrected for multiple testing, a second correction for multiple testing was not applied.

A single-marker quantitative analysis showed that both markers in DCDC2, Rs 793,862 and Rs 807,701, were significantly
Table 2. Single-Marker TDT Analysis for Markers in the VMP/DCDC2 and KIAA0319/TRAP Regions in the Attention-Deficit/Hyperactivity Disorder Sample

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Allele</th>
<th>Frequency</th>
<th>Transmissions</th>
<th>Nontransmissions</th>
<th>$\chi^2$</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMP</td>
<td>Rs 3,829,810</td>
<td>A</td>
<td>.25</td>
<td>97</td>
<td>66</td>
<td>5.896</td>
<td>.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>.75</td>
<td>66</td>
<td>97</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rs 3,178</td>
<td>A</td>
<td>.49</td>
<td>134</td>
<td>98</td>
<td>5.586</td>
<td>.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>.51</td>
<td>154</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCDC2</td>
<td>Rs 793,862</td>
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<td>.35</td>
<td>99</td>
<td>65</td>
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<td></td>
<td></td>
<td>G</td>
<td>.65</td>
<td>65</td>
<td>99</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Rs 807,701</td>
<td>C</td>
<td>.35</td>
<td>128</td>
<td>70</td>
<td>16.990</td>
<td>.000$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>.65</td>
<td>70</td>
<td>128</td>
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</tr>
<tr>
<td>KIAA0319</td>
<td>Rs 4,504,469</td>
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<td>.34</td>
<td>114</td>
<td>112</td>
<td>.018</td>
<td>.893</td>
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<tr>
<td></td>
<td></td>
<td>G</td>
<td>.66</td>
<td>114</td>
<td>112</td>
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<td></td>
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<tr>
<td></td>
<td>Rs 12,194,307</td>
<td>A</td>
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<td>1.330</td>
<td>.249</td>
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<td></td>
<td></td>
<td>G</td>
<td>.63</td>
<td>40</td>
<td>51</td>
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<tr>
<td></td>
<td>Rs 12,213,672</td>
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<td>.37</td>
<td>16</td>
<td>9</td>
<td>1.960</td>
<td>.162</td>
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<td></td>
<td></td>
<td>G</td>
<td>.63</td>
<td>16</td>
<td>9</td>
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<td></td>
<td>Rs 6,935,076</td>
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<td>95</td>
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<td>.363</td>
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<td>.63</td>
<td>101</td>
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<tr>
<td></td>
<td>Rs 2,038,138</td>
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<tr>
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<tr>
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<td>.712</td>
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<td>G</td>
<td>.84</td>
<td>57</td>
<td>61</td>
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</tbody>
</table>

$^a$ p = .000038. Global corrected p = .002, over all 10 markers.

associated with parent-reported IA (Z = 2.374, p = .018; Z = 4.016, p = .00006) and HI (Z = 2.407, p = .016; Z = 3.746, p = .00018) symptoms (Table 3). The marker Rs 793,862 was also associated with teacher-reported IA (z = 2.127, p = .033).

Similarly, Rs 807,701 was associated with both teacher-reported IA (Z = 3.600, p = .0005) and HI (Z = 3.313, p = .001). In VMP, Rs 3,829,810 was significantly associated with both parent-reported IA (Z = 2.434, p = .015) and HI (Z = 2.535, p = .011) symptoms (Table 3). Neither marker was in VMP associated with teacher-reported symptoms (Table 3). In the KIAA0319 TRAP region, Rs 12,194,307 in KIAA0319 was significantly associated with both parent-reported IA (Z = 3.025; p = .002) and HI (Z = 2.175, p = .030) symptoms. This marker was also significantly associated with teacher IA (Z = 2.644, p = .008) and HI (Z = 2.586, p = .010) (Table 3).

Analyses of association of haplotypes with ADHD defined as a categorical trait were also conducted. Markers were grouped as haplotypes on the basis of previous studies of RD: Rs 793,862-Rs 807,701 in DCDC2 (42,43), Rs 3,829,810-Rs 3,178 in VMP (44), Rs 4,504,469-Rs 2,038,137-Rs 2,143,340 in KIAA0319 (36,41), Rs 4,504,469-Rs 6,935,076 in KIAA0319 (39,44), and Rs 4,504,469-Rs 12,194,307-Rs 12,213,672-Rs 6,935,076-Rs 2,038,137-Rs 2,143,340 in KIAA0319/TRAP (44). In DCDC2, two haplotypes, G-T, and A-C, were significantly associated with ADHD ($\chi^2 = 9.248$, p = .002 and $\chi^2 = 10.172$, p = .001, respectively, global $p = .002, 3 d.f.$) (Table 4). In VMP, the G-A haplotype was significantly associated with ADHD ($\chi^2 = 5.245$, p = .022), although the global test was not significant (p = .069) (Table 4).

All three groups of haplotypes across KIAA0319 and TRAP were not associated with ADHD in the categorical analyses of haplotypes (data not shown).

These haplotypes were also tested for association with quantitative measures of ADHD (Table 5). Haplotypes consisting of the two markers in DCDC2 were significantly associated with both parent-reported IA (G-T: Z = -2.978, p = .003; A-C: Z = 3.976, p = .00007; A-T: Z = -2.519, p = .012) and HI (G-T: Z = -2.848, p = .003; A-C: Z = 3.964, p = .00007; A-T: Z = -2.080, p = .038; Table 5). Similarly, haplotypes in DCDC2 were also associated with parent-reported IA (G-T: Z = -2.705, p = .007; A-C: Z = 3.498, p = .0005; A-T: Z = -2.123, p = .034) and HI (G-T: Z = -2.427, p = .015; A-C: Z = 2.752, p = .006; Table 5). When investigated for association with parent-reported ADHD symptoms, haplotypes consisting of the two markers in VMP were significantly associated with IA (G-A: Z = -2.390, p = .017; A-G: Z = 2.092, p = .036) and HI (A-G: Z = 2.099, p = .036) symptoms (Table 5). These haplotypes in VMP were not associated with teacher-reported IA or HI symptoms. No significant evidence for association was found for any haplotypes of markers in the KIAA0319/TRAP regions in the quantitative analyses (data not shown).

Because these genes have previously been implicated as quantitative trait loci for measures of reading, we also analyzed single-word reading (WRMT—Word ID and WRAT—3 Reading) and phonological decoding (WRMT Word Attack) using quantitative analyses. Only Rs 12,194,307 in KIAA0319 was modestly associated with phonological decoding (Z = -2.513, p = .012) (Table 3). All other markers, including those in VMP, DCDC2, and TRAP were not associated with the reading components tested (Table 3).

Discussion

Twin studies have provided estimates of both bivariate heritability and genetic correlations for RD and symptoms of ADHD, particularly inattention, suggesting that some genes will be shared between the two. However, how exactly this manifests in gene function is unknown. That 6p22, particularly DCDC2 and/or KIAA0319 is an RD susceptibility locus has been well supported (36,39–45). There has been some support for VMP (34,40). A previous study by Willcutt and colleagues also linked ADHD to 6p22 (38). The most parsimonious explanation for these findings for both ADHD and RD (38) would be the presence of a pleiotropic gene. To test this hypothesis, we focused on markers previously associated with RD to investigate whether they would also be associated with ADHD and its symptom dimensions.

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Table 3. Single Marker Quantitative Analyses of Attention-Deficit/Hyperactivity Disorder Symptom Counts and Reading Components

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Allele</th>
<th>Frequencies</th>
<th>Zd</th>
<th>Pd</th>
<th>Zf</th>
<th>Pf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent-Reported Symptoms</td>
<td>VMP</td>
<td>A</td>
<td>5</td>
<td>2.434</td>
<td>0.015</td>
<td>2.535</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>DCDC2</td>
<td>C</td>
<td>5</td>
<td>2.374</td>
<td>0.0176</td>
<td>2.407</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>KIAA0319</td>
<td>A</td>
<td>4</td>
<td>-1.349</td>
<td>0.177</td>
<td>-0.344</td>
<td>0.731</td>
</tr>
<tr>
<td></td>
<td>TRAP</td>
<td>A</td>
<td>5</td>
<td>-1.306</td>
<td>0.16</td>
<td>-0.656</td>
<td>0.512</td>
</tr>
</tbody>
</table>

Teacher-Reported Symptoms

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker</th>
<th>Allele</th>
<th>Frequencies</th>
<th>Zd</th>
<th>Pd</th>
<th>Zf</th>
<th>Pf</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMP</td>
<td>A</td>
<td>5</td>
<td>1.420</td>
<td>0.156</td>
<td>1.847</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>DCDC2</td>
<td>C</td>
<td>4</td>
<td>1.217</td>
<td>0.033</td>
<td>1.905</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td>KIAA0319</td>
<td>A</td>
<td>4</td>
<td>3.600</td>
<td>0.0003</td>
<td>3.013</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>TRAP</td>
<td>A</td>
<td>5</td>
<td>2.664</td>
<td>0.008</td>
<td>2.856</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

Reading Components

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker 1</th>
<th>Marker 2</th>
<th>Frequency</th>
<th>Observed</th>
<th>Expected</th>
<th>Var(O-E)</th>
<th>( \chi^2 ) (1 df)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMP</td>
<td>A</td>
<td>G</td>
<td>506</td>
<td>309,580</td>
<td>328,830</td>
<td>70,605</td>
<td>5,245</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>DCDC2</td>
<td>G</td>
<td>T</td>
<td>562</td>
<td>290,750</td>
<td>314,010</td>
<td>58,510</td>
<td>9,248</td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

Our study shows strong association of markers in the 6p22 region with ADHD and the ADHD symptom dimensions, supporting the linkage study of Willcutt and colleagues. However, the determination of the specific ADHD candidate gene is not clear at this time. Association was much stronger in the DCD2/VMP region compared with the KIAA0319/TRAP region. Within the DCD2/VMP region, the markers Rs 793,862 and Rs 807,701 in DCD2 were most strongly associated with ADHD across all analyses conducted. Markers in VMP were also significantly associated with ADHD, albeit not as strongly or consistently as markers in DCD2. In the KIAA0319/TRAP region, one marker in KIAA0319 was associated with symptoms of IA and HI in the single-marker quantitative analysis. However, there was no association with this or any marker in any of the other analyses conducted, making KIAA0319 the weakest of all three candidates for ADHD.

In the VMP/DCDC2 region, it is unsurprising that association findings extend across both genes given that they are adjacent and in a region of high LD. We previously conducted a detailed analysis of intermarker LD across 44 markers in DCD2, VMP, and KIAA0319.

Table 4. Categorical Analysis of Haplotypes in VMP and DCDC2 in the Attention-Deficit/Hyperactivity Disorder Sample

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker 1</th>
<th>Marker 2</th>
<th>Frequency</th>
<th>Observed</th>
<th>Expected</th>
<th>Var(O-E)</th>
<th>( \chi^2 ) (1 df)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMP</td>
<td>A</td>
<td>G</td>
<td>506</td>
<td>309,580</td>
<td>328,830</td>
<td>70,605</td>
<td>5,245</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>T</td>
<td>555</td>
<td>26,249</td>
<td>32,093</td>
<td>5,584</td>
<td>2,692</td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

VMP, global \( \chi^2 \) test, on 3 degrees of freedom = 7.0785, \( p = .069 \). DCDC2: global \( \chi^2 \) test, on 3 degrees of freedom = 15.597, \( p = .002 \).
Table 5. Quantitative Analysis of Haplotypes in VMP and DCDC2 and Attention-Deficit/Hyperactivity Disorder

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Freq</th>
<th>Parent IA</th>
<th>Parent HI</th>
<th>Teacher IA</th>
<th>Teacher HI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z</td>
<td>p</td>
<td>Z</td>
<td>p</td>
<td>Z</td>
</tr>
<tr>
<td>VMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>51</td>
<td>-2.390</td>
<td>.017</td>
<td>-1.845</td>
<td>.065</td>
</tr>
<tr>
<td>A</td>
<td>25</td>
<td>2.092</td>
<td>.036</td>
<td>2.099</td>
<td>.036</td>
</tr>
<tr>
<td>G</td>
<td>24</td>
<td>.537</td>
<td>.091</td>
<td>-0.004</td>
<td>.997</td>
</tr>
<tr>
<td>DCDC2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>.57</td>
<td>-2.978</td>
<td>.003</td>
<td>-2.948</td>
<td>.003</td>
</tr>
<tr>
<td>A</td>
<td>.25</td>
<td>3.976</td>
<td>.00007</td>
<td>3.964</td>
<td>.00097</td>
</tr>
<tr>
<td>G</td>
<td>.13</td>
<td>.563</td>
<td>.507</td>
<td>.741</td>
<td>.459</td>
</tr>
<tr>
<td>A</td>
<td>.05</td>
<td>-2.519</td>
<td>.012</td>
<td>-2.080</td>
<td>.038</td>
</tr>
</tbody>
</table>

HI, hyperactivity/impulsivity; IA, inattention.

KIAA0319, TTKAP, and THEM2 in an independent study sample from Toronto selected for probands with reading difficulties (44).

Intemarker LD was then compared with LD across this region in the imputed HapMap sample. In both samples, high levels of LD were found between the 11 markers tested in VMP and the 8 markers tested in DCDC2. In addition, two of three previously published studies of RD in the region found support for DCDC2. Also, there was some evidence for association of markers in VMP with RD (34,42,43), suggesting that markers in both genes could be in LD with RD risk conferring DNA variants. Overall, the high level of LD between markers in this region will make it difficult to determine both the true candidate gene and the location of causal variants.

Twin studies consistently predict a stronger genetic relationship between RD and IA (12,14). Because 6p22 is an established RD locus, we hypothesized a stronger association with IA. Therefore, it was surprising when we observed equally strong association of markers with both the HI and IA symptom dimensions in the quantitative analyses. A recent twin study showed that although genetic correlations between RD and IA are strong, genetic correlations between RD and HI were not entirely absent (14). This suggests that some RD loci contribute to HI in addition to IA, which appears to be the case on 6p. This study is the first at this locus to partition the ADHD phenotype into its symptom dimensions.

Discrepancies among informants of ADHD behavior have been extensively documented, with twin studies indicating evidence for a common genetic factor underlying parent and teacher ratings of symptoms, together with additional, informant-specific genetic influences (68,69), supporting our analyses of the symptoms separately by informant. However, for the analyses here, the results are fairly consistent between parents' and teachers' ratings of ADHD symptoms, with markers in DCDC2 associated with ADHD symptoms as reported by both informants. The associated marker in VMP, Rs 3,429,810, was significant for parent report of both IA and HI but not significant for teacher report of these symptoms. However, there were trends in the same direction for the teacher-reported symptoms for the same alleles.

We also tested markers for association with measures of reading skills because these markers have all been associated with RD, or quantitative measures of reading, in previous studies. All four markers in VMP (Rs 3,429,810, Rs 3178) and DCDC2 (Rs 793,862 and Rs 807,701), associated with ADHD were not associated with the RD measures tested. The same alleles of Rs 793,862 and Rs 807,701 in DCDC2 have been associated with RD both individually and as a haplotype in two independent German samples (43). However, these results were not replicated in two additional studies of three independent RD samples (40,44). Markers in VMP have also been associated with RD (34,44), including Rs 3178 (44), which was associated with ADHD in our study. However, in both of those studies, support for either DCDC2 (34) or KIAA0319 (44) was stronger. A single marker in KIAA0319 in our study showed marginally significant association with phonological decoding (WRMT Word Attack). This marker was also associated with IA and HI symptoms in the single-marker quantitative analysis but not the other analyses conducted. Results from quantitative analyses should be interpreted cautiously, however, because they can be affected by factors that include the variance and the reliability of the phenotypic measure (70). In addition, only 31 individuals from our ADHD sample also met the criteria for a diagnosis of RD. Therefore, there might not have been enough power in this sample for this particular analysis, especially if this locus contributes to more severe RD as has been previously shown (36,39–43). Thus far, there is no clear and consistent overlap for association of the same markers with symptoms of both ADHD and reading skills in the same sample. Instead, there is evidence of strong association with RD from previous studies (36,39–43) and association with ADHD in our study. Therefore, there is currently not enough evidence to single out one specific pleiotropic gene for ADHD and RD on 6p.

In conclusion, the results from this study suggest that in addition to RD, the 6p22 locus also contributes to ADHD, supporting the 2002 study of Willcutt et al. (38). Our data narrow the search area by suggesting that the causal variants for ADHD might be found in the VMP/DCDC2 region. These data also suggest that the causal variants for ADHD within this region contribute to both IA and HI symptoms. Overall, additional studies with independent samples selected for RD, ADHD, and both disorders will be required before we can assess whether these genes are pleiotropic for both disorders.

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The authors report no biomedical financial interests or potential conflicts of interests.

Supplementary material cited in this article is available online.


