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Investigation of the *DCDC2* intron 2 deletion/compound short tandem repeat polymorphism in a large German dyslexia sample

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Abstract

Dyslexia is a complex disorder manifested by difficulties in learning to read and spell despite conventional instruction, adequate intelligence and sociocultural opportunity. It is among the most common neurodevelopmental disorders with a prevalence of 5–12%. The dyslexia susceptibility locus 2 on chromosome 6p21–p22 is one of the best-replicated linkage regions in dyslexia. On the basis of systematic linkage disequilibrium studies, the doublecortin domain containing protein 2 gene (*DCDC2*) was identified as a strong candidate gene in this region. Data from a US study have suggested a complex deletion/compound short tandem repeat (STR) polymorphism in intron 2 of *DCDC2* as the causative mutation. In this study, we analyzed this polymorphism in 396 German dyslexia trios which included 376 trios previously providing strong support for the *DCDC2* locus. We observed no significant deviation from random transmission, neither for the deletion nor for the alleles of the compound STR. We also did not find the deletion or any of the STR alleles to

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be in linkage disequilibrium with the 2-marker haplotype, which was associated with dyslexia in our sample. We thus conclude that the causative variant/s in *DCDC2* conferring susceptibility to dyslexia in our sample remain/s to be identified.

Keywords

doublecortin domain; dyslexia susceptibility 2; genetic association; reading; spelling

Introduction

Dyslexia is among the most common complex neurodevelopmental disorders with a prevalence of 5–12%, depending on the applied diagnostic criteria (Shaywitz et al., 1990; Katusic et al., 2001). It is manifested by difficulties in learning to read and spell despite conventional instruction, adequate intelligence and sociocultural opportunity. From the nine reported susceptibility loci, the dyslexia susceptibility locus 2 (*DYX2*)on chromosome 6p21–p22 is one of the best replicated linkage regions in dyslexia (Schumacher et al., 2007).

On the basis of systematic linkage disequilibrium studies at *DYX2*, association with two gene clusters, *VMP/DCDC2/KAAG1* and *KIAA0319/TTRAP/THEM2*, was reported (Deffenbacher et al., 2004; Francks et al., 2004; Cope et al., 2005; Meng et al., 2005; Schumacher et al., 2006). In the *VMP/DCDC2/KAAG1* gene cluster, the most convincing association was found for the doublecortin domain containing protein 2 gene (*DCDC2*, MIM 605755) in two independent studies from the US and Germany (Meng et al., 2005; Schumacher et al., 2006). *DCDC2*, typified by two doublecortin peptide domains spanning exons 1–2 and 3–5, is involved in processes of cortical neuronal migration during brain development (Meng et al., 2005).

In an attempt to identify the causative mutation at *DCDC2*, Meng et al. reported a deletion region of 2445bp in intron 2 of *DCDC2* that harbours a compound short tandem repeat (STR) polymorphism (GenBank accession no. BV677278). The compound STR polymorphism is composed of variable copy numbers of $(GAGAGGAAGG\ AAA)_n$ and $(GGAA)_n$ repeat units (Table 1). Transmission disequilibrium tests performed in 153 US dyslexia families yielded significant association (P=0.00002) with quantitative reading performance when combining the deletion and minor alleles of the compound STR marker (minor allele frequency of less than 5%). The fact that the associated STR marker contains putative brain active transcription binding sites supports the possible functional relevance of this complex polymorphism.

As we also have earlier reported evidence for association of dyslexia with *DCDC2* we wanted to investigate whether the intron 2 deletion/compound STR polymorphism would explain our association finding. If this would prove to be the case, the evidence supporting the identification of the first common causative mutation for dyslexia would be strong.

Participants and methods

A total of 396 German trios were included in the analysis. All probands were clinically diagnosed as dyslexic and were selected according to a discrepancy criterion between IQ and spelling. As earlier described (Schulte-Körne et al., 1996) probands had to have an IQ>85, and had to have a discrepancy of at least 1 SD between the observed and expected spelling score predicted by IQ. Families were recruited at the Departments of Child and Adolescent Psychiatry in Marburg and Würzburg. Written informed consent was obtained from all individuals or the parents of children aged younger than 14 years. Most of the families had already been included in our previous study in which *DCDC2* was identified as susceptibility gene for dyslexia (Schumacher et al., 2006). We assessed the deletion/STR alleles using PCR-based and sequencing methods as described in the study by Meng et al. (2005). Statistical analysis was performed using UNPHASED (MRC Biostatistics Unit, Cambridge, UK) 2.4.04 and 3.0.9.

Results and discussion

From the 1188 individuals 2374 alleles were used for statistical calculations. Here one individual failed genotyping, eight individuals were homozygous and 184 individuals were heterozygous for the deletion. In total we were able to identify nine alleles of the compound STR marker and the deletion. We did not find three of the US alleles in our German sample (2, 7, 8) but identified two additional ones (19, 20) that had not been found in the study by Meng et al. (2005). An overview of the different alleles of the STR marker and their frequencies is given in Table 1. Transmission disequilibrium test analysis yielded no significant association for the deletion itself or any of the STR alleles with dyslexia (Table 2). When we applied alternative phenotypic measures, such as phonological awareness and nonword reading, no significant association was obtained that withstood correction for multiple testing (data not shown). When combining the minor alleles (frequency <5%) and the deletion, as proposed by Meng et al. (2005), the results remained nonsignificant (data not shown). As expected by these results, we found that, although there is meaningful, albeit incomplete linkage disequilibrium between our risk haplotype and the deletion, the deletion resides in the great majority of individuals on different haplotypic backgrounds than our previously associated risk haplotype (rs793862–rs807701, A–C) (Schumacher et al., 2006). Given these results we conclude that the variant/s in *DCDC2*, conferring susceptibility to dyslexia in our German sample, remain/s to be identified. An explanation for the conflicting results might be phenotypic differences between ours and the US sample, because of differing ascertainment criteria. Whereas, the selection of probands in the US sample was based on reading performance, we applied a discrepancy criterion between IQ and spelling. The resulting differences in the phenotypic composition of the samples might relate to differences at the genetic level. The fact that we did not observe association with phenotypic measures associated with reading performance, however, is not in support of this hypothesis.

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Table 1

Distribution of the DCDC2 intron 2 deletion/compound STR polymorphism alleles

	Compound STR, accession no. BV677278						Allele frequency[a]	
Alleles	**Repeat unit 1**	**Repeat unit 2**	**SNP1**	**Repeat unit 3**	**Repeat unit 4**	**Repeat unit 5**	**Meng *et al.* *n* = 612 alleles**	**This study *n* = 1582 alleles**
1	(GAGAGGAAGGAAA)2	(GGAA)7		(GGAA)2	(GGAA)4	(GGGA)2	0.624	0.606
2	(GAGAGGAAGGAAA)1	(GGAA)9	DelGAAA	(GGAA)0	(GGAA)4	(GGGA)2	0.003	—
3	(GAGAGGAAGGAAA)1	(GGAA)6		(GGAA)2	(GGAA)4	(GGGA)2	0.060	0.055
4	(GAGAGGAAGGAAA)2	(GGAA)6		(GGAA)2	(GGAA)4	(GGGA)2	0.106	0.106
5	(GAGAGGAAGGAAA)2	(GGAA)8		(GGAA)2	(GGAA)4	(GGGA)2	0.028	0.043
6	(GAGAGGAAGGAAA)2	(GGAA)8		(GGAA)2	(GGAA)3	(GGGA)2	0.039	0.048
7	(GAGAGGAAGGAAA)2	(GGAA)8		(GGAA)1	(GGAA)4	(GGGA)2	0.003	—
8	(GAGAGGAAGGAAA)2	(GGAA)7	DelGAAA	(GGAA)0	(GGAA)4	(GGGA)2	0.003	—
9	(GAGAGGAAGGAAA)1	(GGAA)7		(GGAA)2	(GGAA)4	(GGGA)2	0.005	0.008
10	(GAGAGGAAGGAAA)2	(GGAA)4		(GGAA)2	(GGAA)4	(GGGA)2	0.044	0.043
19	(GAGAGGAAGGAAA)2	(GGAA)9		(GGAA)2	(GGAA)3	(GGGA)2	—	0.003
20	(GAGAGGAAGGAAA)2	(GGAA)9		(GGAA)2	(GGAA)4	(GGGA)2	—	0.004
Deletion	—	—	—	—	—	—	0.085	0.086

[a]Frequency among parents only.

SNP, single nucleotide polymorphism; STR, short tandem repeat.

Table 2

TDT analysis of 396 German trios

	TDT results		
Alleles	**Number transmitted**	**Number not transmitted**	***P* value**
1	150	175	0.165
3	37	31	0.467
4	72	61	0.340
5	32	26	0.430
6	31	28	0.696
9	3	7	0.200
10	32	24	0.284
19	3	1	0.306
20	2	4	0.410
Deletion	52	57	0.632

TDT, transmission disequilibrium test.