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Association of ADHD and the *Protogenin* gene in the chromosome 15q21.3 reading disabilities linkage region

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Abstract

Twin studies indicate genetic overlap between symptoms of attention deficit hyperactivity disorder (ADHD) and reading disabilities (RD), and linkage studies identify several chromosomal regions possibly containing common susceptibility genes, including the 15q region. Based on a translocation finding and association to two specific alleles, the candidate gene, *DYX1C1,* has been proposed as the susceptibility gene for RD in 15q. Previously, we tested markers in *DYX1C1* for association with ADHD. Although we identified association for haplotypes across the gene, we were unable to replicate the association to the specific alleles reported. Thus, the risk alleles for ADHD are yet to be identified. The susceptibility alleles may be in a remote regulatory element, or *DYX1C1* may not be the risk gene. To continue study of 15q, we tested a coding region change in *DYX1C1*, followed by markers across the gene *Protogenin* (*PRTG*) in 253 ADHD nuclear families. *PRTG* was chosen based on its location and because it is closely related to *DCC* and *Neogenin*, two genes known to guide migratory cells and axons during development. The markers in *DYX1C1* were not associated to ADHD when analyzed individually; however, six markers in *PRTG* showed significant association with ADHD as a categorical trait ($P = 0.025–0.005$). Haplotypes in both genes showed evidence for association. We identified association with ADHD symptoms measured as quantitative traits in *PRTG*, but no evidence for association with two key components of reading, word identification and decoding was observed. These findings, while preliminary, identify association of ADHD to a gene that potentially plays a role in cell migration and axon growth.

Keywords

ADHD; association; reading disabilities; 15q21.3

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Attention deficit hyperactivity disorder (ADHD) and reading disabilities [(RD) also known as developmental dyslexia) are common childhood disorders that are often comorbid (Nigg *et al.* 1998, 2002; Pennington *et al.* 1993; Willcutt & Pennington 2000; Willcutt *et al.* 2000). It is estimated that 20–40% of individuals who are diagnosed with ADHD will also be identified to have RD (August & Garfinkel 1990; Faraone *et al.* 1993; Humphries *et al.* 1994; Semrud-Clikeman *et al.* 1992; Srinivas *et al.* 1992; Tannock & Schachar 1996), while in the general population, the prevalence is estimated to be around 3–6%.

Support for genetic overlap can be derived from twin studies that have found evidence for shared genetic factors, particularly for inattention symptoms (Willcutt *et al.* 2000, 2007). The most recent study estimated the genetic correlation between inattention and reading phenotypes and found significant genetic correlation between inattention and reading discrepancy (0.72), orthographic choice (0.71) and phoneme awareness (0.41) (Willcutt *et al.* 2007), which indicates that these phenotypes will share some of their susceptibility genes. Genetic correlations for symptoms of hyperactivity/impulsivity and these reading measures were not as high (0.37, 0.40 and 0.40, respectively) (Willcutt *et al.* 2007).

Further support for shared genetic factors is the overlapping chromosomal regions for ADHD and RD identified by linkage and association studies. These include: 4q12-13 (Arcos-Burgos *et al.* 2004; Fisher *et al.* 2002), 6p21-22 (Cardon *et al.* 1994; Cope *et al.* 2005a; Francks *et al.* 2004; Gayan *et al.* 1999; Paracchini *et al.* 2006), 6q12-14 (Ogdie *et al.* 2003; Petryshen *et al.* 2002), 10cen-q11 (Bakker *et al.* 2003; Loo *et al.* 2004), 15q15-21 (Bakker *et al.* 2003; Chapman *et al.* 2004; Grigorenko *et al.* 1997; Morris *et al.* 2000; Smith *et al.* 1983, 1991), 16p13 (Loo *et al.* 2004; Ogdie *et al.* 2004) and 17p11-q22 (Arcos-Burgos *et al.* 2004; Loo *et al.* 2004; Ogdie *et al.* 2004).

The evidence for linkage in the 15q region for ADHD was from a genome scan in a sample of 164 ADHD-affected sibling pairs from Holland (Bakker *et al.* 2003). The study indicated that a major gene contributing to ADHD is located on chromosome 15q. For RD, the first published linkage study identified evidence for linkage to the chromosome 15 centromeric region using chromosomal heteromorphisms; chromosomal band polymorphisms that are visible using cytogenetic techniques (Smith *et al.* 1983). Evidence for linkage or association of RD and the related phenotype of spelling ability to markers on chromosome 15 was found in additional studies; however, the regions identified in these studies spanned a very large region of the long (q) arm, thus impeding gene identification (Chapman *et al.* 2004; Grigorenko *et al.* 1997; Morris *et al.* 2000; Schulte-Korne *et al.* 1998). A recent study of unselected twins also supports this locus as contributing to reading skills in the population (Bates *et al.* 2007). The overlapping regions of linkage for both RD and ADHD in the 15q region are consistent with the possibility that a gene on chromosome 15q is pleiotropic, contributing both to RD and ADHD.

A translocation breakpoint on 15q in a family cosegregating with RD led to the identification of a gene, *DYX1C1* (also called *EKN1*), implicating this gene as the susceptibility gene on 15q (Taipale *et al.* 2003). In addition to the translocation occurring within intron 8 of this gene, association was also reported in that study. The coding region of the *DYX1C1* gene was screened in 20 Finnish RD subjects and eight changes in the DNA

sequence were identified (Taipale *et al.* 2003). Two of these were reported to be associated with RD; the A allele of a G to A bp change located 3 bp before the beginning of the sequence that codes for the *DYX1C1* protein (−3G/A) and the T allele of a G to T change at position 1249 (1249G/T). Both these changes could potentially result in a change of function (Taipale *et al.* 2003). The −3A could change the efficiency of transcription or translation, and the 1249T results in a premature truncation of the protein by four amino acids. Based on this, the authors of that paper concluded that these two DNA changes contribute to the susceptibility to RD.

The studies in our RD families provided some support for association of *DYX1C1* to RD (Wigg *et al.* 2004); however, the association observed was for different alleles and haplotypes than those reported to be associated to RD in the Finnish sample. Five additional studies have not replicated the association of the −3A or 1249T alleles to RD (Bellini *et al.* 2005; Brkanac *et al.* 2007; Cope *et al.* 2005b; Marino *et al.* 2005; Meng *et al.* 2005a; Scerri *et al.* 2004). These studies in total indicate that these alleles identified as associated (−3A or 1249T) are unlikely to be the functional DNA changes contributing to RD. Given that the coding region has been screened and no further associated variants were found, an explanation for the association to RD is needed. The associated markers may be in linkage disequilibrium (LD) with the susceptibility alleles either in a regulatory region of *DYX1C1* or in another gene in the region. Although the translocation breakpoint was identified as occurring in the *DYX1C1* gene, chromosomal translocations can cause differential expression of multiple genes in the region by changing the position of the genes relative to their normal chromosomal environment. This type of position effect has been documented to occur for a number of chromosomal translocations (Kleinjan & van Heyningen 1998) and thus the susceptibility gene may be located at a distance from *DYX1C1*. Furthermore, this region of 15q21 shows punctate LD patterns with strong LD between markers located at large distances separated by regions of low LD (http://www.hapmap.org/). Thus, the identified association signals in *DYX1C1* could be detecting susceptibility alleles at a large distance from *DYX1C1.*

Given the genetic overlap for ADHD and RD for the 15q region, we previously studied the relationship of the *DYX1C1* to reading phenotypes, ADHD and the inattention and hyperactive/impulsive dimensions of ADHD in a sample of families with ADHD children (Wigg *et al.* 2005). This sample of ADHD families is independent of the sample of RD families that has been previously published (Wigg *et al.* 2004). In the ADHD sample, for the single marker analysis, we did not observe significant biased transmission of the alleles of any of the six markers genotyped. We did find significant biased transmission for one haplotype (χ^2 = 6.926, df = 1, P = 0.009) and biased non-transmission for another (χ^2 = 4.462, df = 1, P = 0.035) when all six markers that were genotyped across *DXY1C1* were analyzed (global χ^2 = 9.312, df = 3, P = 0.025). The haplotypes biased in transmission, however, did not include the −3A and 1249T alleles.

Thus, like RD, we found no support for these specific alleles as contributing to ADHD and we continued to study this region by fine mapping across the 15q region. This study focused on fine mapping the region for the relationship of markers to ADHD and ADHD symptoms. We began by genotyping the six markers originally genotyped in *DYX1C1* in an additional

67 families collected since our first study of this gene and added a new nonsynonymous coding region polymorphism. For study, we also selected the gene *PRTG*, located distally to *DYX1C1*, based on its potential role in axon guidance and neuronal outgrowth and its proximity to *DYX1C1*. This gene, originally annotated as *FLJ25756*, has only recently been characterized in chick embryos and has been renamed *protogenin*: 'proto' for its early expression in the nervous system and 'genin' because of its structural similarity to neogenin (Toyoda *et al.* 2005). *PRTG* is a member of the immunoglobulin superfamily that includes receptors for secreted guidance cues as well as classical neuronal cell adhesion molecules (Toyoda *et al.* 2005). The structure of *PRTG* is more similar to the DCC subgroup of these proteins that includes the receptors *DCC* and *Neogenin*, both known to guide migratory cells and axons during development (Toyoda *et al.* 2005). The reason that this gene stands out as a candidate is that the genes that have provided preliminary evidence as susceptibility genes for RD, *DCDC2*, *KIAA0319* and *ROBO1*, including *DYX1C1* (Fisher & Francks 2006; Galaburda *et al.* 2006; Hannula-Jouppi *et al.* 2005; McGrath *et al.* 2006; Williams & O'Donovan 2006), have a role in neuronal migration or axon guidance (Brose *et al.* 1999; Kidd *et al.* 1999; Meng *et al.* 2005b; Paracchini *et al.* 2006; Seeger *et al.* 1993; Wang *et al.* 2006). While the role of these genes in RD is far from conclusive, the proteins these genes encode may be functionally linked in pathways involved in neuronal migration or axon growth. Previous data indicate that postmortem brains of dyslexic individuals show subtle cortical anomalies involving neuronal migration and axon growth, thus changes in function or expression of these genes offers an intuitively satisfying common mechanism to the neurodevelopmental risk for RD (Galaburda *et al.* 2006).

Based on the potential involvement of *PRTG* in axon growth and its location in a region linked to both ADHD and RD distal to *DYX1C1* on 15q, we tested this gene for association with ADHD and to ADHD symptom dimensions of inattention and hyperactivity/impulsivity in a sample of families with an ADHD proband. We also tested for the relationship of reading component skills using measures of word identification and phonological decoding. We selected 20 single nucleotide polymorphisms (SNPs) across this candidate gene for an association study and genotyped these 20 markers in our Toronto sample of 253 families with an ADHD proband and 47 affected siblings.

Materials and methods

Subjects

The clinical assessment and the diagnostic criteria of the subjects have been described in detail in previous publications (Barr *et al.* 1999, 2000, 2001a,b). Briefly, probands and affected siblings were recruited from the Child Development and Neuropsychiatry Clinics at the Hospital for Sick Children. The selection criteria for inclusion were subjects between the ages of 7 and 16 years that met the Diagnostic and Statistical Manual of Mental Disorder, 4th Edition (DSM-IV) criteria for ADHD. Subjects were excluded if they scored below 80 on both the Performance and Verbal Scales of the Weschler Intelligence Scale for Children (Wechsler 1991), had evidence of neurological or complex medical illness, Tourette syndrome, chronic multiple tics, bipolar affective disorder, psychotic symptoms or had a comorbid anxiety, depressive or developmental disorder that could better account for the

behaviors (as specified by DSM-IV). For the diagnoses of ADHD and information on ADHD symptoms, two informants were used: the parents completed the Parent Interview for Child Symptoms (Ickowicz *et al.* 2006) and the teachers were administered the Teacher Telephone Interview for Children's Academic Performance, Attention, Behavior and Learning: DSM-IV version (R. Tannock, M. Hum, M. Masellis, T Humphries, R. Schachar, Hospital for Sick Children, Toronto, unpublished data). The parents were interviewed about the child's behavior at home by a trained clinician, either a master's level psychologist, social worker or psychiatrist. The 30-min teacher interview, which was conducted by phone by trained professionals (masters or doctoral level psychologists or special education consultants), probed the child's behavior in the school setting. The use of two independent informants ensures that children were at least moderately impaired in two settings. A 6/4 algorithm was used to determine if the subjects met criteria for ADHD; this algorithm specifies that to meet criteria, the child must be identified as having six of nine inattentive or hyperactive/impulsive symptoms as specified by DSM-IV for either the parent or teacher interview, and that the child must exhibit a minimum of four inattentive or hyperactive/impulsive symptoms according to the second informant.

The child was assessed in a full day protocol at the Hospital for Sick Children. The children were free of medication for a minimum of 24 h before assessment. All interviews were recorded so that surveillance was maintained, reliability could be assessed and to prevent criterion shift. Training was provided to the interviewers to ensure that the criterion of 90% symptom agreement was reached. Interviews were conducted independently by separate clinicians that rate the symptoms based on the parent's and teacher's descriptions of the child's observable behavior at home and school, respectively.

The assessment protocol also includes measures of academic achievement, including reading ability. The reading tests and WISC-III were administered by a Master's level psychological assistant supervised by a registered clinical psychologist. The characteristics of this sample for the quantitative measures used in this study are given in Table 1.

The majority of individuals with RD are far more deficient in single-word recognition and its component processes than in comprehension. Thus, measures of word identification and decoding (the understanding of the complex mapping that translates written letters into spoken sounds, measured by the ability to pronounce nonwords) can provide a good estimate of an individual's reading ability (Perfetti 1985). Reading ability was measured using the Wide Range Achievement Test – III (Wilkinson 1993), and Word Attack and Word Identification Subtests of the Woodcock Reading Mastery Test –Revised (Woodcock 1987). In this group of ADHD-affected children, 17% could be classified as having RD defined, for this study, as 1.5 SD below the mean on any one of the three measures of reading or 1 SD below the mean on the average of any two. Thus, at the current time, the sample of children with ADHD + RD is too small for the analysis as a categorical trait.

The study sample was comprised of 253 nuclear families from the Toronto area, including 47 affected siblings. This gave a total of 300 affected children (242 boys and 58 girls). For the affected children, the distribution among the DSM-IV ADHD subtypes was 14% of the predominantly hyperactive/impulsive subtype, 24% of the predominantly inattentive subtype

and 62% of the combined subtype. The majority of the families reported their ethnic background to be of European Caucasian descent, while 10% of families were of other or mixed background, including Chinese, African, Indian and native Canadians.

This protocol was approved by the Hospital for Sick Children's Research Ethics Board, and written informed consent and children's assent was obtained for all participants.

Isolation of DNA and marker genotyping

DNA was extracted from blood lymphocytes using a high salt extraction method (Miller *et al.* 1988). The SNPs across the *PRTG* gene were selected using the Tagger Pairwise Method (de Bakker *et al.* 2005) as implemented on the International HapMap Project Browser (www.hapmap.org) as well as three publicly reported synonymous and nonsynonymous SNPs. For *DYX1C1*, eight SNPs were genotyped, eight of these had been previously genotyped in 186 of the ADHD families (Wigg *et al.* 2005) and the genotyping was updated in our expanded sample. Two additional SNPs, rs600753 and rs16787 in the *DYX1C1* gene were also genotyped. The marker, rs16787, significantly deviated from Hardy–Weinberg disequilibrium in the parental chromosomes and was therefore not included in the analyses. The SNP assays were manufactured by Applied Biosystems as either Assays-On-Demand (predesigned) or as Assays-by-Design (made to order). The 10 μl polymerase chain reaction (PCR) reactions contained 30 ng of genomic DNA, 10 μmol of TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 0.25 μl of the allelic discrimination mix which is a premade mix containing the specific primers (18 μM) and probes (4 μM; Applied Biosystems). The thermal cycling conditions were 95°C for 10 min followed by 40 cycles of 94°C for 15 seconds and an annealing temperature of 60°C for 1 min. Included on each 96-well plate were two negative controls. The end-point data, for each plate, were collected using the ABI 7900HT Sequence Detection System (SDS) with the allelic discrimination analysis mode of the SDS software package version 2.0 (Applied Biosystems).

Statistical analysis

Descriptive statistics given in Table 1 were generated with SPSS 10.1 (http://www.spss.com/). All data were screened for Mendelian errors using PEDSTATS 0.6.11, and MERLIN 1.1.2 was used to detect any crossovers between markers (Abecasis *et al.* 2002). This data set was free of any detectable Mendelian errors and none of the markers used in the analyses deviated from the Hardy–Weinberg equilibrium. The extended transmission disequilibrium test (ETDT 2.1) program (Sham & Curtis 1995) was used to test for the biased transmission of alleles at each marker, based on the categorical diagnosis of DSM-IV ADHD. The ETDT program does not include in the analyses families with a single parent because of the possibility of bias when one of the alleles is rare (Curtis & Sham 1995). Permutation analysis for the categorical results was performed using the UNPHASED 3.0 program. For this analysis, the option, robust permutation (robust to prior linkage) was used for 1000 permutations (Dudbridge 2003). The transmission of the haplotypes was analyzed with the TRANSMIT program (Clayton 1999). The TRANSMIT 2.5 program tests for association between the markers by examining the transmission of haplotypes from parents to affected offspring. We used the robust estimator option, which corrects for prior linkage

and multiple affected siblings in the analysis (Clayton 1999). Haplotypes with frequencies less than 10% were pooled and χ^2 and *P* values are only reported for those with frequencies greater than 10%.

For the analysis of the quantitative measures, the FBAT 1.5.5 program was used (Horvath *et al.* 2001; Laird *et al.* 2000). This statistic is used to assess for the transmission of alleles in relation to continuous phenotypic scores. The statistic implemented in the FBAT program eliminates the need for assumptions about the phenotype distribution (Laird *et al.* 2000) and is not sensitive to the ascertainment strategy (Horvath *et al.* 2001). An offset value was used for the FBAT analyses to mean center all traits (Lange & Laird 2002). Two-sided *P* values were reported for all results. The quantitative analyses results are not corrected for multiple tests because they were secondary analyses to the categorical results. Furthermore, there is no generally agreed upon approach for correction when the phenotypic measures are correlated and the markers are in LD to each other as these are not independent tests. Haploview was used to estimate the LD between markers as measured by D' and r^2 (Barrett *et al.* 2005).

Results

To test for association of the *PRTG* and *DYX1C1* genes in ADHD, we genotyped SNPs across the *PRTG* and *DYX1C1* genes in families with an ADHD proband and affected siblings. Six of the SNPs across the *DYX1C1* gene were updated from previously published results (Wigg *et al.* 2005) with 67 additional ADHD families included in the analyses (total $n = 253$). For the single marker analysis of the seven SNPs in *DYX1C1*, we did not observe biased transmission for any of the alleles using transmission disequilibrium test (TDT). Our previously published results (italicized in Table 2) (Wigg *et al.* 2005) were similar, although we did observe trends for association for the alleles of several of the markers previously and in the larger sample. The marker rs600753 is a non-synonymous SNP (Gly191Glu). No evidence for association was identified for alleles at this marker.

Using a categorical TDT analysis of the *PRTG* gene, five SNPs were significantly associated to ADHD defined as a categorical trait. The most significant marker was rs2414424 with a *P* value of 0.005, this maintained global significance ($P = 0.046$) after permutation testing when considering the 15 markers that were genotyped before identifying this result (Table 2). After observing association of markers in *PRTG*, we genotyped 13 additional markers including two nonsynonymous SNPs, rs16976466 (Ala236Thre) and rs1438914 (Leu1062Ile), and one synonymous SNP, rs11854213 (Asn445Asn). The reported SNP, rs10518816 (Leu826Val), was also genotyped in a sample of 100 unrelated individuals but was not found to be polymorphic. Of the coding SNPs that were polymorphic in our sample, only rs1438914 in *PRTG* showed evidence for biased transmission [T (Leu) allele $\chi^2 = 5.760$, df = 1, $P = 0.016$].

Our previous studies of *DYX1C1* provided evidence for association of haplotypes of the six markers genotyped on the sample at that time (global *P* value = 0.025). We used TRANSMIT to analyze the same haplotypes of the six markers on the larger sample, and with the additional families we observed biased transmission of the same haplotypes (Table

3). The results however were less significant and the global results were no longer significant (χ^2 = 6.809, df = 1, P = 0.078).

Haplotype analysis was performed on the three blocks of LD across the *PRTG* gene (LD shown in Fig. 1, results shown in Table 4). The blocks were defined by the algorithm developed by Gabriel *et al.* (2002), which creates a block if 95% of the informative comparisons are in strong LD. The most significant haplotype was for rs7165971 and rs12591646 (P = 0.007, df = 1), the two markers in block 3 (global χ^2 = 7.296, df = 2, P = 0.026).

Because twin studies indicate a stronger relationship between reading ability and inattention symptoms than with hyperactive/impulsive symptoms (Willcutt *et al.* 2000), we predicted that this locus would contribute to reading scores and more strongly to inattention symptoms than hyperactive/impulsive symptoms. However, this is not what we observed: the results from the quantitative analyses of inattention symptoms, as rated by both parents and teachers, were significant for five of the markers in *PRTG* (Table 5). The results from the analyses of hyperactive/impulsive symptoms were also significant with two of the five markers that were significant for inattention symptoms (Table 5). For the markers in *DYX1C1*, we did not observe significant results, although one marker, rs2007494, showed a trend for association with inattention symptoms as reported by parents (P = 0.051). The quantitative analyses of reading scores for two key reading component skills, word identification and decoding, showed no evidence for significant association to the markers in either gene (data not shown).

Discussion

The 15q region has been linked to both ADHD and RD. In a previous study, we explored the RD candidate gene, *DYX1C1*, and found evidence for association of ADHD with haplotypes of six markers across the gene but not individual markers. In this study, we added additional families and SNPs to our *DYX1C1* analysis and investigated *PRTG* as a potential candidate for ADHD. *PRTG* was chosen based on its location and its predicted involvement in cell migration and axon growth.

Genotypes for markers in the *DYX1C1* gene were genotyped in 253 families, adding 67 families to our original report of this gene and a new nonsynonymous marker was genotyped. None of the seven SNPs that was analyzed individually showed evidence of biased transmission for the single marker analysis, but the same haplotype that was biased in transmission previously was still significant; however, the global analysis of haplotypes was no longer significant.

Markers in *PRTG* were significantly associated with ADHD as a categorical trait, and we further found association with markers in *PRTG* and the inattention and hyperactive/impulsive dimensions of ADHD, analyzed as quantitative traits, with parent-reported inattention showing the most significance results across markers. The parent and teacher ratings for hyperactive/impulsive symptoms were also significant, although not to the same degree as inattention scores. We did not detect any evidence for association with tests of key

reading component skills, word identification or word decoding, with markers in the *PRTG* or *DYX1C1* genes.

The finding of association to ADHD and ADHD symptoms to this region of 15q but no evidence for association of the same markers to measures of reading does not fit the hypothesis of a common gene contributing to both disorders. One possible explanation is that the *PRTG* gene is only related to ADHD and is not related to reading skills. The previous linkage findings for RD and ADHD could be merely coincidental with two independent genes residing in the same chromosomal region. However, our results in an independent sample of children with RD provides evidence for weak association of *PRTG* and reading skills using quantitative analyses and some evidence of association with haplotypes when analyzed using a categorical approach (K. Wigg, Y. Feng, B. Anderson, T. Cate-Carter, J. Archibald, E. Kerr, R. Tannock, M. Lovett, T. Humphries and C. Barr, unpublished data). The lack of association for reading skills using quantitative analyses in our ADHD sample may simply result from insufficient variation of the trait in this sample. The mean of the scores is only slightly below the age norms with a standard deviation of 1 or less for these three measures. Another possibility is that this gene only contributes to reading scores in the low range; however, a recent study of unselected twins provided evidence for linkage of markers in 15q to reading skills, particularly to regular spelling (Bates *et al.* 2007), indicating that the 15q locus contributes to reading skills across the spectrum.

Although less significant than for *PRTG*, haplotype analyses of *DYX1C1* provides some indication of association with ADHD, thus the possibility exists that both genes contribute to ADHD. Although LD between markers in these genes complicate the interpretation of the association findings, both genes should be considered candidates for future studies. Another possibility is that the susceptibility alleles reside in a gene regulatory element located somewhere in this region that contributes to the expression of either one or both the genes.

In the 6p21 region, two genes that are involved in neuronal migration (Paracchini *et al.* 2006; Schumacher *et al.* 2006) have been put forward as strong candidates for RD: *KIAA0319* and *DCDC2*, both supported by several independent association studies (Cope *et al.* 2005a; Deffenbacher *et al.* 2004; Harold *et al.* 2006; Meng *et al.* 2005b; Paracchini *et al.* 2006; Schumacher *et al.* 2006). There is speculation that in fact both genes may be involved (Harold *et al.* 2006), which would provide a stronger linkage signal for the 6p region.

DYX1C1 is indicated to play a role in neuronal migration by *in vivo* RNAi studies (Wang *et al.* 2006). Furthermore, *in utero* RNAi of *DYX1C1* impairs auditory processing of complex stimuli and spatial learning in rats, with the deficits correlated with the location of malformations in either the cortex (auditory) or hippocampus (spatial learning) (Threlkeld *et al.* 2007). To date, there is little information about the expression or biological function of *PRTG* (Toyoda *et al.* 2005; Vesque *et al.* 2006). It can be predicted that this gene is involved in axonal growth based on its similarity to *DCC* and *Neogenin* (Vesque *et al.* 2006). Thus, similar to the 6p21 findings, the possibility of two genes within a linkage peak should be considered and further studies are warranted.

Molecular genetic studies of ADHD have focused on the role of genes involved in neurotransmitter release and response, particularly in the dopaminergic system. This approach has been very successful in identifying genes contributing to ADHD (Faraone *et al.* 2005). However, there are likely to be many ways in which controlled neurotransmitter release, uptake or action is affected. This includes disruption in brain pathways involved in neurodevelopment resulting in changes in the neuronal innervation of critical brain regions. The finding here, that *DYX1C1*, with a role in neuronal migration and/or the *PRTG* gene, suspected to be involved in cell migration and axon growth based on its similarity to *DCC* and *neogenin*, may show genetic variation that contributes to ADHD, opens up interesting avenues of research in ADHD.

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References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet. 2002; 30:97–101. [PubMed: 11731797]

Arcos-Burgos M, Castellanos FX, Pineda D, Lopera F, Palacio JD, Palacio LG, Rapoport JL, Berg K, Bailey-Wilson JE, Muenke M. Attention-deficit/hyperactivity disorder in a population isolate: linkage to loci at 4q13.2, 5q33.3, 11q22, and 17p11. Am J Hum Genet. 2004; 75:998–1014. Epub 2004 Oct 20. [PubMed: 15497111]

August GJ, Garfinkel BD. Comorbidity of ADHD and reading disability among clinic-referred children. J Abnorm Child Psychol. 1990; 18:29–45. [PubMed: 2324400]

Bakker SC, van der Meulen EM, Buitelaar JK, Sandkuijl LA, Pauls DL, Monsuur AJ, van't Slot R, Minderaa RB, Gunning WB, Pearson PL, Sinke J. A whole-genome scan in 164 Dutch sib pairs with attention-deficit/hyperactivity disorder: suggestive evidence for linkage on chromosomes 7p and 15q. Am J Hum Genet. 2003; 72:1251–1260. [PubMed: 12679898]

de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet. 2005; 37:1217–1223. [PubMed: 16244653]

Barr CL, Wigg K, Malone M, Schachar R, Tannock R, Roberts W, Kennedy JL. Linkage study of catechol-O-methyltransferase and attention-deficit hyperactivity disorder. Am J Med Genet. 1999; 88:710–713. [PubMed: 10581494]

Barr CL, Feng Y, Wigg K, Bloom S, Roberts W, Malone M, Schachar R, Tannock R, Kennedy JL. Identification of DNA variants in the SNAP-25 gene and linkage study of these polymorphisms and attention-deficit hyperactivity disorder. Mol Psychiatry. 2000; 5:405–409. [PubMed: 10889551]

Barr CL, Shulman R, Wigg K, Schachar R, Tannock R, Roberts W, Malone M, Kennedy JL. Linkage study of polymorphisms in the gene for myelin oligodendrocyte glycoprotein located on chromosome 6p and attention deficit hyperactivity disorder. Am J Med Genet. 2001a; 105:250–254. [PubMed: 11353444]

Barr CL, Wigg K, Zai G, Roberts W, Malone M, Schachar R, Tannock R, Kennedy JL. Attention-deficit hyperactivity disorder and the adrenergic receptors alpha1C and alpha2C. Mol Psychiatry. 2001b; 6:334–337. [PubMed: 11326305]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Bates TC, Luciano M, Castles A, Coltheart M, Wright MJ, Martin NG. Replication of reported linkages for dyslexia and spelling and suggestive evidence for novel regions on chromosomes 4 and 17. Eur J Hum Genet. 2007; 15:194–203. [PubMed: 17119535]

Bellini G, Bravaccio C, Calamoneri F, Donatella Cocuzza M, Fiorillo P, Gagliano A, Mazzone D, del Giudice EM, Scuccimarra G, Militerni R, Pascotto A. No evidence for association between

dyslexia and DYX1C1 functional variants in a group of children and adolescents from Southern Italy. J Mol Neurosci. 2005; 27:311–314. [PubMed: 16280601]

Brkanac Z, Chapman NH, Matsushita MM, Chun L, Nielsen K, Cochrane E, Berninger VW, Wijsman EM, Raskind WH. Evaluation of candidate genes for DYX1 and DYX2 in families with dyslexia. Am J Med Genet B Neuropsychiatr Genet. 2007; 144:556–560.

Brose K, Bland KS, Wang KH, Arnott D, Henzel W, Goodman CS, Tessier-Lavigne M, Kidd T. Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell. 1999; 96:795–806. [PubMed: 10102268]

Cardon LR, Smith SD, Fulker DW, Kimberling WJ, Pennington BF, DeFries JC. Quantitative trait locus for reading disability on chromosome 6. Science. 1994; 266:276–279. [PubMed: 7939663]

Chapman NH, Igo RP, Thomson JB, Matsushita M, Brkanac Z, Holzman T, Berninger VW, Wijsman EM, Raskind WH. Linkage analyses of four regions previously implicated in dyslexia: confirmation of a locus on chromosome 15q. Am J Med Genet B Neuropsychiatr Genet. 2004; 131:67–75.

Clayton D. A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. Am J Hum Genet. 1999; 65:1170–1177. [PubMed: 10486336]

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, Holmans P, Owen MJ, O'Donovan MC, Williams J. Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet. 2005a; 76:581–591. [PubMed: 15717286]

Cope NA, Hill G, van den Bree M, Harold D, Moskvina V, Green EK, Owen MJ, Williams J, O'Donovan MC. No support for association between dyslexia susceptibility 1 candidate 1 and developmental dyslexia. Mol Psychiatry. 2005b; 10:237–238. [PubMed: 15477871]

Curtis D, Sham PC. A note on the application of the transmission disequilibrium test when a parent is missing [letter]. Am J Hum Genet. 1995; 56:811–812. [PubMed: 7887437]

Deffenbacher KE, Kenyon JB, Hoover DM, Olson RK, Pennington BF, DeFries JC, Smith SD. Refinement of the 6p21.3 quantitative trait locus influencing dyslexia: linkage and association analyses. Hum Genet. 2004; 115:128–138. Epub 2004 May 11. [PubMed: 15138886]

Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol. 2003; 25:115–121. [PubMed: 12916020]

Faraone SV, Biederman J, Lehman BK, Keenan K, Norman D, Seidman LJ, Kolodny R, Kraus I, Perrin J, Chen WJ. Evidence for the independent familial transmission of attention deficit hyperactivity disorder and learning disabilities: results from a family genetic study. Am J Psychiatry. 1993; 150:891–895. [PubMed: 8494064]

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P. Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry. 2005; 57:1313–1323. Epub 2005 Jan 21. [PubMed: 15950004]

Fisher SE, Francks C. Genes, cognition and dyslexia: learning to read the genome. Trends Cogn Sci. 2006; 10:250–257. [PubMed: 16675285]

Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, Newbury DF, Crawford LR, Palmer CG, Woodward JA, Del'Homme M, Cantwell DP, Nelson SF, Monaco AP, Smalley SL. A genomewide scan for loci involved in attention-deficit/hyperactivity disorder. Am J Hum Genet. 2002; 70:1183–1196. [PubMed: 11923911]

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, Cardon LR, Marlow AJ, MacPhie IL, Walter J, Pennington BF, Fisher SE, Olson RK, DeFries JC, Stein JF, Monaco AP. A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. Am J Hum Genet. 2004; 75:1046–1058. [PubMed: 15514892]

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshular D. The structure of haplotype blocks in the human genome. Science. 2002; 296:2225–2229. [PubMed: 12029063]

Galaburda AM, LoTurco J, Ramus F, Fitch RH, Rosen GD. From genes to behavior in developmental dyslexia. Nat Neurosci. 2006; 9:1213–1217. [PubMed: 17001339]

Gayan J, Smith SD, Cherny SS, Cardon LR, Fulker DW, Brower AM, Olson RK, Pennington BF, DeFries JC. Quantitative-trait locus for specific language and reading deficits on chromosome 6p. Am J Hum Genet. 1999; 64:157–164. [PubMed: 9915954]

Grigorenko EL, Wood FB, Meyer MS, Hart LA, Speed WC, Shuster A, Pauls DL. Susceptibility loci for distinct components of developmental dyslexia on chromosomes 6 and 15. Am J Hum Genet. 1997; 60:27–39. [PubMed: 8981944]

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, Kaariainen H, Kere J. The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet. 2005; 1:e50. [PubMed: 16254601]

Harold D, Paracchini S, Scerri T, Dennis M, Cope N, Hill G, Moskvina V, Walter J, Richardson AJ, Owen MJ, Stein JF, Green ED, O'Donovan MC, Williams J, Monaco P. Further evidence that the KIAA0319 gene confers susceptibility to developmental dyslexia. Mol Psychiatry. 2006; 11:1085–1091. 1061. [PubMed: 17033633]

Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype–phenotype associations. Eur J Hum Genet. 2001; 9:301–306. [PubMed: 11313775]

Humphries T, Koltun H, Malone M, Roberts W. Teacher-identified oral language difficulties among boys with attention problems. J Dev Behav Pediatr. 1994; 15:92–98. [PubMed: 8034773]

Ickowicz A, Schachar R, Sugarman R, Chen S, Millette C, Cook L. The parent interview for child symptoms (PICS): a situation-specific clinical-research interview for attention deficit hyperactivity and related disorders. Can J Psychiatry. 2006; 50:325–328.

Kidd T, Bland KS, Goodman CS. Slit is the midline repellent for the robo receptor in Drosophila. Cell. 1999; 96:785–794. [PubMed: 10102267]

Kleinjan DJ, van Heyningen V. Position effect in human genetic disease. Hum Mol Genet. 1998; 7:1611–1618. [PubMed: 9735382]

Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. Genet Epidemiol. 2000; 19(Suppl 1):S36–S42. [PubMed: 11055368]

Lange C, Laird NM. On a general class of conditional tests for family-based association studies in genetics: the asymptotic distribution, the conditional power, and optimality considerations. Genet Epidemiol. 2002; 23:165–180. [PubMed: 12214309]

Loo SK, Fisher SE, Francks C, Ogdie MN, MacPhie IL, Yang M, McCracken JT, McGough JJ, Nelson SF, Monaco AP, Smalley SL. Genome-wide scan of reading ability in affected sibling pairs with attention-deficit/hyperactivity disorder: unique and shared genetic effects. Mol Psychiatry. 2004; 9:485–493. [PubMed: 14625563]

Marino C, Giorda R, Luisa Lorusso M, Vanzin L, Salandi N, Nobile M, Citterio A, Beri S, Crespi V, Battaglia M, Molteni M. A family-based association study does not support DYX1C1 on 15q21.3 as a candidate gene in developmental dyslexia. Eur J Hum Genet. 2005; 13:491–499. [PubMed: 15702132]

McGrath LM, Smith SD, Pennington BF. Breakthroughs in the search for dyslexia candidate genes. Trends Mol Med. 2006; 12:333–341. [PubMed: 16781891]

Meng H, Hager K, Held M, Page GP, Olson RK, Pennington BF, DeFries JC, Smith SD, Gruen JR. TDT-association analysis of EKN1 and dyslexia in a Colorado twin cohort. Hum Genet. 2005a; 118:87–90. [PubMed: 16133186]

Meng H, Smith SD, Hager K, Held M, Liu J, Olson RK, Pennington BF, DeFries JC, Gelernter J, O'Reilly-Pol T, Somlo S, Skudlarski P, Shaywitz SE, Shaywitz BA, Marchione K, Wang Y, Paramasivam M, LoTurco JJ, Page GP, Gruen JR. DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci U S A. 2005b; 102:17053–17058. [PubMed: 16278297]

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16:1215. [PubMed: 3344216]

Morris DW, Robinson L, Turic D, Duke M, Webb V, Milham C, Hopkin E, Pound K, Fernando S, Easton M, Hamshere M, Williams N, McGuffin P, Stevenson J, Krawczak M, Owen MJ, O'Donovan MC, Williams J. Family-based association mapping provides evidence for a gene for reading disability on chromosome 15q. Hum Mol Genet. 2000; 9:843–8. [PubMed: 10749993]

Nigg JT, Hinshaw SP, Carte ET, Treuting JJ. Neuropsychological correlates of childhood attention-deficit/hyperactivity disorder: explainable by comorbid disruptive behavior or reading problems? J Abnorm Psychol. 1998; 107:468–480. [PubMed: 9715582]

Nigg JT, Blaskey LG, Huang-Pollock CL, Rappley MD. Neuropsychological executive functions and DSM-IV ADHD subtypes. J Am Acad Child Adolesc Psychiatry. 2002; 41:59–66. [PubMed: 11800208]

Ogdie MN, Macphie IL, Minassian SL, Yang M, Fisher SE, Francks C, Cantor RM, McCracken JT, McGough JJ, Nelson SF, Nelson SF. A genomewide scan for attention--deficit/hyperactivity disorder in an extended sample: suggestive linkage on 17p11. Am J Hum Genet. 2003; 72:1268–1279. [PubMed: 12687500]

Ogdie MN, Fisher SE, Yang M, Ishii J, Francks C, Loo SK, Cantor RM, McCracken JT, McGough JJ, Smalley SL, Monaco AP, Smalley SL. Attention deficit hyperactivity disorder: fine mapping supports linkage to 5p13, 6q12, 16p13, and 17p11. Am J Hum Genet. 2004; 75:661–668. [PubMed: 15297934]

Paracchini S, Thomas A, Castro S, Lai C, Paramasivam M, Wang Y, Keating BJ, Taylor JM, Hacking DF, Scerri T, Francks C, Richardson AJ, Wade-Martins R, Stein JF, Knight JC, Copp AJ, Loturco J, Monaco AP. The chromosome 6p22 haplotype associated with dyslexia reduces the expression of KIAA0319, a novel gene involved in neuronal migration. Hum Mol Genet. 2006; 15:1659–1666. Epub 2006 Apr 6. [PubMed: 16600991]

Pennington BF, Groisser D, Welsh MC. Contrasting cognitive deficits in attention deficit hyperactivity disorder versus reading disability. Dev Psychol. 1993; 29:511–523.

Perfetti, CA. Reading Ability. Oxford Press; New York: 1985.

Petryshen TL, Kaplan BJ, Hughes ML, Tzenova J, Field LL. Supportive evidence for the DYX3 dyslexia susceptibility gene in Canadian families. J Med Genet. 2002; 39:125–126. [PubMed: 11836362]

Scerri TS, Fisher SE, Francks C, MacPhie IL, Paracchini S, Richardson AJ, Stein JF, Monaco AP. Putative functional alleles of DYX1C1 are not associated with dyslexia susceptibility in a large sample of sibling pairs from the UK. J Med Genet. 2004; 41:853–857. [PubMed: 15520411]

Schulte-Korne G, Grimm T, Nothen MM, Muller-Myhsok B, Cichon S, Vogt IR, Propping P, Remschmidt H. Evidence for linkage of spelling disability to chromosome 15. Am J Hum Genet. 1998; 63:279–282. [PubMed: 9634517]

Schumacher J, Anthoni H, Dahdouh F, Konig IR, Hillmer AM, Kluck N, Manthey M, Plume E, Warnke A, Remschmidt H, Hulsmann J, Cichon S, Lindgren CM, Propping P, Zucchelli M, Ziegler A, Peyrard-Janvid M, Schulte-Korne G, Nothen MM, Kere J. Strong Genetic Evidence of DCDC2 as a Susceptibility Gene for Dyslexia. Am J Hum Genet. 2006; 78:52–62. [PubMed: 16385449]

Seeger M, Tear G, Ferres-Marco D, Goodman CS. Mutations affecting growth cone guidance in Drosophila: genes necessary for guidance toward or away from the midline. Neuron. 1993; 10:409–426. [PubMed: 8461134]

Semrud-Clikeman M, Biederman J, Sprich-Buckminster S, Lehman BK, Faraone SV, Norman D. Comorbidity between ADDH and learning disability: a review and report in a clinically referred sample. J Am Acad Child Adolesc Psychiatry. 1992; 31:439–448. [PubMed: 1592775]

Sham PC, Curtis D. An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. Ann Hum Genet. 1995; 59:323–336. [PubMed: 7486838]

Smith SD, Kimberling WJ, Pennington BF, Lubs HA. Specific reading disability: identification of an inherited form through linkage analysis. Science. 1983; 219:1345–1347. [PubMed: 6828864]

Smith SD, Kimberling WJ, Pennington BF. Screening for multiple genes influencing dyslexia. Read Writing Interdiscipl J. 1991; 3:285–298.

Srinivas NR, Hubbard JW, Quinn D, Midha KK. Enantioselective pharmacokinetics and pharmacodynamics of dl-threo-methylphenidate in children with attention deficit hyperactivity disorder. Clin Pharmacol Ther. 1992; 52:561–568. [PubMed: 1424430]

Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, Lyytinen H, Muller K, Kaaranen M, Lindsberg PJ, Hannula-Jouppi K, Kere J. A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc Natl Acad Sci U S A. 2003; 100:11553–11558. [PubMed: 12954984]

Tannock, R., Schachar, R. Executive dysfunction as an underlying mechanism of behavior and language problems in attention deficit hyperactivity disorder. In: Beitchman, JH.Cohen, N.Konstantareas, MM., Tannock, R., editors. Language, Learning, and Behavior Disorders: Developmental, Biological, and Clinical Perspectives. Cambridge University Press; New York: 1996. p. 128-155.

Threlkeld SW, McClure MM, Bai J, Wang Y, Loturco JJ, Rosen GD, Fitch RH. Developmental disruptions and behavioral impairments in rats following in utero RNAi of Dyx1c1. Brain Res Bull. 2007; 71:508–514. [PubMed: 17259020]

Toyoda R, Nakamura H, Watanabe Y. Identification of protogenin, a novel immunoglobulin superfamily gene expressed during early chick embryogenesis. Gene Expr Patterns. 2005; 5:778–785. [PubMed: 15922677]

Vesque C, Anselme I, Couve E, Charnay P, Schneider-Maunoury S. Cloning of vertebrate Protogenin (Prtg) and comparative expression analysis during axis elongation. Dev Dyn. 2006; 235:2836–2844. [PubMed: 16881056]

Wang Y, Paramasivam M, Thomas A, Bai J, Kaminen-Ahola N, Kere J, Voskuil J, Rosen GD, Galaburda AM, Loturco JJ. DYX1C1 functions in neuronal migration in developing neo-cortex. Neuroscience. 2006; 143:515–522. [PubMed: 16989952]

Wechsler, D. Wechsler Intelligence Scale for Children. 3. Harcourt Brace & Co; San Antonio, TX: 1991.

Wigg KG, Couto JM, Feng Y, Anderson B, Cate-Carter TD, Macciardi F, Tannock R, Lovett MW, Humphries TW, Barr CL. Support for EKN1 as the susceptibility locus for dyslexia on 15q21. Mol Psychiatry. 2004; 9:1111–1121. [PubMed: 15249932]

Wigg K, Couto J, Feng Y, Crosbie J, Anderson B, Cate-Carter TD, Tannock R, Lovett MW, Humphries T, Kennedy JL, et al. Investigation of the relationship of attention deficit hyperactivity disorder to the EKN1 gene on chromosome 15q21. Sci Stud Read. 2005; 9:261–283.

Wilkinson, GS. Wide Range Achievement Test 3-Revision 3. Jastak Associates; Wilmington, DE: 1993.

Willcutt EG, Pennington BF. Comorbidity of reading disability and attention-deficit/hyperactivity disorder: differences by gender and subtype. J Learn Disabil. 2000; 33:179–191. [PubMed: 15505947]

Willcutt EG, Pennington BF, DeFries JC. Twin study of the etiology of comorbidity between reading disability and attention-deficit/hyperactivity disorder. Am J Med Genet. 2000; 96:293–301. [PubMed: 10898903]

Willcutt EG, Pennington BF, Olson RK, Defries JC. Understanding comorbidity: a twin study of reading disability and attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet. 2007; 144:709–714.

Williams J, O'Donovan MC. The genetics of developmental dyslexia. Eur J Hum Genet. 2006; 14:681–689. [PubMed: 16721404]

Woodcock, RW. Woodcock Reading Mastery Tests – Revised. American Guidance Service Inc; Circle Pines, MN, USA: 1987.

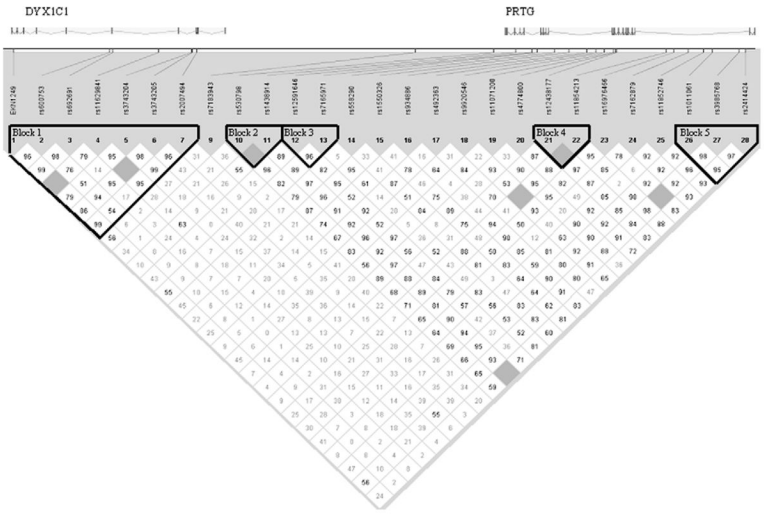

Figure 1.
Linkage disequilibrium and location of SNPs aross *DYX1C1* and *PRTG.*

Table 1

Characteristics of sample for quantitative measures used in this study

	Probands (*n* = 198)		Siblings (*n* = 55)		Probands and siblings (*n* = 253)			
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Skewness**	**Kurtosis**
Parent inattention	5.64	2.09	4.20	2.85	5.20	2.44	−0.50	−0.40
Parent hyperactive/impulsive	5.32	2.51	3.79	2.92	4.85	2.74	−0.21	−1.05
Teacher inattention	5.18	2.32	3.46	2.58	4.65	2.53	−0.42	−0.82
Teacher hyperactive/impulsive	3.98	2.67	2.45	2.63	3.50	2.75	0.24	−1.20
WRAT-III reading	96.90	15.13	97.87	11.38	97.11	14.38	−0.24	0.37
WRMT-R word identification	95.49	15.08	95.15	12.68	95.41	14.52	−0.65	0.75
WRMT-R word attack	93.70	13.90	94.58	12.61	93.91	13.59	−0.58	0.08

Reading scores are standard scores with a mean of 100 and an SD of 15. ADHD symptom scores are up to a maximum of 9. Parent scores were derived from the semistructured parent interview. Teacher scores were derived from the semistructured teacher telephone interview. WRMT, Woodcock Reading Mastery Test – Revised.

Table 2

Categorical analysis of ADHD for the *DYX1C1* and *PRTG* genes

Gene	Name	Position	MAF	Allele*	T		NT		χ^2		*P* value	
DYX1C1	DYX1C1-1249	53510160	0.114	G/T	55	*45*†	55	*32*	0.000	*2.195*	1.000	*0.139*
	rs600753	53546485	0.459	G/A	111		101		0.472		0.492	
	rs692691	53547867	0.411	G/A	130	*96*	125	*77*	0.098	*2.087*	0.754	*0.149*
	rs11629841	53564980	0.338	A/C	131	*84*	120	*79*	0.482	*0.153*	0.488	*0.695*
	rs3743204	53577602	0.220	C/A	97	*70*	78	*52*	2.063	*2.656*	0.151	*0.103*
	rs3743205	53577822	0.084	G/A	42	*26*	37	*22*	0.316	*0.333*	0.574	*0.564*
	rs2007494	53579537	0.250	T/A	90	*73*	70	*53*	2.500	*3.175*	0.114	*0.075*
PRTG	rs7183943	53662358	0.370	T/C	109		101		0.305		0.581	
	rs530798	53692019	0.464	T/C	114		101		0.786		0.375	
	rs1438914	53700167	0.109	T/A	62		38		5.760		0.016	
	rs12591646	53706431	0.225	A/C	92		64		5.026		0.025	
	rs7165971	53708305	0.319	A/G	119		83		6.416		0.011	
	rs558290	53714990	0.338	A/G	84		66		2.160		0.142	
	rs1550326	53720491	0.434	C/A	124		105		1.576		0.209	
	rs934886	53727251	0.393	C/T	114		103		0.558		0.455	
	rs492363	53730834	0.229	T/C	96		86		0.549		0.459	
	rs9920546	53734024	0.429	A/G	101		91		0.521		0.470	
	rs11071200	53737374	0.208	T/G	87		60		4.959		0.026	
	rs4774800	53738233	0.280	A/G	89		72		1.795		0.180	
	rs12438177	53738724	0.392	C/T	99		95		0.082		0.775	
	rs11854213	53757333	0.143	T/C	56		53		0.083		0.773	
	rs16976466	53760089	0.149	G/A	63		57		0.300		0.584	
	rs7162879	53765733	0.260	T/A	102		71		5.555		0.018	
	rs11852746	53771557	0.441	A/G	107		102		0.120		0.729	
	rs1011061	53774790	0.477	T/C	115		112		0.040		0.842	
	rs3985768	53784836	0.468	T/A	102		99		0.045		0.832	
	rs2414424	53787355	0.191	A/G	84		51		8.067		0.005	

MAF, minor allele frequency.

* Overtransmitted alleles are listed first.

† Results in 186 families previously published (Wigg *et al.* 2005) are given in italics.

Table 3

Haplotype analysis of the six SNPs previously genotyped in the *DYX1C1* gene (rs2007494, rs3743205, rs3743204, rs11629841, rs692691 and 1249G/T)

Haplotype						Frequency*	Observed†	Expected‡	Var(O-E)§	χ^2 (df = 1)¶		*P* value	
A	G	C	A	G	G	0.424	296.39	278.18	70.481	4.706	*6.926***	0.030	*0.009*
A	G	C	C	A	G	0.278	185.45	185.67	59.335	0.001	*0.0002*	0.978	*0.989*
T	G	A	A	G	G	0.117	69.169	78.72	32.872	2.775	*4.462*	0.096	*0.035*

Global chi-squared test, on 17 df = 20.569. Chi-squared test on 3 df for haplotypes with frequencies >10% = 6.8097, $P = 0.078$ (previous global $\chi^2 = 9.3124$, $P = 0.025$).

*Only haplotypes with a frequency greater than 0.10 were used in the analysis.

†Test statistic representing the observed number of transmissions.

‡Expected values of the test statistic under the null hypothesis of no linkage or association.

§Variance of (observed − expected) transmissions.

¶(Observed − expected)^2/Var(O-E).

**Previously published results are given in italics.

Table 4

Haplotype analysis of the SNPs in LD blocks

Haplotype		**Frequency**	**Observed**	**Expected**	**Var(O-E)**	**χ^2 (df = 1)**	***P* value**
Haplotype analysis for block 2							
rs1438914	rs530798						
A	T	0.458	274.960	270.19	69.345	0.328	0.567
A	C	0.438	243.350	256.74	66.876	2.679	0.102
T	C	0.103	70.640	62.012	34.926	2.132	0.144
T	T	0.001	1.050	1.06	0.0002522	0.370	0.543
Global chi-squared test, on 3 df = 4.5318, *P* = 0.209.							
Chi-squared test on 2 df for haplotypes with frequency >10% = 3. 6081, *P* = 0.165.							
Haplotype analysis for block 3							
rs7165971	rs12591646						
A	A	0.683	433.920	413	61.162	7.155	0.007
G	C	0.213	108.840	123.84	43.172	5.208	0.023
G	A	0.098	56.149	59.905	25.133	0.561	0.454
A	C	0.006	1.088	3.2557	2.1015	2.237	0.135
Global chi-squared test, on 3 df = 8.7482, *P* = 0.033.							
Chi-squared test on 2 df for haplotypes with frequency > 10% = 7.2969, *P* = 0.026.							
Haplotype analysis for block 4							
rs11854213	rs12438177						
T	C	0.487	202.34	204.75	51.946	0.112	0.738
T	T	0.382	258.33	258.35	53.679	0.000	1.000
C	C	0.130	75.153	72.199	27.099	0.322	0.570
C	T	0.001	0.17926	0.7041	0.24586	1.120	0.290
Global chi-squared test, on 3 df = 1.4814, *P* = 0.224.							
Chi-squared test on 3 df = 1.4814, *P* = 0.224.							
Haplotype		Frequency	Observed	Expected	Var(O-E)	χ^2 (df = 1)	*P* value
Haplotype analysis for block 5							

Haplotype			Frequency	Observed	Expected	Var(O-E)	χ^2 (df = 1)	*P* value
rs2414424	rs3985768	rs1011061						
A	A	T	0.465	271.89	267.45	62.345	0.317	0.573
A	T	C	0.333	213.13	199.63	62.124	2.932	0.087
G	T	C	0.182	92.862	104.33	37.798	3.483	0.062
A	T	T	0.011	4.0352	5.6443	2.5618	1.011	0.315
A	A	C	0.005	0.00981	2.6457	1.2254	5.670	0.017
G	T	T	0.002	0.00022	1.1987	0.5012	2.866	0.090
G	A	T	0.002	0.07118	1.0974	0.4989	2.111	0.146

Global chi-squared test, on 6 df = 15.258, $P = 0.018$.

Chi-squared test on 3 df for haplotypes with frequency >10% = 12.051, $P = 0.007$.

Table 5

Quantitative analysis of ADHD symptoms

	PRTG				
	rs12591646	**rs7165971**	**rs4774800**	**rs7162879**	**rs2414424**
Parent-reported inattention symptoms	0.031 *	0.010	0.085	0.004	0.004
Parent-reported hyperactive/impulsive symptoms	0.016	0.005		0.047	0.091
Teacher reported inattention symptoms	0.045	0.029	0.081	0.030	0.005
Teacher reported hyperactive/impulsive symptoms	0.045	0.042			

* *P* values were calculated using the FBAT program.