Association of the DAT1 genotype with inattentive behavior is mediated by reading ability in a general population sample

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ABSTRACT

Attention deficit hyperactivity disorder (ADHD) and reading disability (RD) frequently co-occur in the child population and therefore raise the possibility of shared genetic etiology. We used a quantitative trait loci (QTL) approach to assess the involvement of the dopamine transporter (DAT1) gene polymorphism in mediating reading disability and poor attention in a general population sample of primary school children aged 6–11 years in the UK. The potential confounding effects of IQ and chronological age were also investigated. We found an independent association between the homozygous DAT1 10/10 repeat genotype and RD that was not accounted for by the level of ADHD symptoms. This finding suggests that the DAT1 gene polymorphism may influence a common neural mechanism underlying both reading acquisition and ADHD symptoms.

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1. Introduction

Children with attention-deficit hyperactivity disorder (ADHD) are characterized by their poor attention to detail, difficulties in maintaining attention over a sustained period of time, and moment-to-moment variability throughout task performance. ADHD frequently co-occurs with reading disability (RD), a disorder that is characterized by impairments in single word reading, reading fluency, and reading comprehension (Catts & Kamhi, 2005). The incidence of RD in samples of children selected for ADHD is estimated to be between 25% and 40% (Semrud-Clikeman et al., 1992), while 15–35% of children with RD will meet the criteria for ADHD (Willcutt & Pennington, 2000). Together, ADHD and RD represent the two most common developmental disorders of childhood, each occurring in approximately 5% of the population, with a higher frequency of boys than girls.

At the cognitive level, although some evidence points to commonalities across and within a range of executive function sub-domains in comorbid RD and ADHD, specifically impairments in working memory (e.g. Cohen et al., 2000; Martinussen & Tannock, 2006; Willcutt et al., 2001; Willcutt, Pennington, Olson, Chhabildas, & Hulslander, 2005), the evidence is not always consistent (de Jong et al., 2009; Marzocchi et al., 2008; Willcutt et al., 2005). In contrast, deficits in response inhibition appear to be specific to ADHD (e.g. Cornish et al., 2005; Rubia et al., 2001, but see Castellanos and Tannock (2002), for a review). One promising cognitive overlap between RD and ADHD recently reported by Willcutt et al. (2010) is a possible shared deficit in speed of processing. Willcutt et al. examined the genetic and environmental etiology of scores on composite measures derived from an extensive battery of cognitive tests administered as part of the Colorado Learning Disabilities Research Center (CLDRC) twin study. The purpose of this study was to tease apart specific neuropsychological processes that account for the phenotypic comorbidity between RD and ADHD. These authors concluded that processing speed was the only cognitive composite that could be explained by common genetic influences that increase susceptibility to both disorders.

At the genetic level, there is converging evidence that the substantive co-morbidity between ADHD and RD is due, in part, to shared (pleiotropic) genetic factors (Gayan & Olson, 2001; Willcutt, Pennington, & DeFries, 2000; Willcutt, Pennington, Olson, & DeFries, 2007). Luca et al. (2007) found that the dopamine receptor D1 gene (DRD1) showed a strong association with inattentive behavior in children selected for reading problems but showed no such relationship with reading disability, suggesting that DRD1 uniquely contributes to inattention. To date, although no specific genes involved in the ADHD + RD phenotype have yet been identified, a number of genome-wide linkage analyses have identified potential loci pleiotropic for ADHD + RD (Bakker et al., 2003; Couto et al., 2009; Gayan et al., 2005; Loo et al., 2004; Stevenson et al., 2005; Wigg et al., 2008).
In the present study, we focus on a possible association between RD, ADHD symptoms and the dopamine transporter (DAT1) gene polymorphism (SLC6A3) located on chromosome 5p15.3. The pharmacological effects of psycho-stimulants have made dopaminergic genes, including DAT1, logical candidates for ADHD. Several studies provide evidence in favor of an association between the 10 repeat-allele and ADHD symptoms (Brookes et al., 2008; Chen et al., 2003; Cornish, Wilding, & Hollis, 2008; Cornish et al., 2005; Doyle et al., 2009; Genro et al., 2008); although non-replications have been reported (Bakker et al., 2003; Langley et al., 2008; Chen et al., 2003; Cornish, Wilding, & Hollis, 2008; Cornish et al., 2005), in the present study we test the hypothesis that this gene may have a moderating rather than direct influence on cognitive impairments (Kebr & Joober, in press). In contrast, no current studies have yet examined the direct association between DAT1 and RD, or between DAT1 and comorbid ADHD + RD. However, previous findings indicate measures of response inhibition load most closely with reading and not attention constructs in experimental studies (Purvis & Tannock, 2000) and in robust ‘error-free’ latent variable analysis (Savage, Cornish, Manly, & Hollis, 2006), indicating a potential important link between DAT1 and reading ability that has hitherto not been explored.

Using a quantitative trait loci (QTL) approach to investigate the link between DAT1 and RD, and having previously found an association between DAT1 and ADHD symptoms in the same sample (Cornish et al., 2005), in the present study we test the hypothesis that this association is not independent of reading disability, and that DAT1 would remain a strong predictor of reading ability after initial controls for variability in ADHD symptoms (based on teacher-ratings of attention problems, and extraneous measures; IQ, chronological age) (Model 1). In contrast, we hypothesized that DAT1 would not be a strong predictor of ADHD symptoms after parallel controls for reading ability and extraneous measures (Model 2).

2. Method

2.1. Participants

In the first stage of the study, we anonymously screened an epidemiological sample of 1776 6-to-11-year-old children from Central England (UK) for symptoms of ADHD using a teacher-rated behavioral questionnaire, the strengths and weaknesses of ADHD–symptoms and normal behavior scale (SWAN; Swanson, McStephen, Hay, & Levy, 2001). The SWAN scale is based on the 18 ADHD symptoms listed in the DSM-IV manual (American Psychiatric Association, 1994). Scoring for each item goes from a low level of problems (3, 2, 1) through average (0) to a high level (–1, –2, –3). Children’s scores ranged from a minimum of –27 to a maximum of 27 for each sub-scale: Inattention (items 0–9) and hyperactivity/impulsivity (items 10–18). This scale allows for a normal distribution of the data and avoids potential psychometric flaws that are associated with skewed distributions. This is especially advantageous when selecting extreme high and low scorers for genetic association studies investigating qualitative trait loci (QTL), as in the present study.

Of the 1776 questionnaires, 92 were excluded from further analysis due to missing or incomplete responses. There were complete questionnaires on 872 boys and 812 girls. SWAN summary (total) scores were normally distributed; Boys: mean = 4.7, SD = 23.1, skewness = –0.11, kurtosis = –0.30; Girls: mean = –9.9, SD = 20.4, skewness = –0.24, kurtosis = –0.30. Because the distribution of SWAN total score in boys was shifted to the right compared to the distribution of scores for girls (such that 86% of the highest 10% SWAN ratings were for boys), only boys were included in the subsequent parts of this study. This produced a total of 126 participants made up of two groups: (1) 58 boys who were rated by teachers above the 90th percentile for Inattentive and/or hyperactivity/impulsivity sub-scale items on the SWAN questionnaire (mean SWAN score of 40.8; SD 1.56; age range 6–11 years; mean age 9 years 5 months); (2) 68 boys who were rated by teachers as below the 10th percentile for inattentive and/or hyperactivity/impulsivity sub-scale items on the SWAN questionnaire (mean SWAN score of –33.1; SD –1.63; age range 6–11 years; mean age 8 years 6 months). Each participant was also rated by the same class teacher using the Conners’ Teacher Rating Scale-Revised: Short version (CTRS-R:S) (Conners, Sitarenios, Parker, & Epstein, 1998a) and by parents using the Conners’ Parent Rating Scale-Revised: Short version (CTRS-R:S) (Conners, Sitarenios, Parker, & Epstein, 1998b).

The ADHD index of the CPRS-R:S and CTRS-R:S contains 12 items that discriminate well between ADHD clinical cases and controls (Conners et al., 1998a, 1998b). High correlations between these two scales have previously been reported (Cornish et al., 2005) and the SWAN has demonstrated good psychometric properties in discriminating between children with and without ADHD (Young, Levy, Martin, & Hay, 2009). None of the children were receiving stimulant medication (e.g. methylphenidate or dexamphetamine). One child was African Caribbean, and one child was Cypriot.

2.2. DNA extraction and genotyping

Buccal cells were harvested in 10 ml sterile saline, and DNA was extracted by alkaline lysis of the cells, as described by Ferrie et al. (1992). The analyses were performed at the Department of Molecular Genetics, Nottingham City Hospital, Nottingham, England, and each individual was genotyped twice. This DNA procedure has been described in detail in Cornish et al. (2005). Genotyping was conducted on 119 out of the 126 eligible participants (boys scoring > 90th and < 90th percentile on the SWAN scale). DNA samples were unobtainable or incomplete from seven participants and these children were excluded from further analysis. Initial analysis was conducted using the three most common genotype groups: 10/10, (n = 62); 9/10 (n = 41), and 9/9 (n = 12). Further analysis was made combining the 9/10 genotype with 3/10, 8/10 and 11/10 to create a heterozygous 10-repeat allele group (n = 45).

2.3. Intellectual ability

All participants were tested (individually) on the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). This test provides a composite IQ score based on four subtests tapping both verbal and performance domains.

2.4. Reading tests

The Neale Analysis of Reading Ability (2nd edition) (NARA, Neale, 1997) was used to measure reading. Children are asked to read aloud a series of graded fiction and non-fiction narratives as speedily and as accurately as possible. Children are also told in advance that they will be asked questions about the narratives they have read. The test provides an age-standardized score measure of reading accuracy (calculated by the number of reading errors to a discontinuation point of 14 errors in a single narrative), rate (based on words read per minute in each narrative) and comprehension (based on the accuracy of subsequent responses to questions about
the passage). Form A was selected as Form B may show a gender skew (Stothard & Hulme, 1992). Reading disability can be defined by deficiencies in one or more of these diverse aspects of reading ability and the standard scores are commonly used to produce a binary variable of good versus poor readers.

### 2.5. Procedure

Following regional and local ethical approval, all participants were tested in the school environment and testing followed the same standardized routine on each occasion.

### 2.6. Statistical methods

We sought to test the hypothesis that DAT1 would remain a strong predictor of reading ability after initial controls for variability in ADHD symptoms, as well as the contrasting hypothesis that DAT1 would remain a strong predictor of ADHD symptoms after initial controls for variability in reading ability. Regression analysis was used to test these hypotheses. Parallel analyses were run with either attention or reading as the DV. As reading measures were continuous, linear regression approaches were used for this DV, whereas binary logistic regression analyses were used when the binary attention group construct operated as the DV. In all analyses, IQ and chronological age were treated as extraneous variables, and were entered as steps 1 and 2 in all analyses, prior to entering of the variables of particular interest at steps 3 and 4.

### 3. Results

The final sample comprised 104 children (three children failed to provide data from the reading measures). Of these, 56 were rated as having ‘high SWAN scores’ (high ADHD symptom group) and 48 were rated as having ‘low SWAN scores’ (low ADHD symptom group). Fifty-nine children had the DAT1 two 10-repeat alleles (10/10) and 45 had the one 10-repeat allele. The 104 children were then classified into good readers and poor readers based on a standard binary classification procedure using the NARA (poor reader = standard score <85). This yielded 32 poor readers on reading accuracy (mean reading accuracy = 73.44, SD = 7.49) and 72 good readers (mean reading ability = 105.03, SD = 5.36). For reading comprehension this same binary classification yielded 31 poor readers on reading comprehension (mean reading comprehension ability = 74.07, SD = 4.39) and 73 good readers (mean reading comprehension ability = 106.03, SD = 11.85). Finally, the same analysis for reading rate yielded 27 poor readers (mean reading rate = 75.04, SD = 11.30) and 77 good readers (mean reading ability = 106.32, SD = 10.30). Further analysis showed that 75.4% of the high ADHD symptom group were poor readers, whereas only 18% of the low ADHD symptom group were poor readers, confirming that there was significant overlap between RD and high levels of ADHD symptoms.

Statistical analysis of the data showed that there was a strong association between reading disability and the presence of the two 10-repeat alleles but not the one 10-repeat allele for reading accuracy, chi square with continuity correction = 7.41, df = 1; P = .005; for reading comprehension, chi square with continuity correction = 8.94; df = 1; P = .003; and for reading rate, chi square with continuity correction = 10.51; df = 1; P = .001. DAT1 allele frequencies for good and poor readers across reading sub-domains are presented in Figs. 1a, 1b, 1c.

We then used a series binary logistic regression to test the two models of reading and ADHD symptom group (see Table 1). All models had excellent goodness of fit (Hosmer–Lemeshow goodness of fit = ns in all cases). We first considered a 4-component predictor model for reading: Chronological age + IQ, +ADHD symptom group + DAT1 (Model 1). In all cases, DAT1 predicted significant additional variation in reading at step 4 of analyses as we predicted. We then considered a 4-component predictor model for ADHD symptom group: Chronological age + IQ +reading + DAT1 (Model 2), where reading comprehension, accuracy and rate were each entered in turn at step 3 and compared. In these analyses, DAT1 did not predict significant additional variation in ADHD symptoms in any case at step 4 of analyses, as we predicted.
Associations between DAT1, reading ability and attention.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Reading comprehension</th>
<th>Reading accuracy</th>
<th>Reading rate</th>
</tr>
</thead>
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<tr>
<td></td>
<td>$R^2$ change</td>
<td>Significance</td>
<td>$R^2$ change</td>
</tr>
<tr>
<td>Model 1: Predicting reading from IQ, Chronological age, attention group, and DAT1 (N = 107 children)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Age</td>
<td>.01</td>
<td>.28</td>
<td>.02</td>
</tr>
<tr>
<td>(2) IQ</td>
<td>.70</td>
<td>.001</td>
<td>.71</td>
</tr>
<tr>
<td>(3) Attention</td>
<td>.08</td>
<td>.041</td>
<td>.07</td>
</tr>
<tr>
<td>(4) DAT1</td>
<td>.05</td>
<td>.016</td>
<td>.02</td>
</tr>
<tr>
<td>H–L fit $P = .58$, ns.</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Dependent variable: Attention

<table>
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<tr>
<th>Step IV</th>
<th>$R^2$ change</th>
<th>Significance</th>
<th>$R^2$ change</th>
<th>Significance</th>
<th>$R^2$ change</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 2: Predicting Swan attention group category from IQ, Chronological age, reading, and DAT1 (N = 107 children)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Age</td>
<td>.06</td>
<td>.010</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(2) IQ</td>
<td>.55</td>
<td>.047</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) ReadingABC</td>
<td>.13</td>
<td>.001</td>
<td>.12</td>
<td>.001</td>
<td>.09</td>
<td>.008</td>
</tr>
<tr>
<td>(4) DAT1</td>
<td>.00</td>
<td>.343</td>
<td>.01</td>
<td>.290</td>
<td>.02</td>
<td>.103</td>
</tr>
<tr>
<td>H–L fit $P = .18$, ns.</td>
<td></td>
<td></td>
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<tr>
<td>Total variance explained</td>
<td>74%</td>
<td></td>
<td>74%</td>
<td></td>
<td>70%</td>
<td></td>
</tr>
</tbody>
</table>

Note: H–L fit = Hosmer–Lemeshow goodness of fit measure.

4. Discussion

In the present study we adopted a quantitative trait loci (QTL) approach to investigate in an epidemiological sample of primary school children the association between the DAT1 10/10 genotype and reading disability. Specifically, we sought to establish whether the DAT1–ADHD association reported in a previous study (Cornish et al., 2005) would remain after controlling for reading ability. We predicted that the genotype association would be more strongly related to reading disability than with high levels of ADHD symptoms. Our findings support this prediction and highlight a strong and direct DAT1-reading link that is inexplicable as a consequence of ADHD symptom levels or extraneous factors such as chronological age, or intellectual ability. Furthermore, while highly specific in nature, the DAT1 – reading association is evident for measures assessing quite diverse aspects of reading ability (accuracy, rate, and comprehension). These findings indicate that the origin of DAT1 involvement is in the need for accurate and efficient word recognition, as this is the behavioral component ability that is shared by all reading measures described here.

In cognitive models of skilled reading, word recognition requires the integration of distributed patterns of activation in biologically-plausible connectionist structures. These structures have been instantiated as explicit computational models which incorporate networks of distributed recognition units with cell-like qualities of activation and inhibition of response to discrete aspects of word stimuli (Jackson & Coltheart, 2001; Harm & Seidenberg, 2004). Furthermore, reducing either the number or specificity of these excitatory and inhibitory pathways simulates behavioral patterns evident in developmental dyslexia (Harm & Seidenberg, 2004). Disruption of attentional mechanisms has been proposed to play a causal role in reading difficulties (Shaywitz & Shaywitz, 2008), and the covariance between reading disability and ADHD inattention symptoms has been shown to be largely driven by genetic factors (Paloyelis, Rijskijk, Wood, Asherson, & Kuntsi, 2010). Although no direct relationship has been established between connectionist models and DAT1, the disruption or reduction in the main lines of communication within dopaminergic neurotransmitter pathways that is partly determined by the presence of the DAT1 10/10 genotype may well provide a highly plausible candidate explanation of disruptions to both reading acquisition and ADHD inattention symptoms.

At the genetic level, previous reports of links between DAT1 and inattentive behavior (Bellgrove et al., 2005, 2009; Cornish et al., 2005; Loo et al., 2003) may reflect variation in neuropsychological processes common to both reading ability and generation of ADHD symptoms. Reading ability may be a more proximal phenotypic measure to these core processes (Purvis & Tannock, 2000) and may therefore explain most of the variance in ADHD symptoms within the co-morbid RD + ADHD phenotype. It is quite possible that some inconsistencies in reported association of DAT1 with the neuropsychological phenotype in ADHD may in fact reflect the uncontrolled influence of reading ability and variation in frequencies of unique shared ADHD genes in the samples examined (see Rommelse et al. (2008), for a review). Our data certainly suggest that further advances in the behavior-genetics of ADHD will require the routine collection of cognitive data on reading ability as well as ADHD symptoms in general population samples. However, it is unclear on the basis of these findings whether a distinct ADHD + RD phenotype shares a pleiotropic gene (DAT1) that also influences common underlying attentional functions such as response inhibition and selective attention as reported in our previous study (Cornish et al., 2005), or may reflect individual differences in processing speed that increase genetic risk for comorbidity between ADHD and RD (Willcutt et al., 2010).

Our findings should be interpreted in view of several limitations. Consistent with the multiple deficit model (Pennington, 2006) which proposes that complex developmental disorders arise from a constellation of unique and shared risk factors, it has emerged that processing speed is the most likely candidate for a shared genetic risk factor for ADHD and RD (Willcutt et al., 2010; McGrath et al., 2011). Therefore it is not entirely clear whether the current findings reflect the uncontrolled influence of processing speed on common genetic influences in the comorbidity between ADHD and RD. However, the previous findings are ambiguous as to whether processing speed plays a causal role or represents a symptom of having RD or ADHD (McGrath et al., 2011). Future molecular genetic studies will need to include specific measures of processing efficiency to further explore the multiple deficit model of comorbid ADHD and RD.

Although the SWAN scale has been reported previously as providing an approximation to a normal distribution with the advantage of sampling at the extreme ends of the ADHD continuum, it should be acknowledged the inherent limitation of using teacher
ratings and the resulting profile only including boys. However, this was an unavoidable consequence of the rightward shift in the distribution of the scores in a population-based sample such that boys represented 86% of the highest 10% of SWAN scores. Nevertheless, it is possible that teacher ratings may have identified more boys when compared to their female counterparts and thus the inclusion of females rated on both parent and teacher ratings will be an important direction for future research. Another limitation is that sampling at the extreme ends of the ADHD behavioral dimension may have obscured any potential DAT1 association based on the eligibility inclusion; however, the inclusion of only the top and bottom 10% of the ADHD distribution for further neuropsychological testing and genotyping has been previously shown to be effective in increasing the likelihood of detecting important associations between DAT1 and variance in ADHD symptoms (Cornish et al., 2005, 2008). Finally, it is not clear from the current findings whether specific environmental factors such as socioeconomic status would have a direct influence on both reading difficulties and ADHD symptoms and thus examining environmental risk factors should be a critical goal of this next generation of research.

In conclusion, our findings report a novel association between DAT1 and reading ability that has not hitherto been explored. Although DAT1 has previously been linked to ADHD, the evidence remains inconsistent both in population and clinical studies, and the current findings further support the utility of a QTL approach for future molecular genetic studies in ADHD. Our results suggest that reading ability may be a useful phenotypic marker for a particular sub-type of ADHD associated with shared genes for RD. Future research needs to elucidate the differing unique and shared gene-environment interactions and developmental trajectories associated with the emergence of RD and ADHD.

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