The KIAA0319-Like (*KIAA0319L*) Gene on Chromosome 1p34 as a Candidate for Reading Disabilities

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Abstract

A locus on chromosome 1p34-36 (*DYX8*) has been linked to developmental dyslexia or reading disabilities (RD) in three independent samples. In the current study, we investigated a candidate gene *KIAA0319-Like* (*KIAA0319L*) within *DYX8*, as it is homologous to *KIAA0319*, a strong RD candidate gene on chromosome 6p (*DYX2*). Association was assessed by using five tagging single nucleotide polymorphisms in a sample of 291 nuclear families ascertained through a proband with reading difficulties. Evidence of association was found for a single marker (rs7523017; $P = 0.042$) and a haplotype ($P = 0.031$), with RD defined as a categorical trait in a subset of the sample ($n = 156$ families) with a proband that made our criteria for RD. The same haplotype also showed evidence for association with quantitative measures of word-reading efficiency (i.e., a composite score of word identification and decoding; $P = 0.032$) and rapid naming of objects and colors ($P = 0.047$) when analyzed using the entire sample. Although the results from the current study are modestly significant and would not withstand a correction for multiple testing, *KIAA0319L* remains an intriguing positional and functional candidate for RD, especially when considered alongside the supporting evidence for its homolog *KIAA0319* on chromosome 6p. Additional studies in independent samples are now required to confirm these findings.

Keywords

Association study; complex trait; QTL; childhood disorder; developmental dyslexia

INTRODUCTION

Developmental dyslexia, also known as specific reading disabilities (RD), is a learning disability that affects at least 3–6% of otherwise normally developing children and often

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persists into adulthood. It is characterized by problematic word recognition, as well as poor spelling and word-decoding abilities (Lyon, 2003). Additional overlapping reading components and skills such as phonological processing, rapid naming, spelling, and orthographic processing, have also been shown to be problematic in RD (Olson et al., 1994; Pennington, 1997; Wolf & Bowers, 1999; Lyon, 2003; Schulte-Korne et al., 2003; Vellutino et al., 2004). RD has neurobiological origins and can occur despite average intelligence and effective classroom instruction (Habib, 2000; Lyon, 2003). Dysfunction of neurobiological systems have been postulated to cause RD, and functional neuroimaging evidence has revealed differences in brain-action profiles when RD and non-RD individuals engage in reading-related tasks (Habib, 2000).

The familial nature of RD has been well documented (Finucci et al., 1976), and evidence from twin studies indicates that RD, reading ability, and its component skills have a substantial genetic influence (DeFries et al., 1987; Olson et al., 1989; Olson, 2002; Harlaar et al., 2005). Heritability estimates for word recognition, phonological decoding, orthographic coding, phoneme awareness, and rapid naming range from 0.55 to 0.72 (Davis et al., 2001; Gayan & Olson, 2001; Olson, 2002). Different pairings of these traits have shown significant bivariate heritability, indicating that some genes may influence multiple component skills (Davis et al., 2001; Gayan & Olson, 2001; Olson, 2002).

Nine chromosomal regions have been reported, by at least one study, to be linked to RD. The regions are on 1p (Rabin et al., 1993; Grigorenko et al., 2001; Tzenova et al., 2004), 2p (Fagerheim et al., 1999; Petryshen et al., 2002), 3p (Nopola-Hemmi et al., 2001; Hannula-Jouppi et al., 2005), 6p (Cardon et al., 1994; Cardon et al., 1995; Grigorenko et al., 1997; Fisher et al., 1999; Gayan & Olson, 1999; Grigorenko et al., 2000; Kaplan et al., 2002; Deffenbacher et al., 2004; Francks et al., 2004), 6q (Petryshen et al., 2001), 15q (Smith et al., 1983; Nothen et al., 1999; Morris et al., 2000; Grigorenko et al., 2001), 11p (Hsiung et al., 2004), 18p (Fisher et al., 2002), and Xq (de Kovel et al., 2004). Some of these linkage findings have been replicated in independent samples, providing stronger evidence to support these chromosomal regions as locations for susceptibility loci. Candidate genes on 15q, 6p, and 3p have recently been reported as being associated with RD by fine-scale linkage disequilibrium mapping or by mapping of chromosomal translocations (Taipale et al., 2003; Deffenbacher et al., 2004; Francks et al., 2004; Wigg et al., 2004; Cope et al., 2005; Hannula-Jouppi et al., 2005; Meng et al., 2005; Schumacher et al., 2006; Luciano et al., 2007).

Suggestive evidence for linkage to chromosome 1p34-36 was first shown in the area around the Rhesus factor (Rh) locus ($Z_{\max} = 0.950-2.33$; $\theta = 0.2$) (Rabin et al., 1993). Alongside this initial finding, a balanced translocation was reported, very close to this region in a family that segregated RD and retarded speech development $t(1;2)(1p22;2q31)$ (Froster et al., 1993). This was taken as suggestive evidence of a susceptibility gene on the distal region of 1p (1p34-36) or 2q (Rabin et al., 1993).

Two additional independent investigations were conducted, and here, too, evidence for linkage of RD to the same region of 1p was found (Grigorenko, 2001; Tzenova et al., 2004). The first study investigated the inheritance of 12 microsatellite markers in eight multiplex

families (165 individuals) with well-documented histories of reading problems. Evidence for linkage was found for phonemic awareness to D1S253-D1S436 ($P = 0.06\text{--}0.01$) and MATN1-PPT ($P = 0.02$), phonological decoding to D1S199-D1S478 ($P < 0.001$), rapid naming of objects and colors to D1S253-D1S507 ($P = 0.05\text{--}0.01$), single-word reading to D1S199-D1S478 ($P = 0.02\text{--}0.001$) and MATN1-PPT ($P = 0.02\text{--}0.04$), and lifetime diagnosis of dyslexia to D1S199-D1S478 ($P < 0.001$) (Grigorenko, 2001). A study conducted on 100 Canadian families with multiple affected children (914 individuals in total) found evidence for linkage of RD defined as a categorical trait to D1S507 (MLS = 3.65), as well as the quantitative traits of spelling (LOD = 3.30) and phonological coding (LOD = 1.13) between D1S552 and D1S1622, which are located close to D1S199, confirming the results of Grigorenko and colleagues (Tzenova et al., 2004).

Although these three studies have found consistent evidence for linkage to 1p34-36, the region remains large, with hundreds of possible candidate genes making fine mapping studies both costly and arduous. Based on recent findings by multiple groups on chromosome 6p, our group identified a strong candidate gene for investigation within this linkage region on chromosome 1p. The susceptibility locus on chromosome 6p had been narrowed to five possible candidate genes (Deffenbacher et al., 2004), all expressed in the brain (Londin et al., 2003). Of these, *KIAA0319* has received the most support (Francks et al., 2004; Cope et al., 2005; Harold et al., 2006a,b; Luciano et al., 2007); however it is not unanimous, as other studies have found association with *DCDC2*, a nearby gene (Meng et al., 2005; Schumacher et al., 2006). The candidate genes on 6p have also been tested for association with RD in our independent sample from Toronto (Couto et al., submitted). We found evidence in support of *KIAA0319* ($P = 0.025\text{--}0.014$, single-marker categorical and quantitative analyses, and haplotype analyses), but not *DCDC2*. The collective evidence for association with *KIAA0319* on 6p prompted us to conduct a search for homologous genes on other loci linked to RD. We found the gene *KIAA0319-Like (KIAA0319L)*, also referred to as polycystic kidney disease-like-1 (*PKDL-1*) on chromosome 1p34.3. According to an NCBI protein-to-protein blast, both *KIAA* genes are 61% similar, with only 6% of the coding sequence lying in gaps.

This gene is located within linkage peaks from three independent studies on chromosome 1p (Rabin et al., 1993; Grigorenko et al., 2001; Tzenova et al., 2004), making it a suitable positional candidate, whereas the homology to *KIAA0319* on 6p, suggests it is a potential functional candidate. For these reasons, we investigated this gene for association with RD in our sample of Canadian families.

MATERIALS AND METHODS

Subjects and Ethnicity

Subjects aged 6–16 that presented with reading problems were recruited from local schools to participate in this study (Wigg et al., 2004). Siblings in the same age range were also recruited, regardless of reading ability. Participants were restricted to families with English as a first language or with at least 5 years of education in an English-speaking school. The sample is currently made up of 291 nuclear families, of which 165 are trios, 77 are families with 2 children, and five families have 3 children. The sample also includes 20 single-parent

families, with 1 child, 23 with 2 children, and one with 3 children. There are, in total, 112 siblings in the sample.

The sample mainly consists of individuals of Northern, Southern, Eastern, and Western European ancestry from the Toronto area. Toronto is the largest city in Canada and has a high influx of immigrants. The Canadian population of European ancestry reflects diverse waves of immigration from continental Europe and the British Isles. Of the parents in our sample, 68.1% describe their parent's (i.e., child's grandparents) ethnicity as European or British. This includes individuals who are of mixed European descent (e.g., father from England and mother from Italy). Another 26% describe their parents as "Caucasian Canadians" without specific ethnic backgrounds. Individuals from South America made up 1.8% of the sample, while 2.9% of the sample was of non-European ancestry and 1.2% was of non-European mixed ethnicity. Of the children in the sample, 38.2% had both parents who came from the same country (e.g., father and mother from Italy), and 61.8% had parents who originated in different countries (e.g., mother from Canada and father from Ireland).

Assessment of Subjects

The reading measures used in this study have been described previously (Wigg et al., 2004). Briefly, a structured interview with parents, Children's Interview for Psychiatric Syndromes (Weller et al., 2000) and a semistructured interview with teachers (Tannock et al., 2002) were conducted to obtain information on symptoms of neurological, medical, and psychiatric disorders, as well as psychosocial stressors. This information was supplemented with the following standardized questionnaires: Conners Parent and Teacher Rating Scales Revised (Conners, 1997) and Ontario Child Health Survey Scales-Revised (Boyle et al., 1993). Subjects were excluded if they showed evidence of neurological 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 (Wechsler, 1991).

Single-word reading, phonological decoding skills, and spelling were assessed using the third edition of the Wide Range Achievement Test (WRAT 3), Woodcock Reading Mastery Tests Revised (WRMT-R), and the Test of Word Reading Efficiency (TOWRE). The WRAT 3 subtests provide assessments of single-word reading and spelling (Wilkinson, 1993). Two subtests of the WRMT-R were used, one to evaluate phonological decoding (Word Attack) and the other to assess single-word reading (Word ID) (Woodcock, 1987). The TOWRE consists of two timed subtests that examine word identification and phonological decoding (Torgesen et al., 1999). The two scores can then be combined to form a composite score for total word-reading efficiency. We chose to use the composite score because the genetic correlation between these two processes is 0.99, indicating that the genes contributing to these skills are almost entirely overlapping (Gayan & Olson, 2001). Rapid naming skills were evaluated by four subtests of the Comprehensive Test of Phonological Processing (CTOPP): rapid digit, letter, object, and color naming (Wagner et al., 1999). These scores were then combined for two different rapid naming composites. Rapid digit and letter naming formed the rapid naming composite (Wagner et al., 1999). Rapid color and object

naming were combined to form the alternative rapid naming composite (Wagner et al., 1999). The first was investigated, since it has been suggested to have a stronger relationship to reading ability, compared to object and color naming (Wagner et al., 1999; Savage & Frederickson, 2005). However, object and color naming was significantly linked to 1p in the study of Grigorenko and colleagues (Grigorenko et al., 2001) and was thus investigated here. The scores from all the above tests were standardized scores, based on age norms. Means and standard deviations for all measures are reported in Supplementary Table 1. Pearson correlations show that all scores are significantly correlated to each other ($P < 0.0001$) (Supplementary Table 2).

Scores on three standardized reading tests were used to classify subjects as “affected” for the categorical analysis. These were the WRMT-R Word Attack, Word Identification, and the WRAT 3 reading subtests. To be classed as affected, subjects had to score 1.5 standard deviations below the population mean (standard score 78 or lower) on two of the three standardized reading tests, or 1 standard deviation (standard score of 85 or lower) on the average of the three. These criteria identify a subset of individuals in our sample whose average scores on the three reading measures fall within the lower 5% tail of normally distributed reading ability in the general population. Out of the 291 subjects and 112 siblings, 156 probands and 25 siblings made the criteria and were used in the categorical analysis.

Isolation of DNA and Marker Genotyping

DNA was extracted directly from blood lymphocytes by using a high-salt extraction method (Miller et al., 1988). According to data provided by the International HapMap project (<http://www.hapmap.org>, Rel #20/phase II Jan 06), the gene *KIAA0319L* is encompassed by one large haplotype block, indicating a region of high linkage disequilibrium. Haplotype blocks were defined by using criteria based on Gabriel et al. (2002). This information was based on genotypes from 44 single-nucleotide polymorphisms (SNPs) in their bank of Centre d’Etude du Polymorphisme Humaine (CEPH) pedigrees of individuals of Northern and Western Europeans from Utah (CEU), available in Release #20/phase II. We note that the CEU information was utilized in the current study, as the ancestry is related to that of our sample. The CEU sample cannot be generalized to reflect the complex ancestry of individuals from Northern, Southern, Eastern, and Western Europe (http://www.hapmap.org/guidelines_hapmap_data.html). The block contained nine haplotypes of frequencies 39.5, 18.6, 14.4, 6.7, 5.8, 5.8, 5.3, 1.3, and 1.3% that could be distinguished by genotyping seven tagSNPs. The SNPs rs2275247, rs917105, rs1203138, rs1203148, rs12408030, and rs7523017 effectively captured haplotypes with frequencies 5% and were chosen for investigation. The tagSNPs were chosen in Haploview (Barrett et al., 2005), using criteria set to display haplotypes with frequencies 1%. In addition to these tagSNPs, four publicly reported nonsynonymous SNPs, rs1635712, rs11551037, rs1361040, and rs12729157, were also genotyped. The location of all markers in the gene is provided in the schematic in Figure 1. Flanking sequence for rs2275247, rs1203138, rs7523017, and rs1151037 in Supplementary Table 3a was ascertained from the UCSC database, based on NCBI build 35 and sent to Applied Biosystems, who then designed the assays (ABI, Foster City, California, USA Assay-By-Design by Applied Biosystems®). Primer and probe sequence are listed in

Supplementary Table 3a. The assays for rs917105, rs1635712, rs1361040, rs12729157, rs1203148, and rs12408030 were predesigned and tested by Applied Biosystems (Assay-On-Demand). The reference sequences for these assays are shown in Supplementary Table 3b. All assays were genotyped by using standard methods with the ABI 7900-HT Sequence Detection System® (Applied Biosystems), using the TaqMan 5' nuclease assay for allelic discrimination.

Screen for Novel DNA Variants in *KIAA0319L*

The gene for *KIAA0319L* consists of 21 exons and has two known isoforms, *a* and *b*. Sequence for *isoform a* is longer and includes isoform *b* and was used as the template for the screen (Figure 1). Exon 1 contains the 5' untranslated region (UTR) and exon 21 encompasses protein coding sequence and the 3' UTR. Since the promoter region for *KIAA0319L* has not been characterized, 610 bp, that includes the full intergenic region upstream of exon 1 and part of the neighboring gene neurochondrin (*NCDN*), was investigated in the screen (Figure 1). Previous investigations indicate that the first 500-bp upstream of the transcription start site contain 58.9% of *cis*-regulatory variants (Rockman & Wray, 2002). Individuals screened included 45 probands that made criteria for RD, a majority of which carried the marker allele (G) for rs7523017 that showed evidence for biased transmission. Some individuals also carried the opposite allele. Three additional individuals were also included that did not make criteria and carried the opposite allele. Gene regions were amplified by using polymerase chain reaction (PCR) and then screened by using denaturing high-performance liquid chromatography (DHPLC) (Supplementary Table 4 for primers and conditions). Samples that showed altered mobility were further analyzed by using direct sequencing.

Statistical Analysis

All markers were tested for Mendelian errors and Hardy-Weinberg equilibrium by using MERLIN (Abecasis et al., 2002). The TDT statistic was calculated by using the extended TDT (ETDT) program for the categorical analysis (Sham & Curtis, 1995). Stepwise regression was conducted in STATA (StataCorpLP, College Station, Texas, USA), using a package designed by David Clayton (<http://www-gene.cimr.cam.ac.uk/clayton/software/>) (Cordell & Clayton, 2002). Association of haplotypes was evaluated by using TRANSMIT (Clayton & Jones, 1999). Haplotypes with frequencies less than or equal to 5% were pooled, and χ^2 and *P*-values are only reported for those with frequencies greater than 0.05. Analysis of the quantitative traits of spelling, total word-reading efficiency, and rapid naming was carried out by using the FBAT program (v1.4) and the HBAT component for the analysis of haplotypes (Laird et al., 2000). This method was used for the quantitative analyses, as the trait does not need to be normally distributed for this statistic. An offset of 100.1 was used to mean center all traits. The additive model was utilized, as it performs well, even in cases where the true model of inheritance is not additive (Horvath et al., 2001).

RESULTS

Ten SNP markers were genotyped across the gene for *KIAA0319L*. Following genotyping in 100 families, all four nonsynonymous SNPs were not polymorphic in our sample and were

not further analyzed. The six tagging SNP markers, rs2275247, rs917105, rs1203138, rs1203148, rs12408030, and rs7523017, were genotyped in all 291 families. The marker, rs2275247, was not in Hardy-Weinberg equilibrium in either the proband ($\chi^2 = 19.47$; $P < 0.0001$) or the parental ($\chi^2 = 26.72$; $P < 0.0001$) chromosomes. No errors in genotyping could be detected by Mendelian errors or crossover within the gene, and sequencing failed to identify any other polymorphism within the region that could interfere with the assay. A low number of observed minor alleles can inflate the type I error rate of χ^2 tests, especially in small samples (Wigginton et al., 2005), and this may explain the deviation from HWE. Therefore, the marker was not included in any further analyses. Using the TDT, we found significant evidence for association with the common allele (G) of the marker rs7523017 and RD ($\chi^2, 1 \text{ df} = 4.122$; $P = 0.042$) (Table 1). These data were not corrected for multiple testing and would not remain significant, if corrected. These tests were carried out in the 156 families, with a proband that made the criteria for RD defined as a categorical trait in our sample. The four other markers tested in the gene showed no significant evidence for association with RD (Table 1).

A stepwise regression analysis (Cordell & Clayton, 2002; Cordell et al., 2004) revealed that the model that best explained affection status of our probands was constructed from four of the previously tested markers. These were rs1203138, rs1203148, rs12408030, and rs7523017 ($P = 0.009$). Haplotypes of the four SNP markers were tested for association by using TRANSMIT (Clayton & Jones, 1999). Significant evidence for association (undertransmission) was found with a haplotype that had a frequency of 6.8% ($\chi^2 = 4.666$; $P = 0.031$). The global test that pooled haplotypes with frequencies under 5% was not significant ($\chi^2, 5 \text{ df} = 5.678$; $P = 0.339$) (Table 2).

The subjects used in the TDT and TRANSMIT analyses made a categorical cut-off for RD. Twin studies indicate that heritability is not only restricted to group deficits, but also applies to a continuum of reading measures (Hohnen & Stevenson, 1999; Gayan & Olson, 2003). Further, this locus has previously been reported to be linked to single-word reading, phonological decoding, phonological awareness, rapid naming, and spelling, suggesting that it contributes to multiple component processes (Grigorenko et al., 2001; Tzenova et al., 2004). For these reasons, the association of key quantitative measures of reading was analyzed to test the relationship of this gene to the phenotypes previously linked to this locus. In addition, this type of analysis allows use of the full sample ($n = 291$ families, 403 individuals), which in some instances, can provide more statistical power. Association of three quantitative measures of reading skills, with both single markers and haplotypes constructed from four markers in *KIAA0319L*, was assessed by using the FBAT program (v1.4) (Laird et al., 2000). Based on the findings of Grigorenko et al. (2001) and Tzenova et al. (2004), we chose measures of spelling, a timed composite measures of word-reading efficiency (composed of phonological decoding and word identification), and two measures of rapid naming. In the single-marker analysis, none of the tagSNPs were associated with the quantitative measures tested; however, there were trends for the marker rs7523017 and the TOWRE total word-reading efficiency scores and rapid naming of the colors and objects (Supplementary Table 5). In the haplotype analysis, we found significant evidence for association (under-transmission) of the 6.8% haplotype with total word-reading efficiency ($Z = 2.142$; $P = 0.032$) and the composite score for rapid object and color naming ($Z = 1.984$; P

= 0.047) (Table 3). A trend toward biased undertransmission was observed for the same 6.8% haplotype and spelling ($P = 0.076$). The letter and digit-naming composite showed no significant evidence for association.

The markers used in this study are not predicted to change the function of this gene; thus, the gene was screened to identify potential functional DNA changes. All coding exons, the 5' and 3' UTR, and the putative proximal promoter of the *KIAA0319L* gene were screened in 48 individuals by using DHPLC, followed by direct sequencing of any PCR product with altered mobility. No novel DNA variants were detected.

DISCUSSION

In this study, we investigated the association of RD with the gene *KIAA0319L*, a positional and potential functional candidate. We found association with a single marker of the five tested, although this P -value will not remain significant if a correction for multiple testing is applied. We also found the association of a multimarker haplotype with both RD defined as a categorical trait and quantitative measures of the reading components. This haplotype, with a frequency of 6.8%, was transmitted less often than expected in the sample (Table 2). A haplotype analysis can be a more statistically powerful test, compared to a single-marker analysis, as it considers information about the ancestral structure of the chromosomes (Akey et al., 2001). Theoretically, the causative variant could have arose somewhere along this haplotype on an ancestral chromosome, and individuals in our sample that carry this particular haplotype have a higher chance of carrying the causative variant(s). However, if there have been multiple risk alleles arising on different haplotypes, these analyses can have less power, as transmission will be split over multiple haplotypes. For this analysis, the global test for association was not significant, even when haplotypes with a frequency of 5% or less were grouped. The global test for association in TRANSMIT undergoes a loss of power as the number of markers that are used in the analysis increases (Clayton & Jones, 1999).

This particular haplotype was also associated with quantitative measures of reading, when analyzed in our full sample. These quantitative analyses provide insights into the role of shared and independent genetic influences on these different reading components. Pearson correlations were highly significant between measures in our sample, indicating these phenotypes are not entirely independent and share variance. As a result, it is expected that there will be shared as well as independent genes contributing to the different reading measures, as indicated by twin studies (Davis et al., 2001; Gayan & Olson, 2001, 2003). The 1p locus was originally identified by using linkage analyses in families selected for reading disability, an approach that might be more likely to identify susceptibility loci contributing to multiple reading processes. These studies also indicated that this locus contributes to a number of reading component processes, which we investigated (Grigorenko, 2001; Tzenova et al., 2004). The study by Grigorenko et al. (2001) showed the strongest evidence for linkage of single-word reading and phonological decoding to the locus on 1p. Our data for the TOWRE composite score, with which the haplotype in *KIAA0319L* is associated, support these findings (Table 3). The strongest evidence for linkage in the Tzenova et al.

(2004) study was for the component of spelling in their quantitative analysis. The trend for association with spelling observed in our data is also supportive.

Previous linkage studies have indicated that rapid naming of objects and colors was significantly linked to this locus (Grigorenko et al., 2001). Rapid naming of letters and numbers was not tested in that study. Tzenova et al. (2004) tested, but did not find, linkage of rapid digit naming in their investigation but did not test for linkage of object and color naming. Our 6.8% haplotype showed marginally significant evidence for association with object and color naming but no association with letter and digit naming. Thus, our study appears to confirm the previous finding of a relationship between this locus to object and color naming but not digit naming. These findings are puzzling, given the current stage of knowledge on the relationship of rapid naming and reading. Dyslexic individuals have difficulties with both naming of objects and colors, and letters and numbers. However, naming of letters and numbers has been shown to be a better predictor of reading difficulties, compared to objects and colors (Wolf et al., 2002). The relationship between these components and phonological processing is still not clear, with some studies suggesting they are independent processes, while others claim they are one in the same (Wolf & Bowers, 1999; Wolf et al., 2002). A stronger relationship between letter and number naming and phonological skills is also supported by twin studies that show a higher bivariate heritability between number and letter naming and decoding (0.25) than for object and color naming and decoding (0.09) (Davis et al., 2001). Thus, the finding of association here with *KIAA0319L*, and linkage to 1p in previous studies, to phonological skills and rapid naming of color and objects, but not digits, is unexpected.

Caution should be used in the interpretation of these results, however, because of the marginal significance level. Further, quantitative analyses are influenced by a number of factors, including the variance and the reliability of the phenotypic measure (Pennington, 1997). Clearly, further confirmation is required before conclusions can be made about the specific contribution of this locus to rapid naming speed.

In order to find functional DNA changes, the protein-coding sequence of the *KIAA0319L* gene was screened; however, no novel DNA changes were found. Further, all nonsynonymous SNPs in the gene from the public databases were not polymorphic in our sample. Therefore, if this gene contributes to the RD phenotype, then the functional DNA change(s) must exist in regions that exercise regulatory control over *KIAA0319L*. Current theories suggest that this is likely the case for most complex traits (Knight, 2005). In addition, our investigation of *KIAA0319* on 6p showed that associated markers from five independent samples, including our own from Toronto, were located either within or in high LD with a region enriched with acetylated histones, a marker for regulatory regions (Couto et al., submitted). Although no novel DNA changes were found in either the putative proximal promoter or the untranslated regions of *KIAA0319L*, areas that usually harbour regulatory elements (Rockman & Wray, 2002; Levine & Tjian, 2003), the risk allele could be located in the intronic regions or regions at a large distance from the gene. Regulatory elements, such as enhancers and insulators, have been known to be found at great distances from genes and even in introns of other genes (Kleinjan & van Heyningen, 2005). However, it is challenging to identify these regions, and at the current time, strong and more consistent

evidence for association of *KIAA0319L* with RD is required before these additional studies are warranted.

A second possibility is that the causal variant(s) could be located in coding regions of neighboring genes. The gene for *KIAA0319L* is in a region of high LD, and the LD block that encompasses this gene also extends across neighboring genes. These include zinc finger protein 262 (*ZNF262*) near the 3' end and neurochondrin (*NCDN*), transcription factor AP-2 epsilon (*TFAP2E*), and proteasome beta 2 subunit (*PSMB2*) near the 5' end. Two, in particular, *ZNF262* and *NCDN*, are both brain expressed and could also be possible candidates.

In this study, we tested the hypothesis that *KIAA0319L* is the RD candidate on chromosome 1p. This follows previous reports of linkage of the 1p locus to RD (Rabin et al., 1993; Grigorenko et al., 2001; Tzenova et al., 2004). Although the results from the current study were modestly significant, the hypothesized gene remains an interesting candidate in light of the supporting evidence for its homolog, *KIAA0319*, on 6p. Functional analyses of *KIAA0319*, using RNA interference in animal models, shows that this gene plays a role in neuronal migration during development (Paracchini et al., 2006). Further characterization of the protein structure revealed that *KIAA0319* contains three main isoforms, one of which is a transmembrane protein that could function in neuronal migration, using the polycystic kidney disease (PKD) domains (Velayos-Baeza et al., 2008). *KIAA0319L* is expressed in the brain and predicted to possess PKD domains (<http://symatlas.gnf.org/>), suggesting that it, too, could be involved in neuronal migration. Dysregulation of neuronal migration has been hypothesized as being the biological mechanism that underpins reading disabilities as not only *KIAA0319*, but also *DCDC2* on 6p and *ROBO1* on chromosome 3, two additional RD candidate genes, have been shown to be involved in neuronal migration and axonal path finding (Hannula-Jouppi et al., 2005; Meng et al., 2005). Thus, the homology of this gene to *KIAA0319* supports it as a candidate, and consistent replications in independent samples are now essential to confirming this initial finding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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(1):97–101. [PubMed: 11731797]
- Akey J, Jin L, Xiong M. Haplotypes vs. single marker linkage disequilibrium tests: what do we gain? *Eur J Hum Genet.* 2001; 9(4):291–300. [PubMed: 11313774]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005; 21(2):263–265. [PubMed: 15297300]

- Boyle MH, Offord DR, Racine Y, Fleming JE, Szatmari P, Sanford M. Evaluation of the revised Ontario Child Health Study scales. *J Child Psychol Psychiatry*. 1993; 34(2):189–213. [PubMed: 8444992]
- Cardon LR, Smith SD, Fulker DW, Kimberling WJ, Pennington BF, DeFries JC. Quantitative trait locus for reading disability on chromosome 6. *Science*. 1994; 266(5183):276–279. [PubMed: 7939663]
- Cardon LR, Smith SD, Fulker DW, Kimberling WJ, Pennington BF, DeFries JC. Quantitative trait locus for reading disability: correction [letter]. *Science*. 1995; 268(5217):1553. [PubMed: 7777847]
- Clayton D, Jones H. Transmission/disequilibrium tests for extended marker haplotypes. *Am J Hum Genet*. 1999; 65(4):1161–1169. [PubMed: 10486335]
- Conners, CK. *Conners' Rating Scales*Revised*. Toronto, Ontario, Canada: Multi-Health Systems Inc; 1997.
- Cope N, Harold D, Hill G, Moskvina V, Stevenson J, Holmans P, et al. Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. *Am J Hum Genet*. 2005; 76(4):581–591. [PubMed: 15717286]
- Cordell HJ, Barratt BJ, Clayton DG. Case/pseudocontrol analysis in genetic association studies: a unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. *Genet Epidemiol*. 2004; 26(3):167–185. [PubMed: 15022205]
- Cordell HJ, Clayton DG. A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. *Am J Hum Genet*. 2002; 70(1):124–141. Epub 2001 Nov 21. [PubMed: 11719900]
- Couto JM, Livne-Bar I, Xu Z, Cate-Carter T, Nathaniel A, Anderson B, et al. Association of Reading Disabilities to a Region Marked by Acetylated H3 Histones in *KIAA0319*. (submitted).
- Davis CJ, Gayan J, Knopik VS, Smith SD, Cardon LR, Pennington BF, et al. Etiology of reading difficulties and rapid naming: the Colorado Twin Study of Reading Disability. *Behav Genet*. 2001; 31(6):625–635. [PubMed: 11838539]
- de Kovel CG, Hol FA, Heister JG, Willems JJ, Sandkuijl LA, Franke B, et al. Genomewide scan identifies susceptibility locus for dyslexia on Xq27 in an extended Dutch family. *J Med Genet*. 2004; 41(9):652–657. [PubMed: 15342694]
- Deffenbacher KE, Kenyon JB, Hoover DM, Olson RK, Pennington BF, DeFries JC, et al. Refinement of the 6p21.3 quantitative trait locus influencing dyslexia: linkage and association analyses. *Hum Genet*. 2004; 115(2):128–138. Epub 2004 May 11. [PubMed: 15138886]
- DeFries JC, Fulker DW, LaBuda MC. Evidence for a genetic aetiology in reading disability of twins. *Nature*. 1987; 329(6139):537–539. [PubMed: 3657975]
- Fagerheim T, Raeymaekers P, Tonnessen FE, Pedersen M, Tranebjaerg L, Lubs HA. A new gene (DYSX3) for dyslexia is located on chromosome 2. *J Med Genet*. 1999; 36(9):664–669. [PubMed: 10507721]
- Finucci JM, Guthrie JT, Childs AL, Abbey H, Childs B. The genetics of specific reading disability. *Ann Hum Genet*. 1976; 40(1):1–23. [PubMed: 962317]
- Fisher SE, Francks C, Marlow AJ, MacPhie IL, Newbury DF, Cardon LR, et al. Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia. *Nat Genet*. 2002; 30(1):86–91. [PubMed: 11743577]
- Fisher SE, Marlow AJ, Lamb J, Maestrini E, Williams DF, Richardson AJ, et al. A quantitative-trait locus on chromosome 6p influences different aspects of developmental dyslexia. *Am J Hum Genet*. 1999; 64(1):146–156. [PubMed: 9915953]
- Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, Cardon LR, et al. 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(6):1046–1058. [PubMed: 15514892]
- Froster U, Schulte-Körne G, Hebebrand J, Remschmidt H. Co-segregation of balanced translocation (1;2) with retarded speech development and dyslexia. *Lancet*. 1993; 342(8864):178–179.
- Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. *Science*. 2002; 296(5576):2225–2229. [PubMed: 12029063]

- Gayan J, Olson RK. Reading disability: evidence for a genetic etiology. *Eur Child Adolesc Psychiatry*. 1999; 8(Suppl 3):52–55. [PubMed: 10638371]
- Gayan J, Olson RK. Genetic and environmental influences on orthographic and phonological skills in children with reading disabilities. *Dev Neuropsychol*. 2001; 20(2):483–507. [PubMed: 11892949]
- Gayan J, Olson RK. Genetic and environmental influences on individual differences in printed word recognition. *J Exp Child Psychol*. 2003; 84(2):97–123. [PubMed: 12609495]
- Grigorenko EL. Developmental dyslexia: an update on genes, brains, and environments. *J Child Psychol Psychiatry*. 2001; 42(1):91–125. [PubMed: 11205626]
- Grigorenko EL, Wood FB, Meyer MS, Hart LA, Speed WC, Shuster A, et al. Susceptibility loci for distinct components of developmental dyslexia on chromosomes 6 and 15. *Am J Hum Genet*. 1997; 60(1):27–39. [PubMed: 8981944]
- Grigorenko EL, Wood FB, Meyer MS, Pauls DL. Chromosome 6p influences on different dyslexia-related cognitive processes: further confirmation. *Am J Hum Genet*. 2000; 66:715–723. [PubMed: 10677331]
- Grigorenko EL, Wood FB, Meyer MS, Pauls JE, Hart LA, Pauls DL. Linkage studies suggest a possible locus for developmental dyslexia on chromosome 1p. *Am J Med Genet*. 2001; 105(1):120–129. [PubMed: 11424982]
- Habib M. The neurological basis of developmental dyslexia: an overview and working hypothesis. *Brain*. 2000; 123(Pt 12):2373–2399. [PubMed: 11099442]
- Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, Kaariainen H, et al. The axon guidance receptor gene *ROBO1* is a candidate gene for developmental dyslexia. *PLoS Genet*. 2005; 1(4):e50. [PubMed: 16254601]
- Harlaar N, Spinath FM, Dale PS, Plomin R. Genetic influences on early word recognition abilities and disabilities: a study of 7-year-old twins. *J Child Psychol Psychiatry*. 2005; 46(4):373–384. [PubMed: 15819646]
- Harold D, Paracchini S, Scerri T, Dennis M, Cope N, Hill G, et al. Linkage disequilibrium structure of KIAA0319 and DCDC2, two candidate susceptibility genes for developmental dyslexia. *Mol Psychiatry*. 2006; 11(12):1061.
- Harold D, Paracchini S, Scerri T, Dennis M, Cope N, Hill G, et al. Further evidence that the KIAA0319 gene confers susceptibility to developmental dyslexia. *Mol Psychiatry*. 2006; 11(12):1085–1091. 1061. [PubMed: 17033633]
- Hohnen B, Stevenson J. The structure of genetic influences on general cognitive, language, phonological, and reading abilities. *Dev Psychol*. 1999; 35(2):590–603. [PubMed: 10082029]
- 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(4):301–306. [PubMed: 11313775]
- Hsiung GY, Kaplan BJ, Petryshen TL, Lu S, Field LL. A dyslexia susceptibility locus (*DYX7*) linked to dopamine D4 receptor (*DRD4*) region on chromosome 11p15.5. *Am J Med Genet*. 2004; 125B(1):112–119. [PubMed: 14755455]
- Kaplan DE, Gayan J, Ahn J, Won TW, Pauls D, Olson RK, et al. Evidence for linkage and association with reading disability on 6p21.3–22. *Am J Hum Genet*. 2002; 70(5):1287–1298. [PubMed: 11951179]
- Kleinjan DA, van Heyningen V. Long-range control of gene expression: emerging mechanisms and disruption in disease. *Am J Hum Genet*. 2005; 76(1):8–32. [PubMed: 15549674]
- Knight JC. Regulatory polymorphisms underlying complex disease traits. *J Mol Med*. 2005; 83(2):97–109. Epub 2004 Dec 9. [PubMed: 15592805]
- 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]
- Levine M, Tjian R. Transcription regulation and animal diversity. *Nature*. 2003; 424(6945):147–151. [PubMed: 12853946]
- Londin ER, Meng H, Gruen JR. A transcription map of the 6p22.3 reading disability locus identifying candidate genes. *BMC Genom*. 2003; 4(1):25.
- Luciano M, Lind H, Duffy DL, Castles A, Wright MJ, Montgomery GW, et al. A haplotype spanning KIAA0319 and TTRAP is associated with normal variation in reading and spelling ability. *Biol Psychiatry*. 2007; 62(7):811–817. [PubMed: 17597587]

- Lyon GR. Part I. Defining dyslexia, comorbidity, teachers' knowledge of language and reading. *Ann Dyslex.* 2003; 53:1–14.
- Meng H, Smith SD, Hager K, Held M, Liu J, Olson RK, et al. DCDC2 is associated with reading disability and modulates neuronal development in the brain. *Proc Natl Acad Sci U S A.* 2005; 102(47):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(3):1215. [PubMed: 3344216]
- Morris DW, Robinson L, Turic D, Duke M, Webb V, Milham C, et al. Family-based association mapping provides evidence for a gene for reading disability on chromosome 15q. *Hum Mol Genet.* 2000; 9(5):843–848. [PubMed: 10749993]
- Nopola-Hemmi J, Myllyluoma B, Haltia T, Taipale M, Ollikainen V, Ahonen T, et al. A dominant gene for developmental dyslexia on chromosome 3. *J Med Genet.* 2001; 38(10):658–664. [PubMed: 11584043]
- Nothen MM, Schulte-Korne G, Grimm T, Cichon S, Vogt IR, Muller-Myhsok B, et al. Genetic linkage analysis with dyslexia: evidence for linkage of spelling disability to chromosome 15. *Eur Child Adolesc Psychiatry.* 1999; 8(Suppl 3):56–59. [PubMed: 10638372]
- Olson, R., Forsberg, H., Wise, B., Rack, J. Measurement of word recognition, orthographic, and phonological skills. In: Lyon, GR., editor. *Frames of Reference for the Assessment of Learning Disabilities.* Baltimore: Paul H. Brookes; 1994. p. 243–247.
- Olson R, Wise B, Conners F, Rack J, Fulker D. Specific deficits in component reading and language skills: genetic and environmental influences. *J Learn Disabil.* 1989; 22(6):339–348. [PubMed: 2738467]
- Olson RK. Dyslexia: nature and nurture. *Dyslexia.* 2002; 8(3):143–159. [PubMed: 12222731]
- Paracchini S, Thomas A, Castro S, Lai C, Paramasivam M, Wang Y, et al. The chromosome 6p22 haplotype associated with dyslexia reduces the expression of KIAA0319, a novel gene involved in neuronal migration. *Hum Mol Genet.* 2006; 15(10):1659–1666. Epub 2006 Apr 6. [PubMed: 16600991]
- Pennington BF. Using genetics to dissect cognition. *Am J Hum Genet.* 1997; 60(1):13–6. [PubMed: 8981941]
- 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(2):125–126. [PubMed: 11836362]
- Petryshen TL, Kaplan BJ, Liu MF, Schmill de French N, Tobias R, Hughes ML, et al. Evidence for a susceptibility locus on chromosome 6q influencing phonological coding dyslexia. *Am J Med Genet (Neuropsychiatric Genet).* 2001; 105:507–517.
- Rabin M, Wen XL, Hepburn M, Lubs HA, Feldman E, Duara R. Suggestive linkage of developmental dyslexia to chromosome 1p34-p36. *Lancet.* 1993; 342(8864):178.
- Rockman MV, Wray GA. Abundant raw material for *cis*-regulatory evolution in humans. *Mol Biol Evol.* 2002; 19(11):1991–2004. [PubMed: 12411608]
- Savage R, Frederickson N. Evidence of a highly specific relationship between rapid automatic naming of digits and text-reading speed. *Brain Lang.* 2005; 93(2):152–159. [PubMed: 15781303]
- Schulte-Korne G, Deimel W, Jungermann M, Remschmidt H. Follow-up of a sample of children with reading-spelling disorders in adulthood. *Z Kinder Jugendpsychiatr Psychother.* 2003; 31(4):267–276. [PubMed: 14694843]
- Schumacher J, Anthoni H, Dahdouh F, Konig IR, Hillmer AM, Kluck N, et al. Strong genetic evidence of DCDC2 as a susceptibility gene for dyslexia. *Am J Hum Genet.* 2006; 78(1):52–62. [PubMed: 16385449]
- Sham PC, Curtis D. An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. *Ann Hum Genet.* 1995; 59(Pt 3):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(4590):1345–1347. [PubMed: 6828864]
- Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, Lyytinen H, et al. 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(20):11553–11558. [PubMed: 12954984]
- Tannock, R., Hum, M., Masellis, M., Humphries, T., Schachar, R. Teacher Telephone Interview for Children's Academic Performance, Attention, Behavior and Learning: DSM-IV Version (TTI-IV). Toronto, Canada: The Hospital for Sick Children; 2002. unpublished document
- Torgesen, JK., Wagner, RK., Rashotte, CA. TOWRE: Test of Word Reading Efficiency. Austin, Texas, USA: Pro-Ed; 1999.
- Tzenova J, Kaplan BJ, Petryshen TL, Field LL. Confirmation of a dyslexia susceptibility locus on chromosome 1p34-p36 in a set of 100 Canadian families. *Am J Med Genet B Neuropsychiatr Genet*. 2004; 127(1):117–124.
- Velayos-Baeza A, Toma C, Paracchini S, Monaco AP. The dyslexia-associated gene KIAA0319 encodes highly N- and O-glycosylated plasma membrane and secreted isoforms. *Hum Mol Genet*. 2008; 17(6):859–871. [PubMed: 18063668]
- Vellutino FR, Fletcher JM, Snowling MJ, Scanlon DM. Specific reading disability (dyslexia): what have we learned in the past four decades? *J Child Psychol Psychiatry*. 2004; 45(1):2–40. [PubMed: 14959801]
- Wagner, R., Torgesen, J., Rashotte, C. Comprehensive Test of Phonological Processing (CTOPP). Austin, Texas, USA: Pro-Ed; 1999.
- Wechsler, D. Wechsler Intelligence Scale for Children*Third Edition. San Antonio, Texas, USA: Harcourt Brace; 1991.
- Weller EB, Weller RA, Fristad MA, Rooney MT, Schecter J. Children's Interview for Psychiatric Syndromes (ChIPS). *J Am Acad Child Adolesc Psychiatry*. 2000; 39(1):76–84. [PubMed: 10638070]
- Wigg KG, Couto JM, Feng Y, Anderson B, Cate-Carter TD, Macciardi F, et al. Support for EKN1 as the susceptibility locus for dyslexia on 15q21. *Mol Psychiatry*. 2004; 13:13.
- Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet*. 2005; 76(5):887–893. [PubMed: 15789306]
- Wilkinson, GS. Wide Range Achievement Test 3–Revision 3. Wilmington, Delaware, USA: Jastak Associates; 1993.
- Wolf M, Bowers PG. The double-deficit hypothesis for the developmental dyslexias. *J Ed Psychol*. 1999; 91(3):415–438.
- Wolf M, Goldberg A, Gidney C, Lovett MW, Cirino P, Morris RD. The second deficit: An investigation of the independence of phonological and naming-speed deficits in developmental dyslexia. *Read Writing Interdisc J*. 2002; 15(1):43–72.
- Woodcock, RW. Woodcock Reading Mastery Tests*Revised. American Guidance Service Inc; Minnesota, USA: 1987.

Figure 1.

A schematic depicting the gene *KIAA0319L isoform a* (NM_024874). Untranslated exons are represented by smaller boxes. There are two known isoforms of the gene, both on the antisense (–) strand. The transcription start sites for both isoforms are at the same position on chromosome 1 (35,692,097, UCSC Human May 2004 (hg17) assembly). The 3′ end for *isoform b* (NM_182686) is in exon 20 of *isoform a*. The schematic also shows the region upstream of *KIAA0319L* that was screened for novel polymorphisms as part of the “putative proximal promoter.” The 610-bp includes the intergenic region between *KIAA0319L* and neurochondrin (*NCDN*) (NM_014284) and part of exon 1 of *NCDN*. Locations of genotyped markers are indicated by arrows. The markers rs1635712, rs11551037, rs12729157, and rs1361040 are all nonsynonymous single-nucleotide polymorphisms that were not polymorphic in our sample.

Table 1Single-Marker Categorical Analysis for 5 tagSNPs in *KIAA0319L*

Marker	Allele ^a	Frequency	Transmissions	Nontransmissions	χ^2	P-value
rs917105	G/C	0.882	33	24	1.421	0.233
rs1203138	T/C	0.110	31	31	0.000	1.000
rs1203148	G/A	0.760	51	45	0.375	0.540
rs12408030	A/G	0.754	63	49	1.750	0.186
rs7523017	G/A	0.931	27	14	4.122	0.042

^aTransmitted allele is indicated first.*P*-values were not corrected for multiple tests; 156 families with a proband that met criteria for RD were used in this analysis.

Table 2

Haplotype Analysis using a Categorical Definition of RD

Marker 1	Marker 2	Marker 3	Marker 4	Frequency	Observed	Expected	Var (O-E)	χ^2 (1 df)	P-value
rs1203138	rs1203148	rs12408030	rs7523017						
2(C)	1(A)	1(A)	1(G)	0.34	110.36	112.78	28.46	0.20	0.64
2(C)	1(A)	2(G)	1(G)	0.23	86.80	80.35	26.06	1.59	0.20
2(C)	2(G)	1(A)	1(G)	0.23	85.17	83.50	28.04	0.09	0.75
1(T)	1(A)	1(A)	1(G)	0.10	39.14	37.47	21.67	0.12	0.71
2(C)	1(A)	1(A)	2(A)	0.06	16.47	24.77	14.75	4.66	0.03
2(C)	2(G)	2(G)	1(G)	0.00	2.15	2.19	0.59	0.00	
1(T)	2(G)	1(A)	1(G)	0.00	2.14	1.97	0.31	0.08	
1(T)	1(A)	2(G)	1(G)	0.00	0.22	1.13	0.86	0.97	
2(C)	2(G)	1(A)	2(A)	0.00	2.25	1.15	1.08	1.13	
2(C)	1(A)	2(G)	2(A)	0.00	0.25	0.13	0.02	0.60	
1(T)	1(A)	1(A)	2(A)	0.00	1.01	0.52	0.24	1.00	

In this analysis, 156 families with a proband that made criteria for RD were used. Global χ^2 , 10 degrees of freedom (df) = 10.268; P = 0.417. Global χ^2 pooling all haplotypes with frequencies less than 5%, 5 df = 5.678; P = 0.339.

Table 3**Haplotype Analysis with Quantitative Measures of Reading**

Haplotype				Freq	fam#	S	E(S)	Var(S)	Z	P
WRAT 3 Spelling										
M1	M2	M3	M4							
2(C)	1(A)	1(A)	1(G)	0.34	161.5	-2457	-2448	16632	-0.07	0.94
2(C)	1(A)	2(G)	1(G)	0.23	122.6	-2055	-1877	15509	-1.43	0.15
2(C)	2(G)	1(A)	1(G)	0.22	120.5	-2032	-1982	15783	-0.40	0.68
1(T)	1(A)	1(A)	1(G)	0.10	80	-722	-788	8744	0.70	0.48
2(C)	1(A)	1(A)	2(A)	0.06	62.3	-480	-650	9232	1.77	0.07
TOWRE-Composite score ^a										
M1	M2	M3	M4							
2(C)	1(A)	1(A)	1(G)	0.34	160.5	-3327	-3197	27092	-0.78	0.43
2(C)	1(A)	2(G)	1(G)	0.23	120.6	-2605	-2385	26271	-1.35	0.17
2(C)	2(G)	1(A)	1(G)	0.22	119.5	-2365	-2401	25327	0.22	0.82
1(T)	1(A)	1(A)	1(G)	0.10	79	-1044	-1104	14593	0.49	0.61
2(C)	1(A)	1(A)	2(A)	0.06	60.4	-525	-768	12870	2.14	0.03
CTOPP-Rapid Naming (Numbers/Letters)										
M1	M2	M3	M4							
2(C)	1(A)	1(A)	1(G)	0.34	151.5	-1748	-1787	14708	0.32	0.74
2(C)	1(A)	2(G)	1(G)	0.23	118.5	-1665	-1533	16117	-1.04	0.29
2(C)	2(G)	1(A)	1(G)	0.22	117.5	-1468	-1409	15524	-0.47	0.63
1(T)	1(A)	1(A)	1(G)	0.10	78	-580	-627	9559	0.47	0.63
2(C)	1(A)	1(A)	2(A)	0.06	58.4	-333	-441	7475	1.25	0.20
CTOPP-Rapid Naming (Colours/Objects)										
M1	M2	M3	M4							
2(C)	1(A)	1(A)	1(G)	0.34	147.5	-2705	-2612	25346	-0.58	0.55
2(C)	1(A)	2(G)	1(G)	0.23	111.8	-2061	-1805	23412	-1.67	0.09
2(C)	2(G)	1(A)	1(G)	0.22	110.3	-1716	-1893	21098	1.22	0.22
1(T)	1(A)	1(A)	1(G)	0.10	71	-851	-838	11848	-0.12	0.90
2(C)	1(A)	1(A)	2(A)	0.06	55.4	-410	-608	9931	1.98	0.04

In this analysis, 403 probands and siblings from 291 nuclear families were used.

^aComposite of scores for single-word reading and phonological decoding.

An offset of 100.1 was used.

M1 = rs1203138; M2 = rs1203148; M3 = 12408030; M4 = rs7523017.

Analyses were conducted on haplotypes with frequencies greater than 1%.