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DCDC2 Genetic Variants and Susceptibility to Developmental Dyslexia

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Abstract

Objective(s)—Developmental Dyslexia is a heritable condition, with genetic factors accounting for 44%–75% of the variance in performance tests of reading component subphenotypes. Compelling genetic linkage and association evidence supports a quantitative trait locus in the 6p21.3 region, which encodes a gene called DCDC2. In the present study, we explored the contribution of two DCDC2 markers to dyslexia, related reading and memory phenotypes in nuclear families of Italian origin.

Methods—303 nuclear families recruited on the basis of having a proband with Developmental Dyslexia have been studied with 6p21.3 markers, BV677278 and rs793862. Marker-trait association was investigated by the quantitative transmission disequilibrium test (QTDT, version 2.5.1) as modelled by Abecasis et al. (2000), which allows for the analyses of quantitative traits. Seven phenotypes were used in association analyses, i.e. word and non-word reading, word and non-word spelling, orthographic choice, memory and the affected status based on inclusion criteria.

Results—QTDT analyses yielded evidence for association between reading skills and the BV677278 deletion (empirical p-values= .025–.029) and between memory and BV677278 allele 10 (empirical p-value= .0001).

Conclusions—Our result adds further evidence in support of DCDC2 contributing to the deficits in Developmental Dyslexia. More specifically, our data support the view that DCDC2 influences both reading and memory impairments thus shedding further light into the etiologic basis and the phenotypic complexity of Developmental Dyslexia.

Keywords

DCDC2; Developmental Dyslexia; Transmission Disequilibrium Test; association study

Introduction

Developmental Dyslexia (DD) is one of the most common neurodevelopmental conditions with a prevalence ranging from 5% to 17.5% depending on the population and inclusion criteria (Shaywitz, 1998, Lindgren et al., 1985). DD is characterized by impaired reading despite normal intelligence and adequate sociocultural opportunities. Heritability ranges from 44% to 75% (Plomin and Kovas, 2005). Prompted by these data, there have been over

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a dozen genetic studies conditioned on categorical and various component subphenotypes of reading and spelling in DD since 1994.

The first evidence for a quantitative-trait locus (QTL) for DD in the 6p21.3 region was found in two independent U.S. samples (Cardon et al., 1994, 1995). Since then, some studies provided significant probabilities of linkage within the same region for a variety of DD-related phenotypes (Fisher et al., 1999; Grigorenko et al., 1997; 2000; 2003; Gayan et al., 1999) while others failed to replicate the original finding (Field and Kaplan, 1998; Petryshen et al., 2000). Successive association studies of DD (Kaplan et al., 2002; Turic et al., 2003; Deffenbacher et al., 2004; Meng et al., 2005; Francks et al., 2004; Cope et al., 2005; Platko et al., 2008) that employed progressively finer maps of the 6p21.3 region, identified multiple candidate DD susceptibility genes. Deffenbacher et al. (2004) integrated linkage and association strategies to detect significant association with five genes - VMP, DCDC2, KIAA0319, TTRAP, and THEM2 - in a selected subset of 114 families. Francks et al. (2004) found significant associations encompassing the first four exons of KIAA0319, the entire TTRAP gene, and the regulatory sequences of THEM2. In an association study of categorically-defined DD in 223 cases, 273 controls and 143 semi-independent triads, Cope et al. (2005) found strong association with markers from KIAA0319. In a study of 153 families with 147 markers distributed through the 1.2 million bases spanning VMP, DCDC2, KIAA0319, TTRAP, and THEM2, Meng et al. (2005) found strong association between markers in DCDC2 and a weighted composite of the reading recognition, comprehension, and spelling subtests of the PIAT (uncorrected p-value = 0.0003) and, by combining rare alleles - including a 2,445 base pair deletion - of a short tandem repeat (STR, BV677278) in intron 2, tested against homonym choice (uncorrected p-value = 0.00002). In 137 triads from Germany, Schumacher et al. (2006) found the strongest association between DD (defined as a spelling disorder) and two DCDC2 markers (uncorrected p-values = 0.011, 0.005) and confirmed with the most severe phenotype in an independent sample of 239 triads. In a case-control sample Platko et al. (2008) found a strong association (uncorrected p-value = 0.00001) between categorically-defined DD and marker D6S299, located ~200kb telomeric of DCDC2 and ~600kb of KIAA0319.

Both genetic linkage and association studies to date have been conditioned on a variety of DD phenotypes including categorical diagnosis based on cutoff values for performance in single word reading or spelling, quantitative scores of reading component subphenotypes of phonological and orthographic processing, assessments of reading deficit severity, and letter/number recall assessments of memory. Focusing on DCDC2, significant associations were found with orthographic, phonological, reading and spelling IQ-adjusted measures of DD in a family sample (highest uncorrected p-value= 0.005 with rs1087266) and in a selected subset (uncorrected p-values= 0.03–0.05 with the BV677278 deletion), whereas no associations were observed with the categorical diagnosis in a case-control sample (Harold et al., 2006). One study failed to detect biased transmission for any polymorphisms of DCDC2 in 191 U.S. families with DD; however for spelling or reading performance, individuals who carried one or two copies of the DCDC2 BV677278 intronic deletion were more severely affected than those who did not (uncorrected p-values= 0.035–0.046; Brkanac et al., 2007). Recently, Ludwig and colleagues (2008) investigated association between DD and DCDC2 intron 2 deletion/STR variants using both categorical and quantitative related traits, but found non-significant results. In contrast, Wilcke and colleagues (2009) found evidence for association in an independent sample of 72 dyslexics from Germany between the DCDC2 intron 2 deletion variant, and a dyseidetic diagnostic subtype (n= 34) which shows prominent problems with visual perception.

The bulk of results reviewed so far shows that DCDC2 likely harbors risk factors for DD and/or DD-related phenotypes. Despite the large amount of data, however, a direct

relationship between genes and measures of specific DD-related cognitive processes, has not emerged consistently across studies. Likewise, the key pathways that connect specific genetic variants to DD phenotypes remain obscure. Moreover, for many studies the sample sizes -especially for the selected samples- were small, so that a few extreme cases could have determined some of the results.

In the present study, we sought to resolve which aspects of the phenotypic profile of DD were unambiguously attributable to DCDC2 effects by exploring the contribution of two among the most replicated markers, i.e. rs793862 and BV677278, to DD and DD-related phenotypes by association analyses in a large sample of nuclear families of Italian origin.

Furthermore, given that memory is impaired in subjects with DD (Vellutino et al., 2004; Swanson, 2006), we hypothesized that DCDC2 might also be a candidate gene for short-term and working memory as a cognitive correlate of DD, although the meaning of the relationship with reading performance is still a subject of debate (Kercher and Sandoval 1991; Gathercole et al. 2006; Cohen-Mimran and Sapir 2007; Swanson and Jerman 2007; van Leeuwen et al., 2009).

Methods

Subjects

The nuclear families in this study have never been studied with 6p21.3 markers. They were recruited on the basis of having a proband with DD (sibling affected status was free to vary) as part of an ongoing project on the genetics of DD at Eugenio Medea Scientific Institute (Marino et al., 2004; 2005). DD was defined by the following criteria: 1) either accuracy or speed 2.0 SD at or below expected grade level on any text, word or non-word reading tests (Cornoldi and Colpo, 1986; Sartori et al., 1995), 2) total IQ above 85 (Wechsler, 1981), and 3) absence of neurological or sensorial disorders. Subjects were given a full description of the experimental procedures and then asked for written informed consent. DNA was extracted from blood or mouthwash samples as previously described (Marino et al., 2004). The protocol was approved by the Scientific Review Board and the Ethical Committee of the Eugenio Medea Scientific Institute.

Neuropsychological measures

A battery of psychometric tests was administered to probands and siblings and scores were standardized against a normative control data set (Sartori et al., 1995; Reynolds and Bigler, 1994). The following neuropsychological measures were used for genetic analyses. Word reading (WR) and non-word reading (NWR) were assessed by the single unrelated word and single unrelated non-word reading subtests from the Battery for the Assessment of Developmental Reading and Spelling Disorders (Sartori et al., 1995), whereby both the number of errors (accuracy) and the time required to complete the task (speed) were measured. Word spelling (SWS), non-word spelling (NWS) and orthographic coding (OC) were assessed by the number of errors in the untimed single unrelated word, single unrelated non-word and sentences containing homophones writing under dictation subtests from the Battery for the Assessment of Developmental Reading and Spelling Disorders (Sartori et al., 1995). Single letter forward span (SLFS) and single letter backward span (SLBS) were assessed with the Italian version of the Test of Memory and Learning (TOMAL) (Reynolds and Bigler, 1994). These letter span tasks require immediate recall, respectively forward and backward, of strings of letters that are read aloud by the operator and strings that are increasingly longer at each step; scores are computed based on the number of correct letters recalled in the correct order for each string.

Seven phenotypes were used in association analyses: WR, NWR, WS, NWS, OC and the affected status based on inclusion criteria. The final phenotype was a composite measure comprised of the SLBS and SLFS tasks. The z-scores from these two tasks were averaged to yield a memory composite score (MEMO). Accuracy and speed were averaged for both the WR and the NWR tasks.

Genotyping

The compound STR, BV677278, in intron 2 was genotyped by sequencing PCR products generated with forward primer (TGTTGAATCCCAGACCACAA) and reverse primer (ATCCCGATGAAATGAAAAGG). The sequencing method has been described elsewhere (Meng et al., 2005). Chromatograms were analyzed and alleles assigned with Mutation Surveyor version 3.1 (SoftGenetics, State College), by comparing samples to reference traces after alignment. The 2,445bp BV677278 deletion was genotyped by allele-specific PCR amplification with a combination of three primers in one reaction as previously described (Meng et al., 2005). Table 1 shows the distribution of DCDC2 BV677278 and rs793862 markers in the sample.

Statistical analysis

Marker-trait association was investigated by the quantitative transmission disequilibrium test (QTDT, version 2.5.1) as modelled by Abecasis and colleagues (2000), which allows for the analyses of quantitative traits. To avoid potential population stratification, we performed the within-family association test. We adopted the orthogonal model carried out in a variance component framework for our analyses. Single-marker TDT analyses were performed for DD as a discrete trait, and for WR, NWR, WS, NWS, OC and MEMO as quantitative traits with both BV677278 and rs793862 markers. For the multiallelic STR marker BV677278, only those alleles for which more than 30 informative nuclear families were available in our sample were tested, as suggested by Abecasis and colleagues (2000). Empirical p-values are reported for the QTDT tests and were computed from 10,000 Monte-Carlo permutations. Bonferroni correction for multiple testing would be too conservative for multiple correlated traits and non-independent genetic markers (Deffenbacher et al., 2004; Franck et al., 2004; Cope et al., 2005; Meng et al., 2005; Schumacher et al., 2006; Brkanac et al., 2007). To allow for comparison across studies, BV677278 alleles were also analyzed according to the Meng et al. (2005) approach, i.e. the deletion and alleles whose frequencies were below .07 were combined. Genotype error checking and Hardy–Weinberg equilibrium analyses were completed in PEDSTATS.

Results

The sample consisted of 303 unrelated nuclear families with 404 offspring; except for 37 families that had only one parent available, all parents were represented, yielding a total sample of 973 subjects, all of Italian ancestry. Complete neuropsychological measures were available for 297 offspring (210 probands and 87 siblings). The probands' mean age was 10.8 ± 2.6 years; 75.9% were males and the mean total IQ was 101.55 ± 11.94 .

Table 2 shows the descriptive statistics of the neuropsychological measures used in the genetic analyses. Ten families were excluded from the analyses due to mendelian errors. Genotype distribution in the parents did not significantly deviate from Hardy-Weinberg equilibrium.

No significant association was found between either BV677278 or rs793862 and DD as a discrete trait. Quantitative analyses yielded evidence for association between WR and the BV677278 deletion (informative families= 56; chi-square= 5.01; degrees of freedom= 279;

nominal p-value= .048; empirical p-value= .025; genetic effect= -1.316), and between NWR and the BV677278 deletion (informative families= 56; chi-square= 4.78; degrees of freedom= 279; nominal p-value= .042; empirical p-value= .029; genetic effect= -1.071). Evidence for association was also found between MEMO and BV677278 allele 10 (informative families= 38; chi-square= 14.97; degrees of freedom= 217; nominal p-value= .00001; empirical p-value= .0001; genetic effect= -1.219). No associations were evident between rs793862 and any measure. The combination of minor alleles and the deletion, as proposed by Meng et al. (2005) yielded a trend toward significance for the discrete trait (informative families= 126; T= -.5; degrees of freedom= 126; empirical p-value= .063) and a strong association with MEMO (informative families= 110; chi-square= 10.92; degrees of freedom= 217; nominal p-value= .00001; empirical p-value= .0003; genetic effect= -.48).

Discussion

We tested for association between two markers within DCDC2 and reading, spelling and memory measures in a sample of DD nuclear families of Italian ancestry. QTD T analyses yielded significant associations between measures of letter recall (MEMO) and phonological skills (WR, NWR), and DCDC2 variation at the BV677278 polymorphic site. The inheritance of risk alleles worsens the offspring's performance by 1.219 SD in memory tasks and by 1.316 SD and 1.071 SD in, respectively, word and non-word reading tasks. As such, our result adds further evidence in support of DCDC2 contributing to the deficits in DD. More specifically, our data support the view that DCDC2 influences interindividual variation in multiple DD-related phenotypes thus shedding further light into the etiologic basis and the phenotypic complexity of DD.

Most marker-trait association studies of DD have focused on phonological processing as a core deficit which has been typically probed by word recognition, phoneme awareness, phonological decoding and spelling tasks. Some of these phenotypes were among those found to be associated with DCDC2 in this study as well as in previous positive association studies of DD (Deffenbacher et al., 2004; Meng et al., 2005; Harold et al., 2006) providing further support that DCDC2 influences phonological components of the reading process. Furthermore, we showed that co-existing deficits in DD, specifically memory impairments, are explained by the same genetic risk, adding evidence for a genetic pleiotropic hypothesis for DD related phenotypes. Therefore, in genetic studies of DD, placing emphasis on the decomposition of the phenotypic profile into different components and conditioning association on additional traits, such as memory, may ultimately result in a more powerful strategy for gene finding.

To assess memory we used forward and backward span tasks which are tests of short-term memory (STM). Backward span tasks also capture some working memory (WM) because they have a processing request, although a simple transposition of order is insufficient to totally move these tasks to the WM category. Indeed, processes related to STM and WM have been one of the most investigated issues in children with DD (Swanson et al., 2004). Memory impairments have been consistently found in DD – with moderate to high effect sizes for verbal STM (-.39 to -1.10) and verbal WM (-.37 to -.84). These impairments persist lifelong suggesting a deficit rather than a developmental delay model (Swanson et al., 2009). Genetic studies have shown that variation in reading performance is explained by specific genes as well as by a set of genes in common with STM and WM; STM and WM factors together contribute 29% of the genetic variability in reading performance (van Leeuwen et al., 2009). Therefore, a common genetic factor could account for storage and manipulation of phonological relevant information, which are important aspects of STM and WM as well as fundamental steps in the reading acquisition process, and consistent with the

results of this study showing both memory and phonological deficits associated with DCDC2.

It is noteworthy that association between DD genes and memory have so far been found solely in Italian and German samples (Marino et al., 2007; Ludwig et al., 2009; Dahdouh et al., 2009), which share transparent orthography-based languages, characterized by unambiguous phoneme-grapheme correspondence. Given the evidence that phonological skills are largely connected to the language structure - that is, the more regular the orthography the less phonological effort that is required in learning to read - we might expect that pure phonological genetically-driven impairments would find a more favorable environmental niche in regular rather than irregular languages. Therefore, marker-trait associations may vary owing to factors that are connected not only to the underlying genetic effect, but also to the linguistic environment, which may cause stratification of the genetic risk across language groups. In such a context, genetic susceptibility in transparent orthography-based language groups is more likely to be driven by additional deficits besides the phonological ones, and memory impairment arises as an additional, perhaps more sensitive, phenotype to be used in association studies.

We did not find significant support for association of reading, spelling or memory phenotypes with rs793862, one of the two most replicated polymorphisms in DCDC2/DD association studies. DD is a heterogeneous disorder and, although this marker has been replicated three times (Schumacher et al., 2006; Harold et al., 2006; Wilcke et al., 2009), negative associations with DD have also been reported (Cope et al., 2005; Brkanac et al., 2007).

It has been suggested that BV677278 has an effect on gene expression regulation (Meng et al., 2005; Meng et al., 2011). Although it is tempting to hypothesize that the associations we found are based on functional mechanisms, BV677278 associated alleles could rather be in linkage disequilibrium with other causative variants. Since the coverage of the DCDC2 region by the markers employed in this study is limited, caution should be applied in addressing the functional mechanism underlying this association.

In summary, DCDC2 has an effect on both phonological and memory components of reading, with effect size influenced by the linguistic environment in which they are assessed. In light of these new data further work is needed to explain differences in phenotypes, allelic variants, and levels of significance across studies that have showed a positive association between DCDC2 and DD. Sample ascertainment, selection and size, high correlation between statistical tests, and genotype/phenotype characterization of samples have been managed differently by different groups. These methodological differences hamper a unitary interpretation of the finding, and resolution of these discrepancies is a significant challenge facing DD researchers. Nevertheless, the reported interaction with KIAA0319 along with a functional role in neuronal migration as well as in cortical and hippocampal layer formation (Meng et al., 2005; Burbidge et al., 2008) suggest there are key DCDC2 molecular networks yet to be discovered. We still need to learn much more about the biology of DCDC2 before we can begin to turn these data into a complete understanding of the complex pathways between lexinome genes, language, reading and fluency.

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Table 1Frequencies of the *DCDC2* markers, BV677278 and rs793862 variants in our sample

Marker/allele	Frequency among probands	Frequency among parents
BV677278		
1	.597	.609
2	-	-
3	.081	.070
4	.086	.098
5	.045	.037
6	.062	.065
7	-	-
8	-	-
9	.002	.002
10	.049	.038
15	.002	.004
17	.002	.002
18	.007	.005
19	.005	.005
20	-	.000
Deletion	.063	.067
rs793862		
minor allele	.291	.297

Table 2
Descriptive statistics of neuropsychological measures in the sample with complete phenotypic and genetic information (n= 297)

TRAIT	Probands n=210					Siblings n=87				
	Min	Max	Mean (SD)	Skewness (SD)	Kurtosis (SD)	Min	Max	Mean (SD)	Skewness (SD)	Kurtosis (SD)
WR Acc	-14.78	.79	-2.74 (2.43)	-1.70 (.17)	4.49 (.34)	-9.33	.85	-1.05 (2.04)	-2.16 (.26)	5.01 (.52)
WR Speed	-17.03	1.28	-3.43 (2.77)	-1.41 (.17)	3.95 (.34)	-22.31	1.17	-1.47 (3.24)	-3.89 (.26)	20.81 (.52)
NWR Acc	-18.11	1.26	-2.25 (2.24)	-2.25 (.17)	12.40 (.34)	-8.10	1.26	-.45 (1.69)	-2.01 (.26)	4.98 (.52)
NWR Speed	-19.00	1.33	-2.93 (2.65)	-1.69 (.17)	6.61 (.34)	-14.64	1.74	-1.44 (2.55)	-2.58 (.26)	9.04 (.52)
WS	-26.23	.93	-3.00 (4.29)	-2.98 (.18)	11.43 (.36)	-17.64	.93	-.87 (2.84)	-3.92 (.27)	18.37 (.53)
NWS	-6.31	1.83	-.55 (1.57)	-1.51 (.18)	2.42 (.36)	-4.46	1.83	.09 (1.09)	-1.94 (.27)	5.63 (.53)
OC	-19.30	3.25	-4.39 (4.09)	-1.24 (.19)	1.48 (.37)	-18.08	.75	-2.27 (3.31)	-2.19 (.26)	6.31 (.52)
SLBS	-2.67	2.67	-.63 (.76)	.67 (.21)	2.96 (.41)	-3.33	1.67	-.57 (.99)	-.77 (.27)	1.95 (.54)
SLFS	-2.33	1.00	-1.08 (.92)	.21 (.20)	-1.02 (.40)	-3.33	1.00	-.86 (.95)	-.67 (.27)	.89 (.54)

WR= word reading; NWR= non-word reading; WS= word spelling; NWS= non-word spelling; OC= orthographic choice; SLBS= single letter backward span; SLFS= single letter forward span; Acc= accuracy