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A Family-Based Association Analysis and Meta-Analysis of the Reading Disabilities Candidate Gene *DYX1C1*

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Abstract

Reading disabilities (RD) have a significant genetic basis and have shown linkage to multiple regions including chromosome 15q. Dyslexia susceptibility 1 candidate gene 1 (*DYX1C1*) on chromosome 15q21 was originally proposed as a candidate gene with two potentially functional polymorphisms at the −3G/A and 1249G/T positions showing association with RD. However, subsequent studies have yielded mixed results. We performed a literature review and meta-analysis of the −3G/A and 1249G/T polymorphisms, including new unpublished data from two family-based samples. Ten markers in *DYX1C1* were genotyped in the two independently ascertained samples. Single marker and −3G/A:1249G/T haplotype analyses were performed for RD in both samples, and quantitative trait analyses using standardized reading-related measures was performed in one of the samples. For the meta-analysis, we used a random-effects model to summarize studies that tested for association between −3G/A or 1249G/T and RD. No significant association was found between the *DYX1C1* SNPs and RD or any of the reading-related measures tested after correction for the number of tests performed. The previously reported risk haplotype (−3A:1249T) was not biased in transmission. A total of 9 and 10 study samples were included in the meta-analysis of the −3G/A and 1249G/T polymorphisms, respectively. Neither polymorphism reached statistical significance, but the heterogeneity for the 1249G/T polymorphism was high. The results of this study do not provide evidence for association between the putatively functional SNPs −3G/A and 1249G/T and RD.

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Keywords

dyslexia; chromosome 15q; *EKN1*; genetics

INTRODUCTION

Specific reading disabilities (RD), or developmental dyslexia, refer to an unexpected and specific difficulty in learning to read despite normal or above average intelligence, education, and socioeconomic opportunity. RD is likely caused by a dysfunction of neural systems involved in cognitive skills required for reading [Habib, 2000]. A core deficit observed in dyslexics involves the processing of phonemes, the most basic unit of speech sounds, which persist into adulthood [Bruck, 1992; Shaywitz et al., 1999; Ramus et al., 2003]. Individuals with RD have difficulty segmenting words into their phonological elements, impairing their ability in learning to read. Several other reading components have been shown to be impaired in individuals with RD including orthographic coding, single-word reading, rapid automatic naming, and spelling [Denckla and Rudel, 1976; Paulesu et al., 2001; Francks et al., 2002]. With an estimated prevalence ranging from 5% to 17.5% in school-aged children, RD is the most common learning disability and affects four out of five individuals with learning disabilities [Shaywitz et al., 1990; Shaywitz, 1998; Katusic et al., 2001]. There is substantial evidence to suggest that RD is both familial and heritable. RD has been shown to cluster in families [Hallgren, 1950; Finucci et al., 1976; Volger et al., 1984] and twin studies estimate the heritability of reading components in the range of 0.30–0.72 [DeFries et al., 1987; Gayan and Olson, 1999; Wadsworth et al., 2000; Gayan and Olson, 2001; Harlaar et al., 2005].

Linkage studies have identified at least nine susceptibility regions (designated as DYX1–9), including DYX1 on chromosome 15q15.2–21.2 which has been replicated in multiple linkage studies [Grigorenko et al., 1997; Schulte-Korne et al., 1998; Morris et al., 2000; Chapman et al., 2004; Schumacher et al., 2008]. One gene in this region, dyslexia susceptibility 1 candidate gene 1 (*DYX1C1*) on chromosome 15q21 (also known as *EKN1*) is among the most well-studied RD candidate genes. *DYX1C1* was originally proposed as a candidate gene based on a t(2;15)(q11;q21) chromosomal translocation that cosegregated with RD in three affected members of a six-member, two generation Finnish family [Taipale et al., 2003]. One additional offspring in the family also carried the translocation, but his RD affection status was unknown due to low verbal and non-verbal performance on cognitive achievement tests. In the same study, a case–control association analysis was performed using a sample of Finnish dyslexics and a control group comprising nondyslexics and anonymous blood donors. Two single nucleotide polymorphisms (SNPs) at the −3G/A (rs3743205) and 1249G/T (rs57809907) positions of the transcript showed significant association, with the minor −3A and 1249T alleles increasing risk for RD. The frequency of the −3A:1249T haplotype was significantly greater in the dyslexic group (14/106 cases) compared to the controls (10/192 nondyslexic and anonymous controls). In addition, an analysis of nine informative family trios found significant overtransmission of the −3A:1249T haplotype to affected offspring.

The results of the Finnish study were particularly promising because of the proposed functionality of the two significant SNPs. The −3G/A position is located in the predicted binding sequence of the Elk-1, HSTF, and TFII-I transcription factors, and was also predicted to affect the Kozak sequence which plays a role in initiating translation. The 1249T variant introduces a premature stop codon, shortening the protein from 420 to 416 amino acids although a latter study indicated that the truncation may not affect function [Wang et al., 2006]. However, there were considerable study design issues that may have affected the results of the initial study by Taipale et al. [2003]. The association analysis included 58 dyslexics and 61 nondyslexics from 20 unrelated families, and a replication set of 3 families and 33 unrelated dyslexic–nondyslexic couples. The χ^2 or Fisher's exact test was used to test for association between the genotyped *DYX1C1* SNPs and RD, and Bonferroni correction was applied to account for multiple testing. The transmission disequilibrium test (TDT) was used to assess the transmission of the risk haplotype −3A:1249T in nine informative trios. Because the analytic strategy used in the study did not take into account the greater allele sharing in the related individuals taken from the same families, the observed results may be biased toward positive spurious associations. In addition, the significant over-transmission of the −3A:1249T haplotype was based on an analysis of only nine informative trios, and these significant findings may be due to the small sample size.

In addition to the significant associations, functional studies have supported a potential role for *DYX1C1* in RD, as *DYX1C1* has been implicated in neural migration. RNA interference (RNAi) of *DYX1C1* in rats disrupted the migration of neurons, causing malformations in the neocortex and hippocampus [Wang et al., 2006; Rosen et al., 2007]. This is consistent with studies of post-mortem dyslexic brains which have reported ectopias and dysplasias, signs of abnormal neural migration [Galaburda et al., 1985; Humphreys et al., 1990]. *DYX1C1* RNAi-treated rats also performed significantly poorer in spatial working memory and auditory processing tasks [Threlkeld et al., 2007; Szalkowski et al., 2011]. Wang et al. [2006] showed that RNAi rescue experiments using the shortened 416 amino acid version of *DYX1C1* predicted by the 1249T variant restored normal neural migration, indicating that this polymorphism was not sufficient to alter the function of *DYX1C1*, at least as measured by this specific assay in rats. Thus the reported role of *DYX1C1* in neural migration is consistent with the neurobiology of RD, although the 1249T allele may not be detrimental to gene function. The initial association findings, in combination with the functional evidence, generated strong interest in *DYX1C1* as a candidate gene.

Previous association studies have illustrated that although *DYX1C1* has been widely studied, the results have been inconsistent and often conflicting, and the status of this candidate gene remains unclear. In a family-based association study by Wigg et al. [2004], a different marker rs11629841 was found to be significantly associated with RD which was in high LD with both −3G/A and 1249G/T. Brkanac et al. [2007] found association with the 1249G/T polymorphism, but it was the major G allele that was overtransmitted, rather than the T allele which showed association in the study by Taipale et al. [2003]. A number of other studies did not find association between *DYX1C1* SNPs and RD [Bellini et al., 2005; Cope et al., 2005; Marino et al., 2005; Saviour et al., 2008; Newbury et al., 2011; Venkatesh et al., 2011].

In addition, previous studies have tested for association between *DYX1C1* SNPs and quantitative reading and language measures. Wigg et al. [2004] found that the major −3G variant was significantly associated with nine measures of reading skills including phonological awareness, word identification, decoding, rapid automatized naming, language ability, and verbal short-term memory. Marino et al. [2007] found the opposite allele −3A was significantly associated with lower performance in a short-term memory test. Other studies did not find association between *DYX1C1* SNPs and quantitative reading tasks [Scerri et al., 2004; Meng et al., 2005]. Two independent Australian cohort studies also performed quantitative analyses of reading and reading related measures in population-based samples [Bates et al., 2010; Paracchini et al., 2011]. Bates et al. [2010] found three SNPs that were significantly associated with different measures: rs685935 with short-term memory; rs17819126 with irregular reading, nonword reading, and irregular spelling; and rs3743204 with nonword reading. Paracchini et al. [2011] found that spelling was associated with the three markers rs7174102, rs8043049, and rs8037376, and reading associated with rs8040756.

The purpose of this study was to evaluate the cumulative epidemiological evidence of *DYX1C1* as a RD candidate gene. First, we performed a family-based association analysis using two independent samples, one of which was an extension of the sample used in Wigg et al. [2004]. Second, we summarized and evaluated the −3G/A and 1249G/T SNPs through a literature review and meta-analysis of genetic association studies. Our aim was to determine whether specific SNPs in *DYX1C1* were associated with RD, or with reading-related quantitative measures.

MATERIALS AND METHODS

Family-Based Association Analysis

Subjects and assessment—Toronto sample—The first sample included families recruited primarily from the Greater Toronto Area and surrounding regions, each with at least one child exhibiting reading difficulties. Probands ranged from 6 to 16 years old, and siblings within the same range were invited to participate in the study regardless of reading ability. The total sample consisted of 1,416 individuals from 421 nuclear families, consisting of 421 probands and 167 siblings. Of the 421 nuclear families, 148 were previously analyzed in an association analysis of *DYX1C1* [Wigg et al., 2004].

Probands and siblings in the Toronto sample were tested for IQ, reading, and language measures, which have all been described previously [Wigg et al., 2004; Couto et al., 2010; Elbert et al., 2011]. The Wechsler Intelligence Scale for Children (WISC-III or WISC-IV) was used to assess intelligence and cognitive ability, and children were excluded from the study if they scored <80 on the Verbal and Performance Scales of the WISC-III, or on the Verbal Comprehension and Perceptual Reasoning Indices of the WISC-IV [Wechsler, 1991, 2003]. The Wide Range Achievement Test-III (WRAT-III) [Wilkinson, 1993], Woodcock Reading Mastery Test-Revised (WRMT-R) [Woodcock, 1987], and Comprehensive Test of Phonological Processing (CTOPP) [Wagner et al., 1999] were used to measure reading, spelling, phonological awareness, and rapid naming skills. Receptive and expressive language ability was assessed using the Clinical Evaluation of Language Fundamentals—3rd

edition (CELF-3) [Semel et al., 1995]. Three subtests that measured single word and nonword reading; WRAT-III Reading, WRMT-R Word ID, and WRMT-R Word Attack, were used to determine RD affection status for the categorical analysis. Probands and siblings were considered to have RD if they scored 1.5 standard deviations below the mean on two of the three measures or one standard deviation below the mean on the average of all three. For the analysis of the quantitative traits, scores from the WRAT-III, WRMT-R, CTOPP, and CELF-3 subtests were used as the quantitative phenotypes and information from all of the 588 subjects in the sample was included.

To screen for symptoms of possible neurological, medical, and psychiatric disorders, parents were interviewed using the structured Children's Interview for Psychiatric Syndromes (ChIPS) [Weller et al., 2000], and teachers were interviewed using the semi-structured Teacher Telephone Interview [Tannock et al., 2002]. This information was supplemented with two standardized questionnaires: the Conners Parent and Teacher Rating Scales—Revised [Conners, 1997] and Ontario Child Health Survey Scales—Revised [Boyle et al., 1993]. Subjects were excluded if there was evidence of neurological or chronic medical illness, bipolar affective disorder, psychotic symptoms, Tourette syndrome or chronic multiple tics.

Subjects were either native English speakers or were educated for at least 5 years in an English-speaking school. The ethnicity of the sample was primarily Caucasian, with 68.1% of individuals reporting European descent and 26% reporting "Caucasian Canadian" background. The remaining subjects reported South American (1.8%), non-European (2.9%), or non-European mixed background. The protocol for the Toronto sample was approved by The Hospital for Sick Children Research Ethics Board, and written informed consent was obtained from all participants.

Subjects and assessment—Calgary sample—The second sample genotyped for this study consisted of nuclear families with affected siblings from Calgary, Alberta. Full descriptions of the recruitment and assessment of the 95 families have previously been described [Field and Kaplan, 1998; Petryshen et al., 2001; Hsiung et al., 2004; Tzenova et al., 2004]. In contrast to previous studies, which included members of extended families, only members of the nuclear family were used in the study. Most of the affected sibpair families were ascertained through a proband >8 years of age who attended a special school for children with learning disabilities. A few affected sibpair families derived from extended families were close (non-sibling) relatives of the proband but only one affected sibpair family per extended family was utilized. For the 95 families in which DNA was available, 86 families included at least two children who were diagnosed with RD, with a total of 207 affected children. In the Calgary sample, RD was diagnosed based on the word attack subtests from the Woodcock Reading Mastery Test and the Revised Woodcock–Johnson Psychoeducation Test, both which comprise phonological coding tasks. Subjects <18 years of age who performed ≥2 years below the age norm were considered affected. Full-scale IQ was estimated using a short form of the Wechsler Intelligence Scale for Children for subjects 8–16 years of age [Wechsler, 1974] and a short form of the Wechsler Adult Intelligence Scale for adult subjects [Wechsler, 1981].

The sample was also primarily white Caucasian, with 4 of the original 100 multi-generational families containing one unaffected non-European parent. The protocol for the Calgary sample was approved by the University of Calgary Ethics Review Board and written informed consent was obtained from all participants.

SNP genotyping for the Toronto and Calgary samples—For both the Toronto and Calgary samples, genomic DNA was extracted from whole blood using a high-salt extraction method [Miller et al., 1988]. A total of 10 markers were genotyped in the Toronto and Calgary samples. Six of the markers, including rs2007494, rs57809907 (1249G/T), rs3743205 (−3G/A), rs3743204, rs11629841, and rs692691 were previously genotyped in a subset of the Toronto sample which included 148 families [Wigg et al., 2004]. For this study, the remaining 273 families of the Toronto sample were genotyped for the six SNPs. In the Calgary sample, these six SNPs were genotyped in all 95 families.

Four additional markers, rs600753, rs16787, rs17819126, and rs12899331 were also selected for genotyping in the entire Toronto and Calgary samples. The markers rs600753 and rs16787 were previously found to be significant in several combinations of haplotypes [Dahdouh et al., 2009]. The rs1789126 marker was selected because of a positive association in an Australian sample [Bates et al., 2010], and the rs1289331 was selected as it was suggested to be the binding site of the SP1 transcription factor [Tapia-Paez et al., 2008].

All 10 markers were genotyped using the ABI 7900-HT Sequence Detection System (Applied Biosystems, Foster City, CA) using the TaqMan®5′ nuclease assay for allelic discrimination. The 1249G/T, rs600753, rs3743204, rs2007494, rs16787, and rs12899331 were genotyped using Assay-by-Design probes while rs692691, rs11629841, and rs17819126 were genotyped using Assay-on-Demand probes. The −3G/A polymorphism was previously genotyped using a restriction fragment length polymorphism assay [Wigg et al., 2004]. Later, Assay-by-Design probes were available and were used to genotype the remaining sample.

Genotyping was carried out in 96-well plates consisting of two negative controls. Ten microliters of PCR reactions were performed with the following reagents: 30 ng of genomic DNA, 10 μmol of TaqMan® Universal PCR Master Mix (Applied Biosystems), and 0.25 μl of allelic discrimination mix (Applied Biosystems) with 36 μM of each primer and 8 μM of each probe. The reaction conditions consisted of the following steps: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 94°C for 15 sec and an annealing temperature of 59°C for 1 min. Plates were then read with the ABI 7900-HT Sequence Detection System using the allelic discrimination end-point analysis mode of the SDS software package, v2.0 (Applied Biosystems). Samples with undetermined or unclear calls were genotyped for a second time.

Statistical analysis—The genotyped samples were checked for genotyping and family structure errors using Haploview v4.2 and Merlin v1.1.2, which determines unlikely crossover events suggesting erroneous genotypes [Abecasis et al., 2002; Barrett et al., 2005]. Non-Mendelian inheritance was resolved before analysis by genotyping families a second time and all markers were tested for Hardy–Weinberg Equilibrium. Haploview v4.2 was also used to perform the TDT for association between single SNPs and categorical RD [Barrett et

al., 2005]. Bonferroni correction was used to account for multiple testing, with a type I error rate of 0.05. To account for SNPs in LD for the Bonferroni correction, we used the SNP SpD tool to determine the number of independent tests [Nyholt, 2004]. For the single SNP association analysis with RD, the threshold for statistical significance was corrected for the number of independent SNPs ($P = 0.05/8 = 0.006$), while the quantitative trait analysis was corrected for both the number of independent SNPs ($P = 0.05/8 = 0.006$) and number of independent quantitative measures ($P = 0.006/7 = 0.0009$).

The transmission of the −3G/A:1249G/T haplotypes was analyzed using TRANSMIT v2.5.4 with the robust estimator option [Clayton and Jones, 1999]. In the Toronto sample, association with quantitative measures was assessed using scores from nine reading and language tests: WRAT-III reading and spelling; WRMT-3 word ID and word attack; CTOPP nonword repetition, phonological awareness and rapid digit naming; and CELF-3 receptive and expressive language. The program FBAT v.2.0.3 was used to carry out the quantitative trait analyses [Laird et al., 2000]. For all results, the two-tailed *P* values are reported.

Literature Review and Meta-Analysis

Search strategy—For the literature review and meta-analysis, we included the −3G/A and 1249G/T SNPs because they were the most commonly genotyped across *DYX1C1* association studies and were proposed as having a functional role. We searched MEDLINE, EMBASE, Scopus, BIOSIS, and HuGE Navigator with the keywords "*DYX1C1*," "*EKN1*," "dyslexia," and "reading disabilities" for studies examining an association between categorical RD and the −3G/A or 1249G/T variants. The HuGE navigator was used to search for genome wide association studies (GWAS) using the search terms "dyslexia" and "reading disabilities," and we filtered the results to only GWAS. Genetic linkage studies, conference proceedings, review articles, functional studies were excluded from the meta-analysis. The reference lists from all *DYX1C1* association studies and review articles were examined for additional studies.

Studies that met the inclusion criteria used either a case–control or family-based association design. The following information was collected from each study: country and language, sample size, ethnicity of sample, definition of cases and controls, and whether SNPs were in Hardy–Weinberg Equilibrium. For case–control studies, allele frequencies for the two SNPs were obtained for the cases and controls. For family-based studies, the allele frequencies and the number of transmissions for each allele were obtained for both SNPs. Information that was not provided in the articles was requested from the corresponding authors. If a study used both case–control and TDT analyses, only the TDT data were used because it is robust to population stratification, whereas the case–control design is not.

Meta-analysis—Each study was summarized by constructing two-by-two tables from case–control studies and one-by-two tables from TDT studies. To synthesize the case–control and TDT studies, the method of Kazeem and Farrall [2005] was used. The odds ratios and 95% confidence intervals for individual studies were calculated, with each study given a weight based on the inverse of its variance. Studies using larger samples would carry greater weight as their variances would be smaller. Heterogeneity between studies was

assessed using the Cochran's Q statistic and the I^2 statistic [Higgins and Thompson, 2002]. For the pooled estimate of the odds ratio and its 95% confidence interval, a fixed- [Kazeem and Farrall, 2005] or random-effects [DerSimonian and Laird, 1986] model could be applied. As the 1249G/T SNPs showed evidence of between-study heterogeneity (P = 0.037), we used the random-effects model. Although the −3G/A SNP did not show statistically significant evidence of heterogeneity, the random-effects model was still applied. Since there is evidence for genetic heterogeneity in RD [Pennington et al., 1991; Fisher and DeFries, 2002], which may suggest different effect sizes between populations, the random-effects model was more appropriate than the fixed-effects model, which assumes a common genetic effect [Cohn and Becker, 2003]. In addition, the random-effects model is more robust and generally preferred because it accounts for between-study heterogeneity but also gives similar results to fixed-effects models in the absence of heterogeneity [Kavvoura, 2008].

Subgroup analyses were subsequently performed within the meta-analysis to explore potential sources of heterogeneity. In the first, studies were restricted to white Caucasian populations. In the second, studies that included phonological awareness or decoding tasks in the diagnosis of RD were analyzed together. The third subgroup analysis was restricted to English speaking subjects and English language reading tests.

Publication bias was assessed with the Egger test which uses a linear regression approach to test for funnel plot asymmetry [Egger et al., 1997]. In one study there was an absence of the 1249T allele in the control group [Saviour et al., 2008]. To calculate a non-zero odds ratio, we increased the allele count to 1. However, for the Egger test, an allele count of 0 was used to prevent substantial inflation of the standard error. The catmap package was used to assess between-study heterogeneity, calculate the pooled ORs, and carry out the sensitivity analysis [Nicodemus, 2008]. The open-source statistical program R v2.8.1 was used to run the catmap package. For the calculation of the Egger test, we used the metabias package in STATA v11.1 (StataCorp LP, College Station, TX).

A power analysis of the pooled sample was performed using the Genetic Power Calculator [Purcell et al., 2003]. Although there was no option that combined case–control and TDT samples, we estimated and compared the power of the individual modules. The total sample size was pooled using the cases from the case–control and TDT studies, as the power between the same number of family trios and cases (with 1:1 ratio with controls) is virtually equal [McGinnis et al., 2002]. Both the −3G/A and 1249G/T SNPs were hypothesized as being the causal alleles, and the D'-value was set to 1, with the marker allele frequency set equal to the risk allele frequency. A dominant model was used as this model of inheritance reflected previous association studies. The RD prevalence risk used was 0.1, based on the estimates provided in the literature [Shaywitz et al., 1990]. For the case–control module, a case–control ratio of 1 was used, and the unselected controls option was selected because more than 50% of the pooled control group were unscreened controls. The type I error rate was defined at 0.05 and the threshold for acceptable power was 80%.

The Venice criteria were used to evaluate the credibility of the evidence for association between RD and the −3G/A or 1249G/T variants [Ioannidis et al., 2008]. Three criteria are

taken into consideration including the amount of evidence (e.g., sample size with the risk allele, power, and false-discovery rate), extent of replication (e.g., results from meta-analysis and between-study heterogeneity), and protection from bias (e.g., genotyping errors and population stratification). Each criterion is categorized and using a semi-quantitative index, a final grade of “strong,” “medium” or “weak” is given to the strength of the evidence.

RESULTS

Association Analysis

Of the 10 markers that were genotyped, six were located within introns and four within exons (Fig. 1). The parental minor allele frequencies were similar in both the Toronto and Calgary samples for all 10 markers (Table I). The LD between the SNPs for both samples was also comparable (Fig. 2). For the single SNP analysis, no marker showed significantly biased transmission with RD in either sample. For the haplotype analysis, there was significant undertransmission of the rare −3A/1249G haplotype in the Toronto sample (frequency = 0.013, $P = 0.002$), but this rare haplotype was not observed in the Calgary sample (Table II). For the quantitative trait analysis, three measures showed association at a P-value of <0.05 in the Toronto sample: CELF-III Receptive language and rs17819126 ($P = 0.034$), CTOPP Phonological awareness and rs17819126 ($P = 0.044$), and CELF-III expressive language and rs11629841 ($P = 0.034$). However, these results were not significant after Bonferroni correction.

Literature Review and Meta-Analysis

Including the unpublished data from the Toronto and Calgary samples, 11 studies met inclusionary criteria for the literature review and meta-analysis. Later, one study was excluded because we were unable to contact the corresponding authors to obtain the number of transmitted and untransmitted alleles for the −3G/A and 1249G/T polymorphisms [Cope et al., 2005], and two studies were excluded because subsets of the samples were duplicated in a subsequent study included in this meta-analysis [Wigg et al., 2004; Marino et al., 2005]. A total of eight studies, which analyzed 10 independently ascertained samples, were included in the meta-analysis. Three GWAS were identified using RD samples but were not included. Two of the studies tested for association with non-reading phenotypes. The third study performed a quantitative trait analysis, comparing readers of high and low ability selected from a representative UK sample [Meaburn et al., 2008].

Characteristics of the 10 samples are summarized in Supplementary Table I. All 10 samples were genotyped for the 1249G/T SNP, and 9 of the 10 samples were genotyped for the −3G/A SNP. The study design distribution included five case–control and four family-based for the −3G/A, and six case–control and four family-based for the 1249G/T. In one study, one of the groups of cases was nested within a case–cohort study [Newbury et al., 2011]. Cases and probands were recruited from different sources including hospitals, research centers, and institutions [Taipale et al., 2003; Bellini et al., 2005; Newbury et al., 2011]; regular schools [Brkanac et al., 2007; Saviour et al., 2008; Venkatesh et al., 2011] (Toronto sample, this study); schools for the learning disabled [Saviour et al., 2008; Venkatesh et al., 2011] (Calgary sample, this study); as a part of a case–cohort study [Newbury et al., 2011];

or a combination of referrals from teachers and paediatricians [Marino et al., 2007]. The Toronto sample also recruited families through websites, regional organizations for learning disabilities, referrals from general education teachers, special education teachers, speech/language pathologists, and psychologists. For the case–control studies, controls were selected from the proband's partner and/or family [Taipale et al., 2003], anonymous blood donors [Taipale et al., 2003], unselected European controls from the European Collection of Cell Cultures [Newbury et al., 2011] unmatched healthy individuals [Bellini et al., 2005], unmatched individuals with no history of reading, spelling or other academic difficulties [Saviour et al., 2008], or group-age-matched individuals without RD [Venkatesh et al., 2011]. All of the case–control studies tested for association by comparing the allele frequencies between groups, and no genotype analyses were performed. The ethnicity of the samples included white Caucasians only [Taipale et al., 2003; Bellini et al., 2005; Marino et al., 2007; Newbury et al., 2011], primarily white Caucasian (>90%) [Brkanac et al., 2007] (Toronto and Calgary samples, this study) or Indian only [Saviour et al., 2008; Venkatesh et al., 2011].

The RD diagnosis in all studies was based on significantly low reading ability, with 8 of the 10 studies reporting the specific tests that were administered to the subjects. The psychometric tests that were used to measure reading ability and the cutoffs used for diagnosis varied between and among languages. All of the samples, with the exception of the Calgary sample, used low IQ as an exclusion criterion with the minimum IQ ranging from 80 to 90. One sample analyzed in the study by Newbury et al. [2011] used an IQ discrepancy as part of the RD diagnosis. The most common test that was used to assess intelligence was the Wechsler Intelligence Scale for Children. Five of the samples also reported the use of neurological, medical or sensorial disorders as exclusionary criteria, although the specific disorders that were screened varied across the samples [Bellini et al., 2005; Brkanac et al., 2007; Marino et al., 2007; Newbury et al., 2011] (Toronto sample, this study).

Within the pooled case–control studies, a total of 786 cases and 756 controls were included. For one study, the allele frequencies in two independent samples were compared to the same group of controls but for the meta-analysis, the controls were only counted once [Newbury et al., 2011]. Within the family-based studies, a total of 940 affected subjects were included. Of the studies that were included in the analysis, we were able to contact all corresponding authors and confirm that both SNPs were in Hardy–Weinberg equilibrium except for Taipale et al. [2003].

In the nine samples that were analyzed for the −3G/A polymorphism only the study by [Taipale et al., 2003] showed a statistically significant difference. For the 1249G/T studies, three studies showed significant association. Two studies showed significant association with the T allele including Taipale et al. [2003] and Marino et al. [2007]. In contrast, the study by Brkanac et al. [2007] found that the T allele carried a decreased risk for RD. Using the random-effects model, there was no statistically significant evidence of an association between RD and the −3G/A (OR = 1.16, 95% CI: 0.900, 1.490) or 1249G/T polymorphisms (OR = 1.26, 95% CI: 0.981, 1.624), with significance at $P<0.05$ (Figs. 3 and 4). The level of heterogeneity for both SNPs was medium to high, with an I^2 value of 34% (95% CI: 1, 55)

for the −3G/A and 50% (95% CI: 28, 64) for the 1249G/T. There was no evidence of publication bias for either −3G/A ($P = 0.445$) or 1249G/T ($P = 0.313$). The four additional subgroup analyses did not yield considerably different results.

Using the Venice criteria to evaluate the credibility of the evidence, a “weak” grade was given for both the association between −3G/A and 1249G/T polymorphisms and RD. There was a potential for information bias introduced by genotyping error and misclassification bias in the studies which could have affected the results, while heterogeneity was moderate for the 33G/A and high for the 1249G/T polymorphism.

DISCUSSION

DYX1C1 is a prominent RD candidate gene and has been extensively studied. Although two potentially functional SNPs previously showed significant association with RD in the initial study, subsequent studies yielded contradictory results. We performed a family-based association analysis, expanding on the sample that was used by Wigg et al. [2004]. With 273 additional families and an increase from 101 to 272 subjects meeting the categorical cut off, we tested whether the previous reported associations remained significant, in addition to testing four new additional SNPs. A second independent sample from Calgary was also used to test for categorical association with single SNPs and the −3G/A:1249G/T haplotypes. The rs11629841 marker, which previously showed significantly biased transmission, was not significant in the two samples. For the quantitative trait analysis, the previous associations between the −3G variant and all nine reading measures also did not remain statistically significant in this study. Although we found three reading/language measures with $P < 0.05$, they were not statistically significant when corrected for the multiple tests performed.

In the Toronto sample, there was a statistically significant undertransmission of the −3A/1249G haplotype, differing from the −3G/1249G haplotype that was overtransmitted in the previous study. However, this haplotype was rare in both the samples, found in only 0.013 of parental chromosomes in the Toronto sample and absent in the Calgary sample. Although it is possible that this may suggest a protective effect, it is more likely that these results were due to the relative frequencies as the −3A/1249G, −3A/1249T, −3G/1249T haplotypes were all rare in both samples (all <0.06).

We also conducted a meta-analysis of the two significant *DYX1C1* SNPs reported by Taipale et al. [2003]. Similar to other meta-analyses of genetic association studies, the initial study showed strong association, while subsequent studies tended to find marginal to no significance [Ioannidis et al., 2001]. With 786 cases, 756 controls, and 940 family trios, the meta-analysis did not find significant association between −3G/A or 1249G/T and RD. Combining the cases and family trios, the sample had >80% power to detect an odds ratio as small as 1.3 for both the −3G/A and 1249G/T SNPs, if such an effect existed. The case–control and TDT modules calculated virtually the same power estimate when the cases and the family-trios were equal. However, the between-study heterogeneity likely reduced the true statistical power.

The level of between-study heterogeneity was moderate to high for both SNPs, which may reflect differences in study design, phenotype definition, quality control methods for genotyping, and population stratification. Most studies defined RD as having significantly poor reading skills with normal IQ except for the Calgary sample which did not use IQ to define RD. However, there were differences in the tests administered and the cut-offs used to determine diagnosis. For example, in the studies that used the WISC-III, the cutoff for normal IQ ranged from 80 to 90. Some studies indicated that they excluded neurological, medical or psychiatric disorders. The sources that were used to recruit subjects differed between studies. In addition, among the case–control studies, different types of controls were used including unselected, unmatched, and matched individuals. These differences in the cases and controls may have affected the results of the individual studies, and contributed to the observed between-study heterogeneity.

The quality control measures for the included studies were largely unknown which could create a potential for bias. Misclassification of both genotypes and phenotypes may have affected the results, but the approximate effect is difficult to predict. Population stratification was less likely because the cases and controls were of the same ethnicity, and family-based designs are robust to such problems. Narrowing the studies to only white Caucasian subjects and narrowing the RD phenotype in the subgroup analyses did not yield a significantly different result.

Apart from the Toronto and Calgary samples, we included only published studies, and there was a possibility that unpublished data were not considered in this meta-analysis. If there were unpublished results, however, these would have likely contributed a negative result due to publication biases [Thornton and Lee, 2000]. Of the studies that met inclusionary criteria, one independent study was excluded from the analysis because the specific data were unavailable. This study did not find an association between the two SNPs and RD, and would have also likely contributed a negative result.

Applying the Venice criteria, the strength of the cumulative evidence for an association between −3G/A or 1249G/T and RD was “weak.” With a risk allele count of 207 for −3G/A and 306 for 1249G/T, the sample size was considered to be of moderate size (“B” rating). However, there were considerable differences in RD phenotype definition, study populations, and study findings, and a null overall effect was found in the meta-analysis (“C” rating). There was also a potential for bias which could have affected the results (“B” rating).

Overall, we did not find strong evidence for an association between *DYX1C1* SNPs and RD. In the Toronto sample, previous associations were no longer significant when a larger sample was tested, and an analysis of an independent sample from Calgary did not yield any significant results. The meta-analysis did not find an association between −3G/A or 1249G/T and RD, and there was weak credibility of evidence for both SNPs due to the high between-study heterogeneity. These results also highlight the importance of assessing the associations of other RD candidate genes, as they are also likely to face similar issues of between-study heterogeneity and replication. Thus, to differentiate true associations from

spurious findings, it may be useful to analyze the body of literature through systematic reviews and meta-analyses.

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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]

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]

Bates TC, Lind PA, Luciano M, Montgomery GW, Martin NG, Wright MJ. Dyslexia and DYX1C1: Deficits in reading and spelling associated with a missense mutation. Mol Psychiatry. 2010; 15(12): 1190–1196. [PubMed: 19901951]

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(3):311–314. [PubMed: 16280601]

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]

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 Part B. 2007; 144B(4):556–560. [PubMed: 17450541]

Bruck M. Persistence of dyslexics' phonological awareness deficits. Dev Psychol. 1992; 28:874–886.

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 Part B. 2004; 131B(1):67–75. [PubMed: 15389770]

Clayton D, Jones H. Transmission/disequilibrium tests for extended marker haplotypes. Am J Hum Genet. 1999; 65(4):1161–1169. [PubMed: 10486335]

Cohn LD, Becker BJ. How meta-analysis increases statistical power. Psychol Methods. 2003; 8(3): 243–253. [PubMed: 14596489]

Conners, CK. Conners' rating scales—Revised. Toronto, Canada: Multi-Health Systems Inc; 1997.

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. 2005; 10(3):237–238. [PubMed: 15477871]

Couto JM, Livne-Bar I, Huang K, Xu Z, Cate-Carter T, Feng Y, Wigg K, Humphries T, Tannock R, Kerr EN, Lovett MW, Bremner R, Barr CL. Association of reading disabilities with regions marked by acetylated H3 histones in KIAA0319. Am J Med Genet Part B. 2010; 153B(2):447–462. [PubMed: 19588467]

Dahdouh F, Anthoni H, Tapia-Paez I, Peyrard-Janvid M, Schulte-Korne G, Warnke A, Remschmidt H, Ziegler A, Kere J, Muller-Myhsok B, Nothen MM, Schumacher J, Zucchelli M. Further evidence for DYX1C1 as a susceptibility factor for dyslexia. Psychiatr Genet. 2009; 19(2):59–63. [PubMed: 19240663]

DeFries JC, Fulker DW, LaBuda MC. Evidence for a genetic aetiology in reading disability of twins. Nature. 1987; 329(6139):537–539. [PubMed: 3657975]

Denckla MB, Rudel RG. Rapid "automatized" naming (R.A.N): Dyslexia differentiated from other learning disabilities. Neuropsychologia. 1976; 14(4):471–479. [PubMed: 995240]

DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7(3):177–188. [PubMed: 3802833]

Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997; 315(7109):629–634. [PubMed: 9310563]

Elbert A, Lovett MW, Cate-Carter T, Pitch A, Kerr EN, Barr CL. Genetic variation in the KIAA0319 5′ region as a possible contributor to dyslexia. Behav Genet. 2011; 41(1):77–89. [PubMed: 21207242]

Field LL, Kaplan BJ. Absence of linkage of phonological coding dyslexia to chromosome 6p23–p21.3 in a large family data set. Am J Hum Genet. 1998; 63(5):1448–1456. [PubMed: 9792873]

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, DeFries JC. Developmental dyslexia: Genetic dissection of a complex cognitive trait. Nat Rev Neurosci. 2002; 3(10):767–780. [PubMed: 12360321]

Francks C, MacPhie IL, Monaco AP. The genetic basis of dyslexia. Lancet Neurol. 2002; 1(8):483–490. [PubMed: 12849333]

Galaburda AM, Sherman GF, Rosen GD, Aboitiz F, Geschwind N. Developmental dyslexia: Four consecutive patients with cortical anomalies. Ann Neurol. 1985; 18(2):222–233. [PubMed: 4037763]

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]

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(1):27–39. [PubMed: 8981944]

Habib M. The neurological basis of developmental dyslexia: An overview and working hypothesis. Brain. 2000; 123(Pt12):2373–2399. [PubMed: 11099442]

Hallgren B. Specific dyslexia (congenital word-blindness); a clinical and genetic study. Acta Psychiatr Neurol Suppl. 1950; 65:1–287. [PubMed: 14846691]

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]

Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21(11): 1539–1558. [PubMed: 12111919]

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]

Humphreys P, Kaufmann WE, Galaburda AM. Developmental dyslexia in women: Neuropathological findings in three patients. Ann Neurol. 1990; 28(6):727–738. [PubMed: 2285260]

Ioannidis JP, Boffetta P, Little J, O'Brien TR, Uitterlinden AG, Vineis P, Balding DJ, Chokkalingam A, Dolan SM, Flanders WD, Higgins JP, McCarthy MI, McDermott DH, Page GP, Rebbeck TR, Seminara D, Khoury MJ. Assessment of cumulative evidence on genetic associations: Interim guidelines. Int J Epidemiol. 2008; 37(1):120–132. [PubMed: 17898028]

Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nat Genet. 2001; 29(3):306–309. [PubMed: 11600885]

Katusic SK, Colligan RC, Barbaresi WJ, Schaid DJ, Jacobsen SJ. Incidence of reading disability in a population-based birth cohort, 1976–1982, Rochester, Minn. Mayo Clin Proc. 2001; 76(11):1081–1092. [PubMed: 11702896]

Kavvoura FKIJP. Methods for meta-analysis in genetic association studies: A review of their potential and pitfalls. Hum Genet. 2008; 123(1):1–14. [PubMed: 18026754]

Kazeem GR, Farrall M. Integrating case–control and TDT studies. Ann Hum Genet. 2005; 69(Pt3): 329–335. [PubMed: 15845037]

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]

Marino C, Citterio A, Giorda R, Facoetti A, Menozzi G, Vanzin L, Lorusso ML, Nobile M, Molteni M. Association of short-term memory with a variant within DYX1C1 in developmental dyslexia. Genes Brain Behav. 2007; 6(7):640–646. [PubMed: 17309662]

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(4):491–499. [PubMed: 15702132]

McGinnis R, Shifman S, Darvasi A. Power and efficiency of the TDT and case–control design for association scans. Behav Genet. 2002; 32(2):135–144. [PubMed: 12036111]

Meaburn EL, Harlaar N, Craig IW, Schalkwyk LC, Plomin R. Quantitative trait locus association scan of early reading disability and ability using pooled DNA and 100K SNP microarrays in a sample of 5760 children. Mol Psychiatry. 2008; 13(7):729–740. [PubMed: 17684495]

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. 2005; 118(1):87–90. [PubMed: 16133186]

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, 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(5):843–848. [PubMed: 10749993]

Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, Richardson AJ, Walter J, Stein JF, Talcott JB, Monaco AP. Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. Behav Genet. 2011; 41(1):90–104. [PubMed: 21165691]

Nicodemus KK. Catmap: Case–control and TDT meta-analysis package. BMC Bioinformatics. 2008; 9:130. [PubMed: 18307795]

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004; 74(4):765–769. [PubMed: 14997420]

Paracchini S, Ang QW, Stanley FJ, Monaco AP, Pennell CE, Whitehouse AJ. Analysis of dyslexia candidate genes in the Raine cohort representing the general Australian population. Genes Brain Behav. 2011; 10(2):158–165. [PubMed: 20846247]

Paulesu E, Demonet JF, Fazio F, McCrory E, Chanoine V, Brunswick N, Cappa SF, Cossu G, Habib M, Frith CD, Frith U. Dyslexia: Cultural diversity and biological unity. Science. 2001; 291(5511): 2165–2167. [PubMed: 11251124]

Pennington BF, Gilger JW, Pauls D, Smith SA, Smith SD, DeFries JC. Evidence for major gene transmission of developmental dyslexia. JAMA. 1991; 266(11):1527–1534. [PubMed: 1880884]

Petryshen TL, Kaplan BJ, Liu MF, Schmill de French N, Tobias R, Hughes ML, Field LL. Evidence for a susceptibility locus on chromosome 6q influencing phonological coding dyslexia. Am J Med Genet. 2001; 105:507–517. [PubMed: 11496366]

Purcell S, Cherny SS, Sham PC. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 2003; 19(1):149–150. [PubMed: 12499305]

Ramus F, Rosen S, Dakin SC, Day BL, Castellote JM, White S, Frith U. Theories of developmental dyslexia: Insights from a multiple case study of dyslexic adults. Brain. 2003; 126(Pt4):841–865. [PubMed: 12615643]

Rosen GD, Bai J, Wang Y, Fiondella CG, Threlkeld SW, Loturco JJ, Galaburda AM. Disruption of Neuronal Migration by RNAi of Dyx1c1 Results in Neocortical and Hippocampal Malformations. Cereb Cortex. 2007; 17(11):2562–2572. [PubMed: 17218481]

Saviour P, Kumar S, Kiran U, Ravuri RR, Rao VR, Ramachandra NB. Allelic variants of DYX1C1 are not associated with dyslexia in India. Indian J Hum Genet. 2008; 14(3):99–102. [PubMed: 20300304]

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(11):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(1):279–282. [PubMed: 9634517]

Schumacher J, Konig IR, Schroder T, Duell M, Plume E, Propping P, Warnke A, Libertus C, Ziegler A, Muller-Myhsok B, Schulte-Korne G, Nothen MM. Further evidence for a susceptibility locus contributing to reading disability on chromosome 15q15–q21. Psychiatr Genet. 2008; 18(3):137–142. [PubMed: 18496212]

Semel, E., Wing, E., Secord, W. Clinical evaluation of language fundamentals—Third edition (CELF-3). San Antonio, TX: The Psychological Corporation; 1995.

Shaywitz SE. Dyslexia. N Engl J Med. 1998; 338(5):307–312. [PubMed: 9445412]

Shaywitz SE, Fletcher JM, Holahan JM, Shneider AE, Marchione KE, Stuebing KK, Francis DJ, Pugh KR, Shaywitz BA. Persistence of dyslexia: The Connecticut Longitudinal Study at adolescence. Pediatrics. 1999; 104(6):1351–1359. [PubMed: 10585988]

Shaywitz SE, Shaywitz BA, Fletcher JM, Escobar MD. Prevalence of reading disability in boys and girls. Results of the Connecticut Longitudinal Study. JAMA. 1990; 264(8):998–1002. [PubMed: 2376893]

Szalkowski CE, Hinman JR, Threlkeld SW, Wang Y, LePack A, Rosen GD, Chrobak JJ, LoTurco JJ, Fitch RH. Persistent spatial working memory deficits in rats following in utero RNAi of Dyx1c1. Genes Brain Behav. 2011; 10(2):244–252. [PubMed: 20977651]

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(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

Tapia-Paez I, Tammimies K, Massinen S, Roy AL, Kere J. The complex of TFII-I, PARP1, and SFPQ proteins regulates the DYX1C1 gene implicated in neuronal migration and dyslexia. FASEB J. 2008; 22(8):3001–3009. [PubMed: 18445785]

Thornton A, Lee P. Publication bias in meta-analysis: Its causes and consequences. J Clin Epidemiol. 2000; 53(2):207–216. [PubMed: 10729693]

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(5):508–514. [PubMed: 17259020]

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 Part B. 2004; 127B(1): 117–124. [PubMed: 15108193]

Venkatesh SK, Siddaiah A, Padakannaya P, Ramachandra NB. An examination of candidate gene SNPs for dyslexia in an Indian sample. Behav Genet. 2011; 41(1):105–109. [PubMed: 21203818]

Volger GP, DeFries JC, Decker SN. Family history as an indicator of risk for reading disability. J Learn Disabil. 1984; 17(10):616–618. [PubMed: 6512408]

Wadsworth SJ, Olson RK, Pennington BF, DeFries JC. Differential genetic etiology of reading disability as a function of IQ. J Learn Disabil. 2000; 33(2):192–199. [PubMed: 15505948]

Wagner, R., Torgesen, J., Rashotte, C. Comprehensive test of phonological processing (CTOPP). Austin, TX: Pro-Ed; 1999.

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 neocortex. Neuroscience. 2006; 143(2):515–522. [PubMed: 16989952]

Wechsler, D. Manual for the Wechsler intelligence scale for children-Revised. New York: Psychological Corporation; 1974.

Wechsler, D. Wechsler intelligence scale for children. 3. San Antonio, TX: Harcourt Brace & Co; 1991.

Wechsler, D. Wechsler intelligence scale for children. 4. San Antonio, TX: Psychological Corporation; 2003.

Wechsler, DI. Examiner's manual: Wechsler adult intelligence scale—Revised. New York: Psychological Corporation; 1981.

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, Tannock R, Lovett MW, Humphries TW, Barr CL. Support for EKN1 as the susceptibility locus for dyslexia on 15q21. Mol Psychiatry. 2004; 9(12):1111–1121. [PubMed: 15249932]

Wilkinson, GS. Wide range achievement test 3—Revision 3. Wilmington, DE: Jastak Associates; 1993.

Woodcock, RW. Woodcock reading mastery tests—Revised. Circle Pines, MN: American Guidance Service Inc; 1987.

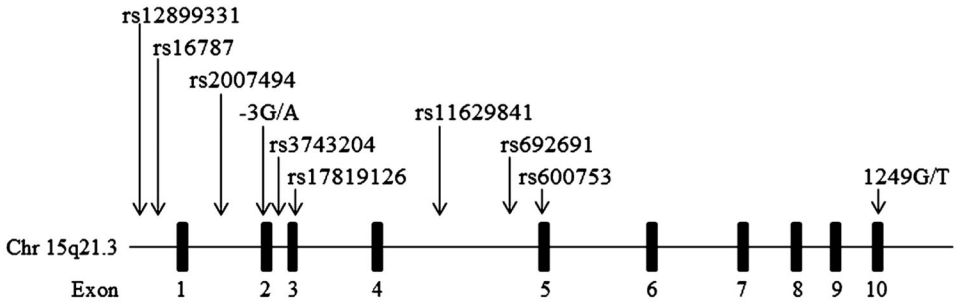

FIG. 1.
Relative positions of the genotyped DYX1C1 markers.

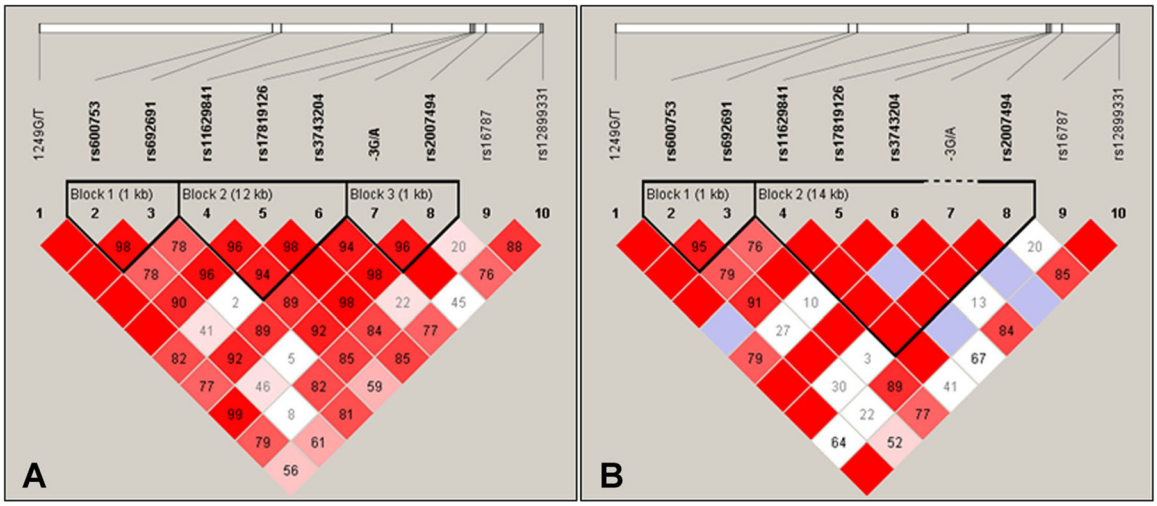

FIG. 2.

Inter-marker LD across the genotyped DYX1C1 SNPs in the Toronto (A) and Calgary (B) samples. The red unnumbered boxes represent inter-marker D' = 1 and are indicative of high LD. The LD blocks in each sample are outlined in bold.

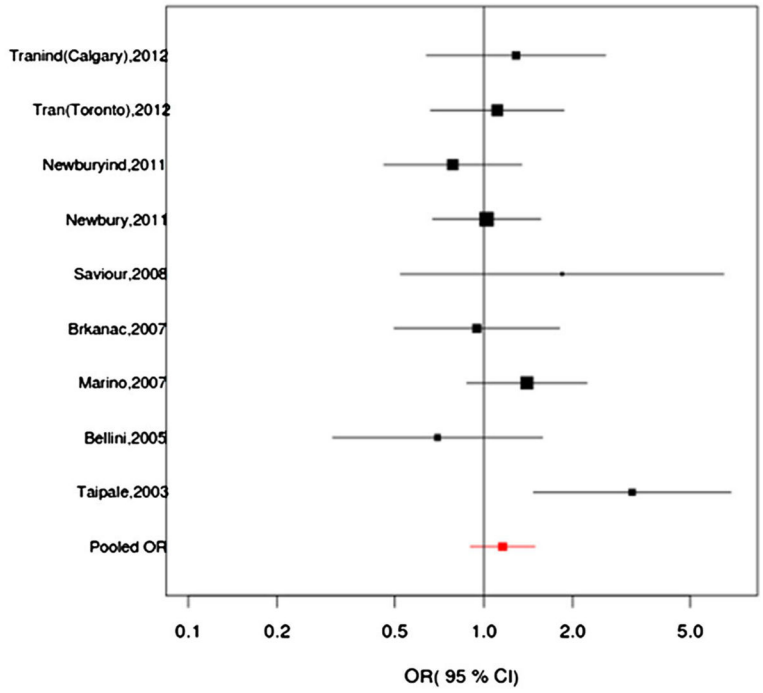

FIG. 3.

−3A random-effects model meta-analysis. The relative weights of the samples are represented by the boxes, and the 95% confidence intervals are represented by the lines.

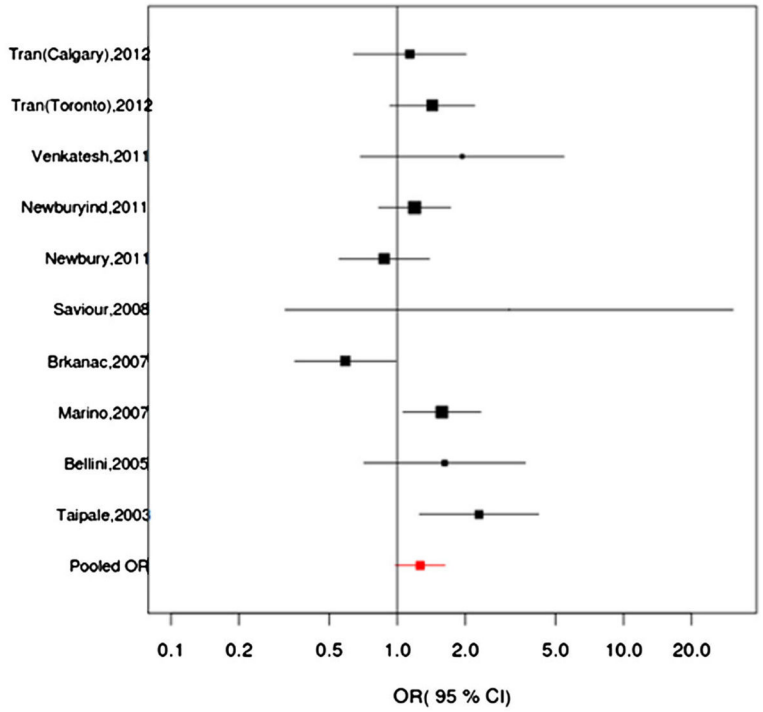

FIG. 4.

1249T random-effects model meta-analysis. The relative weights of the samples are represented by the boxes, and the 95% confidence intervals are represented by the lines.

TABLE I

Single SNP Analysis for the Toronto and Calgary Samples

Marker	Transmission ratio (allele)[a]	Minor allele frequency (allele)	χ^2	*P*-value[b]
Toronto sample				
rs12899331	86(A):76(G)	0.229 (G)	0.617	0.432
rs16787	62(A):55(C)	0.164 (C)	0.419	0.518
rs2007494	90(A):79(T)	0.247 (A)	0.716	0.398
−3G/A	32(A):30(G)	0.070 (A)	0.158	0.691
rs3743204	90(T):82(G)	0.235 (T)	0.372	0.542
rs17819126	33(T):23(C)	0.067 (T)	1.786	0.181
rs11629841	108(G):94(T)	0.344 (G)	0.970	0.325
rs692691	131(T):102(C)	0.404 (T)	3.609	0.058
rs600753	119(T):97(C)	0.494 (C)	2.241	0.134
1249G/T	50(T):35(G)	0.093 (T)	2.722	0.099
Calgary sample				
rs12899331	80(A):64(G)	0.261 (G)	1.778	0.182
rs16787	45(C):39(A)	0.174 (C)	0.429	0.513
rs2007494	59(A):53(T)	0.203 (A)	0.321	0.571
−3G/A	18(G):14(A)	0.049 (A)	0.500	0.480
rs3743204	66(T):62(G)	0.203 (T)	0.125	0.724
rs17819126	26(T):23(C)	0.075 (T)	0.184	0.668
rs11629841	80(G):80(T)	0.337 (G)	0.000	1.000
rs692691	83(T):68(C)	0.388 (T)	1.490	0.222
rs600753	75(T):65(C)	0.488 (C)	0.714	0.398
1249G/T	25(T):22(G)	0.076 (T)	0.191	0.662

[a] Alleles refer to the plus-strand, except for the −3G/A and 1249G/T polymorphisms which refer to the transcript.

[b] Threshold for significance set at 0.006.

TABLE II

Haplotype Analysis for the Toronto and Calgary Samples

Haplotype	Frequency	Observed	Expected	Var (O-E)	Chisq (1df)	*P*-value
Toronto sample[a]						
−3G 1249G	0.895	479.97	480.30	25.64	0.004	0.949
−3A 1249G	0.013	0.03	4.38	1.99	9.540	0.002
−3G 1249T	0.039	20.87	20.55	9.74	0.011	0.918
−3A 1249T	0.052	39.13	34.77	15.16	1.254	0.263
Calgary sample[b]						
−3G 1249G	0.923	376.12	375.58	14.21	0.021	0.884
−3A 1249G	0	—	—	—	—	—
−3G 1249T	0.028	17.88	13.65	6.13	2.910	0.088
−3A 1249T	0.049	14.00	18.77	9.11	2.500	0.114

[a] Global chisquared test, on three degrees of freedom = 10.907 ($P = 0.012$).

[b] Global chisquared test, on two degrees of freedom = 5.0643 ($P = 0.079$).