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Association of short-term memory with a variant within *DYX1C1* in developmental dyslexia

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A substantial genetic contribution in the etiology of developmental dyslexia (DD) has been well documented with independent groups reporting a susceptibility locus on chromosome 15q. After the identification of the *DYX1C1* gene as a potential candidate for DD, several independent association studies reported controversial results. We performed a family-based association study to determine whether the *DYX1C1* single nucleotide polymorphisms (SNPs) that have been associated with DD before, that is SNPs '−3GA' and '1249GT', influence a broader phenotypic definition of DD. A significant linkage disequilibrium was observed with 'Single Letter Backward Span' (SLBS) in both single-marker and haplotype analyses. These results provide further support to the association between DD and *DYX1C1* and it suggests that the linkage disequilibrium with *DYX1C1* is more saliently explained in Italian dyslexics by short-term memory, as measured by 'SLBS', than by the categorical diagnosis of DD or other related phenotypes.

Keywords: Developmental dyslexia, *DYX1C1*, Genetic association, Short-term memory

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Following earlier descriptions (Hallgren 1950) of familial aggregation, substantial heritability has been reported for developmental dyslexia (DD, Fisher & De Fries 2002), with data suggesting that at least some of the genes responsible for normal variation of reading ability are also responsible for reading disability (Plomin & Kovas 2005). Current molecular genetic evidence points to several linkage signals of moderate effect for DD: such signals have been replicated, for example those on chromosomes 1p (Grigorenko *et al.* 2001; Rabin *et al.* 1993; Tzenova *et al.* 2004), 2p (Fagerheim *et al.* 1999; Kaminen *et al.* 2003; Petryshen *et al.* 2002), 6p (Cardon *et al.* 1994, 1995; Fisher *et al.* 1999; Gayan *et al.* 1999; Grigorenko *et al.* 1997; Kaplan *et al.* 2002; Petryshen *et al.* 2001) and 15q (Chapman *et al.* 2004; Grigorenko *et al.* 1997; Schulte-Korne *et al.* 1998; Smith *et al.* 1983, 1991), while other positive studies, like those on chromosome 3p (Nopola-Hemmi *et al.* 2001), 3q (Fisher *et al.* 2002), 7q (Kaminen *et al.* 2003), 13q (Igo *et al.* 2006), 18p (Fisher *et al.* 2002) and Xq (De Kovel *et al.* 2004) have not been replicated. While genetic heterogeneity is – as for many complex disorders – highly probable for DD (Fisher & De Fries 2002), success in replication of genetic findings can crucially depend upon diagnosis, inclusion criteria and ascertainment methods, which may vary across different studies.

In the case of DD, some authors suggest that different writing systems (specifically, logographic vs. alphabetic) may lead to different neurofunctional organization patterns, as shown by neuroimaging data (Siok *et al.* 2004). Other studies, however, bring evidence in favour of unitary mechanisms despite orthographic heterogeneity, at least within languages of the alphabetic scripts (Marino *et al.* 2004; Paulesu *et al.* 2001).

Chromosome 15 has been claimed as potentially involved in the aetiology of DD since the early 1980s. The original positive linkage finding on chromosome 15 originated from a study of American families segregating DD (Smith *et al.* 1983) but in the following attempts to replicate their findings in larger samples the same authors found first weaker (Smith *et al.* 1986), and then negative LOD scores, and raised questions about the optimal criteria for linkage in presence of heterogeneity (Smith *et al.* 1990). Three independent groups successively failed to replicate the Smith *et al.* (1983) chromosome 15 linkage findings in different populations (Bisgaard *et al.* 1987; Cardon *et al.* 1994; Rabin *et al.* 1993).

The successive introduction of a 'cognitive dissection' of the dyslexic phenotype (Grigorenko *et al.* 1997) established a landmark towards reducing the quote of noise introduced by etiological heterogeneity. Linkage in this area was then replicated between a discrete component of the dyslexic phenotype, that is 'Single Word Reading' (SWR), and marker D15S143 at 45.6 cM (measures are given in Kosambi centimorgans according to the Marshfield human linkage map; http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp), a finding further supported by quantitative linkage analysis in an independent sample (Chapman *et al.* 2004). Several linkage scans of DD and reading-related traits followed but they failed to find a linkage peak in this candidate area, once again suggesting

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etiologic heterogeneity for this susceptibility locus (De Kovel *et al.* 2004; Fisher *et al.* 2002; Kaminen *et al.* 2003; Nopola-Hemmi *et al.* 2001; Raskind *et al.* 2005), while association studies were positive on chromosome 15q, with linkage disequilibrium reported for haplotypes D15S146/D15S214/D15S994 at 39.72–40.25 cM (Morris *et al.* 2000) and D15S214/D15S508/D15S182 at 40.25 cM (Marino *et al.* 2004).

To further complicate the picture, some studies lend support to the hypothesis that the susceptibility locus for DD on chromosome 15q has pleiotropic properties which would bridge DD to several developmental disorders, such as Attention Deficit Hyperactivity Disorder (ADHD) (Bakker *et al.* 2003; Loo *et al.* 2004), Speech Sound Disorder (SSD, Smith *et al.* 2005) and Spelling Disorder (Schulte-Korne *et al.* 1998), all conditions for which a shared genetic etiology with DD has been recognized (Plomin & Kovas 2005; Willcutt *et al.* 2000).

A groundbreaking advancement was introduced by the finding of translocations on 15q associated with DD in two families (Nopola-Hemmi *et al.* 2000). The breakpoint of the translocation t(2;15)(q11;q21) was localized to a region bound by D15S143 and D15S1029 (at 45.6 and 47.85 cM, respectively) and disrupted a gene, *DYX1C1*, whose rarer alleles for single nucleotide polymorphisms (SNPs) 1249GT and −3GA were soon thereafter found associated to DD (Taipale *et al.* 2003). A number of studies followed, all of which failed in finding an association with SNPs −3GA and 1249GT of *DYX1C1* (Bellini *et al.* 2005; Cope *et al.* 2005; Marino *et al.* 2005; Meng *et al.* 2005), while two found an over-transmission of the common alleles and haplotypes (Scerri *et al.* 2004; Wigg *et al.* 2004) and thus failed to replicate the Taipale *et al.* (2003) study in the classical sense.

Interestingly, Wigg *et al.* (2004) adopted broad selection criteria to define DD, in that probands were included if their full-scale IQ was above 80 and they performed below 1.5 standard deviation (SD) on the 'word attack'/'word identification'/'wide range achievement' tests, or the average of the three scored below 1 SD (Wigg *et al.* 2004).

The bulk of these data suggests that a susceptibility variant for DD on chromosome 15 is likely but because of substantial etiological heterogeneity only a small proportion of the dyslexic families may carry such risk allele.

Together with quantitative analyses of reading-related traits, the 'phenotype dissection' strategy can increase the power for detecting the linkage/association signals, and while the *DYX1C1*'s relationship with the susceptibility locus on chromosome 15 has not yet been figured out, *DYX1C1* remains a good candidate gene for DD when the phenotypic definition is slightly broadened.

In the present study, we wanted to test the association between the markers of *DYX1C1* (−3GA and 1249GT) found associated in the original Taipale *et al.* (2003) study with a) a diagnosis of DD more leniently defined than in our previous negative study (Marino *et al.* 2005); b) two measures of short-term memory (STM) that indeed play a relevant role in DD (Howes *et al.* 1999) and that, to a different extent, were found associated with *DYX1C1* (Wigg *et al.* 2004) and c) two ecological measures of Text Reading (TR).

Methods

Sample

This study is part of an ongoing project on the genetics of reading disabilities at the Department of Child Psychiatry and Rehabilitation Centre at the Eugenio Medea Institute, Bosisio Parini, Italy. Our standard ascertainment scheme has been reported in detail elsewhere (Marino *et al.* 2004, 2005). Briefly, the subjects are recruited if they have reading difficulties as referred by paediatricians or school teachers. After parental informed consent, the subjects undergo an extensive medical assessment and a battery of tests which include several reading tasks standardized on the Italian population (Cornoldi & Colpo 1986; Sartori *et al.* 1995) and the Wechsler Intelligence Scale for Children, Revised (Wechsler 1981). Blood or mouthwash samples were obtained from all probands and from one or both biological parent.

The inclusion criteria for the present study were 1) either accuracy or speed ≤ -1.0 SD on timed TR tests, timed SWR or timed 'Single Non-Word Reading' (SNWR), 2) a full-scale IQ above 80 and 3) absence of neurological or sensorial disorders.

Because the scope of the present investigation was a wider characterization of DD with phenotypes spanning a theoretical range of reading-related neuropsychological processes, subjects who met the selection criteria and had previously participated in our studies underwent new neuropsychological assessments (vide infra).

Parents were also asked to allow the participation of the probands' biological full-siblings if they were older than 6 and younger than 18 years, and if they had no history of neurological or sensorial disorders.

Sibs underwent the same neuropsychological assessments administered to probands, except for the Wechsler Intelligence Scale for Children, Revised (Wechsler 1981), of which only two subtests were administered (i.e. vocabulary and block design) that show a high correlation (r) with, respectively, Verbal IQ ($r = 0.82$) and Performance IQ ($r = 0.7$) (Wechsler 1981).

If the mean score of vocabulary and block design subtests was above 7 (i.e. −1 SD) – regardless of their reading performance – sibs were included in the study and their mouthwash was obtained to collect DNA.

Phenotype definitions

The phenotypes considered in this study were:

1 TR, assessing reading abilities for meaningful material. It was measured by the 'test for speed and accuracy in reading, developed by the MT group' (Cornoldi & Colpo 1986). Texts increase in complexity with grade level. Both the number of errors (accuracy) and the time required to complete the task (speed) are measured. Grade norms are provided for number of errors and speed (seconds per syllable).

2 SWR, measured by the Single Unrelated Word Reading Subtest of the 'Battery for the Assessment of Developmental Reading and Spelling Disorders' (Sartori *et al.* 1995). This test consists of four lists of 24 words. Both the number of errors (accuracy) and the time required to complete the task (speed) were measured. Grade norms are provided.

3 SNWR, defined as the ability to apply the correct grapheme/-phoneme correspondence rules to the pronunciation of non-words. It was measured by the 'Non-Word Reading Subtest' of the 'Battery for the Assessment of Developmental Reading and Spelling Disorders' (Sartori *et al.* 1995) which consists of three lists of 16 non-words. Both the number of errors (accuracy) and the time required to complete the task (speed) were measured. Grade norms are provided.

4 'Single Word Spelling' (SWS), defined as the ability to correctly write words under dictation. It was measured by the number of errors in the untimed 'Single Unrelated Word Writing Subtest' of the 'Battery for the Assessment of Developmental Reading and Spelling Disorders' (Sartori *et al.* 1995). Grade norms are provided.

5 'Single Non-Word Spelling' (SNWS), defined as the ability of writing pronounceable non-words measured by the number of errors in the untimed 'Non-Word Writing Subtest' of the 'Battery for the

Assessment of Developmental Reading and Spelling Disorders'(Sartori *et al.* 1995). Grade norms are provided.

6 'Orthographic Coding' (OC) defined as the ability to reproduce specific orthographic patterns. It was measured by recording the number of errors in writing under dictation 'Sentences Containing Homophones', of the 'Battery for the Assessment of Developmental Reading and Spelling Disorders' (Sartori *et al.* 1995). Grade norms are provided.

7 'Auditory STM' as measured by two tests: the 'Single Letter Forward Span' (SLFS) and the 'Single Letter Backward Span' (SLBS) of the Italian version of the 'Test of Memory and Learning' (Reynolds & Bigler 1994). These tasks require immediate recall, respectively, forward and backward, of strings of letters that are read aloud by the operator. The strings are increasingly longer at each step. Scores are computed based on the number of correct letters recalled in the correct order for each string. Age norms are provided.

The raw scores obtained by children in each task were age standardized according to the normative data available for the Italian general population (Sartori *et al.* 1995).

Before running the genetic analyses, to correct the age-standardized scores' deviations from normal distributions, data were further transformed through the appropriate phenotype-based extension of the family-based association test (PBAT) option (*z*-score transformation) (Lange *et al.* 2004).

This protocol was approved by the Scientific Institute Eugenio Medea Ethics Board.

Statistical analysis

The Transmission Disequilibrium Test (TDT) is a test of linkage in the presence of association that was originally developed for dichotomous traits (Spielman *et al.* 1993). The Family-Based Association Tests (FBAT) methodology was successively introduced as an extension of the original TDT to deal with quantitative phenotypes (Laird *et al.* 2000); the FBAT statistics computes the distribution of the offspring's genotypes conditioning on parental genotypes or family genotype configuration, assuming that H_0 is true (Laird *et al.* 2000).

In this study we adopted the FBAT methodology to calculate the FBAT statistics as implemented in the PBAT; the PBAT software package version 2.6 is available at http://www.biostat.harvard.edu/~clange/default.htm (Lange *et al.* 2004; Steen & Lange 2005). The PBAT software estimates the heritability (*h*) value for the selected phenotypes which defines the proportion of phenotypic variance explained by the analysed marker; a negative sign denotes a negative correlation between the phenotype and the number of the transmitted alleles.

Single-marker and haplotype analyses with the two SNPs were performed first for DD as a categorical diagnosis, and then for the quantitative traits.

For each SNP, the informative families were those with at least one heterozygous parent, while the informative transmissions were those from a heterozygous parent to each affected offspring, that is one informative family might have more than one informative transmission, depending on the number of offspring.

Although the susceptibility locus for reading-related traits may become more easily detectable once the variance shared with general intelligence is removed (Chapman *et al.* 2004; Francks *et al.* 2004), no such covariation was entered in the genetic analyses because in our sample the phenotypes' scores were not correlated to the full-scale IQ (the only marginally significant correlations were observed between full-scale IQ and 'OC' and 'SWS' with a $r = 0.24$ and $r = 0.21$, respectively).

We applied the Bonferroni correction for multiple testing and used a threshold to infer statistical significance of $P = 0.002$ for the single-marker quantitative analyses (11 phenotypes $\times$ two SNPs), of 0.025 for the single-marker categorical analyses (one phenotype for two SNPs) and of 0.0125 for the haplotype analysis (four combinations of haplotypes with two SNPs).

The offset option was chosen in order to maximize the power of the FBAT statistic (Lange *et al.* 2004). An additive genetic model was assumed throughout, as it performs well across a range of possible genetic models (Lange *et al.* 2004).

Genotyping

Laboratory procedure for DNA sequencing has been described in details elsewhere (Marino *et al.* 2005). Subjects were scored for polymorphisms at −3GA and 1249GT.

Results

The total sample consisted of 194 triads, 18 dyads and 59 sibs (Table 1). Complete information on the neuropsychological assessment was obtained for 164 subjects (114 probands and 50 sibs). Of the total 271 probands and sibs, 158 had already participated in our first association study on *DYX1C1* (Marino *et al.* 2005), and 113 (54 probands and 59 sibs) have never been included in any previous molecular genetic study.

Table 2 shows the descriptive statistics of the reading-related phenotypes. Some subjects scored at the very low negative extreme of the distribution depending on markedly low performances relatively to the mean for the corresponding age group. None of the phenotypes distributions showed either the 'floor' or the 'ceiling' effect. Phenotypic measures in the whole group are highly correlated (Table 3).

A trend towards association was found between −3GA and DD as a categorical diagnosis ($P = 0.0675$, 57 informative families) with a preferential transmission of the 'A' allele in 72 informative transmissions (42 transmitted vs. 30 not transmitted 'A' alleles). No statistically significant association was found between 1249GT and DD (Table 4).

Table 1: Mean scores (SD) and distribution of age, IQ and sex in the total sample

	Total, $n = 271$	Probands, $n = 212$	Siblings, $n = 59$
Males (%)	203 (75)	166 (78)	37 (63)
Age in months	135.85 (36.33)	131.70 (32.54)	146.58 (43.18)
Full-scale IQ	—	101.08 (11.22)	—
Verbal IQ	—	96.32 (11.51)	11.14 (3.9)*
Performance IQ		105.98 (13.14)	11.07 (3.11)†

Normalized scores for vocabulary and block design subtests have mean = 10 and SD = 3.

*Vocabulary subtest only.

†Block design subtest only.

Table 2: Descriptive statistics of the neuropsychological measures in the sample with complete phenotypic and genetic information

	Probands and sibs, $n = 164$					Probands, $n = 114$					Sibs, $n = 50$				
	Min	Max	Mean (SD)	Skew.	Kurtosis	Min	Max	Mean (SD)	Skew.	Kurtosis	Min	Max	Mean (SD)	Skew.	Kurtosis
TR accuracy	−9.96	1.05	−1.33 (1.67)	−1.62	4.44	−9.96	0.90	−1.72 (1.70)	−1.66	4.59	−4.46	1.05	−0.46 (1.19)	−1.54	2.76
TR, speed	−24.69	2.16	−1.76 (3.74)	−4.26	20.64	−24.69	0.45	−2.37 (4.14)	−3.99	16.98	−12.25	2.16	−0.38 (2.03)	−4.53	24.66
SWR, accuracy	−12.66	0.79	−1.94 (2.30)	−1.55	3.31	−12.66	0.67	−2.44 (2.21)	−1.61	4.01	−9.33	0.79	−0.75 (2.07)	−2.51	6.90
SWR, speed	−11.88	0.99	−2.66 (2.65)	−0.95	0.74	−11.47	0.69	−3.40 (2.47)	−0.70	0.28	−11.88	0.99	−0.93 (2.23)	−3.04	12.09
SNWR, accuracy	−18.11	1.26	−1.48 (2.38)	−2.53	13.97	−18.11	1.26	−2.00 (2.39)	−2.87	17.21	−8.10	1.26	−0.24 (1.84)	−2.56	7.68
SNWR, speed	−19.00	2.38	−2.30 (2.70)	−1.97	8.39	−19.00	1.18	−2.92 (2.70)	−2.17	10.07	−9.00	2.38	−0.85 (2.06)	−2.20	6.70
SWS	−26.23	0.93	−1.97 (3.50)	−3.44	17.24	−26.23	0.93	−2.58 (3.80)	−3.35	15.69	−9.78	0.93	−0.60 (2.17)	−3.01	9.62
SNWS	−6.31	1.83	−0.15 (1.37)	−1.73	3.96	−6.31	1.83	−0.35 (1.42)	−1.64	3.61	−4.00	1.83	0.29 (1.12)	−2.08	5.88
OC	−19.30	3.25	−3.07 (3.56)	−1.79	4.52	−19.30	3.25	−3.80 (3.84)	−1.61	3.55	−9.60	0.75	−1.55 (2.21)	−1.56	3.24
SLBS	−2.34	3.10	−0.49 (0.97)	1.01	2.53	−2.34	3.10	−0.54 (0.98)	1.02	2.65	−2.34	3.10	0.38 (0.96)	1.05	2.83
SLFS	−2.34	3.10	−1.03 (1.07)	1.26	2.94	−2.34	3.10	−1.17 (1.12)	1.38	2.77	−2.34	3.10	−0.76 (0.90)	1.57	6.00

Measures are in SD units, relative to the age-appropriate Italian population norm.

Haplotype analyses for DD showed an over-transmission of the variant of interest −3A/1249T (P-value = 0.04681, 44 informative families) which neared the statistical significance (Table 4).

Quantitative analyses yielded a significant evidence for linkage disequilibrium between the rarer 'A' allele of −3GA and SLBS ($P = 0.0011$, with 35 informative families) (Table 5); the proportion of variance explained by the −3A allele is 1.4% and the number of transmitted alleles positively correlates with the phenotype ($h = 0.014$).

On the basis of this result we performed the haplotype analyses for SLBS, and found a statistically significant evidence for association (overall P-value = 0.03919) clearly supported by overrepresentation of the −3A/1249T haplotype ($P = 0.01137$) (Table 6).

Discussion

We were able to find a significant linkage disequilibrium in both single-marker and haplotype analyses between SLBS and the two SNPs of *DYX1C1*, that is −3GA and 1249GT. In the present study, the linkage disequilibrium was supported by the rarer variants of the two polymorphisms, those for which a positive association was found in the original finding by Taipale *et al.* (2003) and for which a functional role has been hypothesized on the basis of bioinformatics predictions (Taipale *et al.* 2003).

The associations between DD/reading-related measures and the polymorphisms of *DYX1C1* have not always been reported for the rarer alleles. In a sample of DD families, Scerri *et al.* (2004) found an association between poorer performance at 'Ortographic Choice' and both 1249G allele and −3G/1249G haplotype. Likewise, Wigg *et al.* (2004) found associations between the most common allele (−3G) and haplotype (3G/1249G) and DD/reading-related measures. Current plausible interpretations for such conflicting results include the possibility that neither the common nor the rare variants of the, respectively, −3GA and the 1249GT, polymorphisms of *DYX1C1* are causative per se but are possibly cosegregating with the actual risk alleles, and the divergent patterns of allelic associations among different studies reflect the haplotypic structure in different population because of stratification effects.

Our results are in harmony with the finding by Wigg *et al.* (2004) in families segregating DD of an association between the allele −3G of *DYX1C1* and phonological STM, measured by the Non-Word Repetition (NWR) task, that scores the ability to repeat unfamiliar polysyllabic sequences. Both tasks, that is NWR and SLBS, tap similar cognitive pathways within the STM system and they are both relevant to DD (Howes *et al.* 1999; Newbury *et al.* 2005), although NWR indexes a more specialized STM system compared with SLBS because it measures the ability to retain phonological information over brief intervals.

Less directly, but perhaps more intriguingly, our findings are also consistent with a linkage peak between D15S1017 (45.62 cM) and D15S1029 (47.85 cM), the same region where *DYX1C1* lies, again with the phenotype NWR although in families segregating SSD (Smith *et al.* 2005). The same

Table 3: Spearman's correlations of the neuropsychological measures in the sample with complete phenotypic and genetic information

	TR, accuracy	TR, speed	SWR, accuracy	SWR, speed	SNWR, accuracy	SNWR, speed	SWS	SNWS	OC	SLBS	SLFS
TR, accuracy		0.64**	0.55**	0.59**	0.53**	0.54**	0.37**	0.39**	0.35**	0.08	0.30**
TR, speed			0.56**	0.78**	0.37**	0.75**	0.37**	0.31**	0.33**	0.09	0.30**
SWR, accuracy				0.60**	0.63**	0.46**	0.43**	0.35**	0.51**	0.28**	0.33**
SWR, speed					0.40**	0.87**	0.42**	0.34**	0.31**	0.07	0.24*
SNWR, accuracy						0.26**	0.48**	0.34**	0.58**	0.14	0.36**
SNWR, speed							0.27**	0.30**	0.21**	0.14	0.23**
SWS								0.40**	0.46**	0.16	0.30**
SNWS									0.33**	0.25**	0.11
OC										0.13	0.27**
SLBS											0.43**
SLFS											

**Two tailed *P*-values < 0.01.

Table 4: Single-marker and haplotype analyses between −3GA and 1249GT and the categorical diagnosis of DD by PBAT

Markers	Alleles	Frequency*	Informative families	*P*-value, FBAT
-3GA	G	0.91	57	0.0675
-3GA	A	0.09	57	0.0675
1249GT	G	0.87	80	0.1173
1249GT	T	0.13	80	0.1173
−3GA/1249GT	G.G.	0.86	83	0.0819
−3GA/1249GT	A.G.	0.02	17	0.2557
−3GA/1249GT	G.T.	0.06	44	0.9829
−3GA/1249GT	A.T	0.06	42	0.0468

The nominal alpha level for significance was set at 0.025 for the single-marker analysis and at 0.0125 for the haplotype analysis.
*Allelic and haplotypic frequencies in parents.

authors (Smith *et al.* 2005) suggest that their findings are a further support to the association of DD with *DYX1C1* (Taipale *et al.* 2003), and that SSD – as measured by NWR – may indeed constitute a more salient phenotype than DD itself.

Table 5: Single-marker analyses between both −3GA and 1249GT of *DYX1C1* and the 11 phenotypes by PBAT

Phenotype	Marker	Allele	Informative families	*P*-value, FBAT
TR, accuracy	−3GA	G	35	0.0249
TR, speed				0.0432
SWR, accuracy				0.0509
SWR, speed				0.0641
SNWR, accuracy				0.0483
SNWR, speed				0.0991
SWS				0.0144
SNWS				0.0242
OC				0.0531
SLBS				0.0051
SLFS				0.0259
TR, accuracy	−3GA	A	35	0.008
TR, speed				0.0155
SWR, accuracy				0.0214
SWR, speed				0.0264
SNWR, accuracy				0.0186
SNWR, speed				0.0468
SWS				0.0042
SNWS				0.0103
OC				0.0255
SLBS				0.0011
SLFS				0.0076
TR, accuracy	1249GT	G	40	0.0091
TR, speed				0.0198
SWR, accuracy				0.0136
SWR, speed				0.0128
SNWR, accuracy				0.0171

The nominal alpha level for significance was set at 0.002.

Table 6: Haplotype analysis for −3GA/1249GT with the SLBS phenotype

Haplotype combination	Haplotype frequency*	Informative families	*P*-value FBAT
−3G/1249G	0.86	31	0.0980
−3A/1249G	0.01	7	0.6028
−3G/1249T	0.06	10	0.879
−3A/1249T	0.07	20	0.0114

The nominal alpha level for significance was set at 0.0125.

*Allelic and haplotypic frequencies in parents.

Seven of 11 phenotypes and DD as a categorical trait had been already investigated in our previous association study (Marino *et al*. 2005) and the *P*-values we had found were of about one order of magnitude higher than in the present study. In a sample otherwise substantially overlapping for the number of informative families, two basic changes were introduced which could explain such discrepancy. First, more lenient criteria to define DD were adopted. While for *KIAA0319*, a candidate gene for DD on chromosome 6, the statistical evidence for linkage was stronger for the most severe dyslexic phenotype (Francks *et al*. 2004), for *DYX1C1* the adoption of broader diagnostic criteria might have contributed to enhance the association signal, possibly a hint that *DYX1C1* influences a wider span of severity along the continuum of reading ability. Second, different analytical approaches were used in the two studies, as only affected siblings were included in the sample of the prior study (Marino *et al*. 2005), whereas now also unaffected siblings were incorporated and contributed to the statistics through the offset option (Lange *et al*. 2002). We plan to assess the usefulness of these explanations in the next future through different approaches which will further enable to appreciate the trade-offs between different analytical methods and will possibly generate further heuristic implications.

The measure for which we found the association (SLBS) was not tested in our previous report (Marino *et al*. 2005), precluding any direct comparison of the data.

Although an influence of reading ability cannot be ruled out for a task such as STM, this task is less likely to be influenced by a language's orthographic structure. As such, STM seems a more sensitive measure for the Italian, transparent orthography-based language, and more suitable for genotype–phenotype correlation studies on DD because it allows for cross-linguistic comparisons of the results. Short-term memory (STM) is implicated in the transient storing of all the relevant representations, thus allowing the grapheme-to-phoneme conversion and phonemes assembly, necessary to read pronounceable non-words and, possibly infrequent words.

While the present findings need replication and their ultimate meaning will be better understood when the true function of *DYX1C1* becomes clear, our data show that finer phenotyping can help to understand the relationships between variation along reading-related traits and specific candidate genes.

References

Bakker, S.C., van der Meulen, E.M., Buitelaar, J.K., Sandkuijl, L.A., Pauls, D.L., Monsuur, A.J, van 't Slot, R., Minderaa, R.B., Gunning, W.B., Pearson, P.L. & Sinke, R.J. (2003) A whole-genome scan in 164 Dutch sib pairs with attention-deficit/hyperactivity disorder: suggestive evidence for linkage on chromosomes 7p and 15q. *Am J Hum Genet* **72**, 1251–1260.

Bellini, G., Bravaccio, C., Calamoneri, F., Donatella Cocuzza, M., Fiorillo, P., Gagliano, A., Mazzone, D., Del Giudice, E.M., Scuccimarra, G., Militerni, R. & Pascotto, A. (2005) No evidence for association between dyslexia and *DYX1C1* functional variants in a group of children and adolescents from Southern Italy. *J Mol Neurosci* **27**, 311–314.

Bisgaard, M.L., Eiberg, H., Moller, N., Niebuhr, E. & Mohr, J. (1987) Dyslexia and chromosome 15 heteromorphism: negative LOD score in a Danish material. *Clin Genet* **32**, 118–119.

Cardon, L.R., Smith, S.D., Fulker, D.W., Kimberling, W.J., Pennington, B.F. & DeFries, J.C. (1994) Quantitative trait locus for reading disability on chromosome 6. *Science* **266**, 276–279. Erratum in: (1995) *Science* **268**, 1553.

Chapman, N.H., Igo, R.P., Thomson, J.B., Matsushita, M., Brkanac, Z., Holzman, T., Berninger, V.W., Wijsman, E.M. & Raskind, W. (2004) Linkage analyses of four regions previously implicated in dyslexia: confirmation of a locus on chromosome 15q. *Am J Med Genet B Neuropsychiatr Genet* **131**, 67–75.

Cope, N.A., Hill, G., van den Bree, M., Harold, D., Moskvina, V., Green, E.K., Owen, M.J., Williams, J. & O'Donovan, M.C. (2005) No support for association between dyslexia susceptibility 1 candidate 1 and developmental dyslexia. *Mol Psychiatry* **10**, 237–238.

Cornoldi, C. & Colpo, G. (1986) *Gruppo MT: Prove di lettura*. Organizzazioni Speciali, Firenze.

De Kovel, C.G., Hol, F.A., Heister, J.G., Willemen, J.J., Sandkuijl, L.A., Franke, B. & Padberg, G.W. (2004) Genomewide scan identifies susceptibility locus for dyslexia on Xq27 in an extended Dutch family. *J Med Genet* **41**, 652–657.

Fagerheim, T., Raeymaekers, P., Tonnessen, F.E., Pedersen, M., Tranebjaerg, L. & Lubs, H.A. (1999) A new gene (DYX3) for dyslexia is located on chromosome 2. *J Med Genet* **36**, 664–669.

Fisher, S.E. & De Fries, J.C. (2002) Developmental dyslexia: genetic dissection of a complex cognitive trait. *Nat Rev Neurosci* **3**, 767–780.

Fisher, S.E., Marlow, A.J., Lamb, J., Maestrini, E., Williams, D.F., Richardson, A.J., Weeks, D.E., Stein, J.F. & Monaco, A.P. (1999) A quantitative-trait locus on chromosome 6p influences different aspects of developmental dyslexia. *Am J Hum Genet* **64**, 146–156.

Fisher, S.E., Francks, C., Marlow, A.J., MacPhie, I.L., Newbury, D.F., Cardon, L.R., Ishikawa-Brush, Y., Richardson, A.J., Talcott, J.B., Gayan, J., Olson, R.K., Pennington, B.F., Smith, S.D., DeFries, J.C., Stein, J.F. & Monaco, A.P. (2002) Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia. *Nat Genet* **30**, 86–91.

Francks, C., Paracchini, S., Smith, S.D., Richardson, A.J., Scerri, T.S., Cardon, L.R., Marlow, A.J., MacPhie, I.L., Walter, J., Pennington, B.F., Fisher, S.E., Olson, R.K., DeFries, J.C., Stein, J.F. & Monaco, A.P. (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. *Am J Hum Genet* **75**, 1046–1058.

Gayan, J., Smith, S.D., Cherny, S.S., Cardon, L.R., Fulker, D.W., Brower, A.M., Olson, R.K., Pennington, B.F. & DeFries, J.C. (1999) Quantitative-trait locus for specific language and reading deficits on chromosome 6p. *Am J Hum Genet* **64**, 157–164.

Grigorenko, E.L., Wood, F.B., Meyer, S.B., Hart, L.A., Speed, W.C. & Shuster, A. (1997) Susceptibility loci for distinct components of developmental dyslexia on chromosome 6 and 15. *Am J Hum Genet* **60**, 27–39.

Grigorenko, E.L., Wood, F.B., Meyer, M.S., Pauls, J.E., Hart, L.A. & Pauls, D.L. (2001) Linkage studies suggest a possible locus for developmental dyslexia on chromosome 1p. *Am J Med Genet* **105**, 120–129.

Hallgren, B. (1950) Specific dyslexia (congenital word-blindness); a clinical and genetic study. *Acta Neurol Scan Suppl* **65**, 1–287.

Howes, N.L., Bigler, E.D., Lawson, J.S. & Burlingame, G.M. (1999) Reading disability subtype and the test of memory and learning. *Arch Clin Neuropsychol* **14**, 317–339

Igo, R.P., Jr, Chapman, N.H., Berninger, V.W., Matsushita, M., Brkanac, Z., Rothstein, J.H., Holzman, T., Nielsen, K., Raskind, W.H. & Wijsman, E.M. (2006) Genomewide scan for real-word reading subphenotypes of dyslexia: novel chromosome 13 locus and genetic complexity. *Am J Med Genet B Neuropsychiatr Genet* **141**, 15–27.

Kaminen, N., Hannula-Jouppi, K., Kestila, M., Lahermo, P., Muller, K., Kaaranen, M., Myllyluoma, B., Voutilainen, A., Lyytinen, H., Nopola–Hemmi, J. & Kere, J. (2003) A genome scan for developmental dyslexia confirms linkage to chromosome 2p11 and suggests a new locus on 7q32. *J Med Genet* **40**, 340–345.

Kaplan, D.E., Gayan, J., Ahn, J., Won, T.W., Pauls, D., Olson, R.K., DeFries, J.C., Wood, F., Pennington, B.F., Page, G.P., Smith, S.D. & Gruen, J.R. (2002) Evidence for linkage and association with reading disability on 6p21.3-22. *Am J Hum Genet* **70**, 1287–1298.

Laird, N.M., Horvath, S. & Xu, X. (2000) Implementing a unified approach to family-based tests of association. *Genet Epidemiol* **19**(Suppl. 1), S36–S42.

Lange, C., DeMeo, D. & Laird, N.M. (2002) Power and design considerations for a general class of family-based association tests: quantitative traits. *Am J Hum Genet* **71**, 1330–1341.

Lange, C., DeMeo, D., Silverman, E., Weiss, S. & Laird, N.M. (2004) PBAT: tools for family-based association studies. *Am J Hum Genet* **74**, 367–369.

Loo, S.K., Fisher, S.E., Francks, C., Ogdie, M.N., MacPhie, I.L., Yang, M., McCracken, J.T., McGough, J.J., Nelson, S.F., Monaco, A.P. & Smalley, S.L. (2004) Genome-wide scan of reading ability in affected sibling pairs with attention-deficit/hyperactivity disorder: unique and shared genetic effects. *Mol Psychiatry* **9**, 485–493.

Marino, C., Giorda, R., Vanzin, L., Nobile, M., Lorusso, M.L., Baschirotto, C., Riva, L., Molteni, M. & Battaglia, M. (2004) A locus on 15q15-15qter influences dyslexia: further support from a transmission/disequilibrium study in an Italian speaking population. *J Med Genet* **41**, 42–46.

Marino, C., Giorda, R., Lorusso, M.L., Vanzin, L., Salandi, N., Nobile, M., Citterio, A., Beri, S., Crespi, V., Battaglia, M. & Molteni, M. (2005) A family-based association study of the *DYX1C1* gene on 15q21.1 in Developmental Dyslexia. *Eur J Hum Genet* **13**, 491–499.

Meng, H., Hager, K., Held, M., Page, G.P. Olson, R.K., Pennington, B.F., DeFries, J.C., Smith, S.D. & Gruen, J.R. (2005) TDT-association analysis of EKN1 and dyslexia in a Colorado twin cohort. *Hum Genet* **118**, 87–90.

Morris, D.W., Robinson, L., Turic, D., Duke, M., Webb, V. & Milham, C. (2000) Family-based association mapping provides evidence for a gene for reading disability on chromosome 15q. *Hum Mol Genet* **9**, 843–848.

Newbury, D.F., Bishop, D.V.M. & Monaco, A.P. (2005) Genetic influences on language impairment and phonological short-term memory. *Trends Cogn Sci* **9**, 528–534.

Nopola-Hemmi, J., Taipale, M., Haltia, T., Lehesjoki, A.E., Voutilainen, A. & Kere, J. (2000) Two translocations of chromosome 15q associated with dyslexia. *J Med Genet* **37**, 771–775.

Nopola-Hemmi, J., Myllyluoma, B., Haltia, T., Taipale, M., Ollikainen, V., Ahonen, T., Voutilainen, A., Kere, J. & Widen, E. (2001) A dominant gene for developmental dyslexia on chromosome 3. *J Med Genet* **38**, 658–664.

Paulesu, E., Demonet, J.F., Fazio, F., McCrory, E., Chanoine, V., Brunswick, N., Cappa, S.F., Cossu, G., Habib, M., Frith, C.D. & Frith, U. (2001) Dyslexia: cultural diversity and biological unity. *Science* **291**, 2165–2167.

Petryshen, T.L., Kaplan, B.J., Fu Liu, M., de French, N.S., Tobias, R., Hughes, M.L. & Field, L.L. (2001) Evidence for a susceptibility locus on chromosome 6q influencing phonological coding dyslexia. *Am J Med Genet* **105**, 507–517.

Petryshen, T.L., Kaplan, B.J., Hughes, M.L., Tzenova, J. & Field, L.L. (2002) Supportive evidence for the DYX3 dyslexia susceptibility gene in Canadian families. *J Med Genet* **39**, 125–126.

Plomin, R. & Kovas, Y. (2005) Generalist genes and learning disabilities. *Psychol Bull* **131**, 592–617.

Rabin, M., Wen, X.L. & Hepburn, M. (1993) Suggestive linkage of developmental dyslexia to chromosome 1p34-p36. *Lancet* **342**, 178.

Raskind, W.H., Igo, R.P., Chapman, N.H., Berninger, V.W., Thomson, J.B., Matsushita, M., Brkanac, Z., Holzman, T., Brown, M. & Wijsman, E.M. (2005) A genome scan in multigenerational families with dyslexia: identification of a novel locus on chromosome 2q that contributes to phonological decoding efficiency. *Mol Psychiatry* **10**, 699–711.

Reynolds, C.R. & Bigler, E.D. (1994) *Test of Memory and Learning (TOMAL)*. PRO-ED, Austin, TX. Italian edition: Ianes, D.. (ed) (1995) *Test di Memoria e Apprendimento (TEMA)*. Centro studi Erickson, Trento, Italy.

Sartori, G., Job, R. & Tressoldi, P.E. (1995) *Batteria per la valutazione della dislessia e della disortografia evolutiva*. Organizzazioni Speciali, Firenze.

Scerri, T.S., Fisher, S.E., Francks, C., MacPhie, I.L., Paracchini, S., Richardson, A.J., Stein, J.F. & Monaco, A.P. (2004) Putative functional alleles of *DYX1C1* are not associated with dyslexia susceptibility in a large sample of sibling pairs from the UK. *J Med Genet* **41**, 853–857.

Schulte-Korne, G., Grimm, T., Nothen, M.M., Muller-Myhsok, B., Cichon, S. & Vogt, I.R. (1998) Evidence for linkage of spelling disability to chromosome 15. *Am J Hum Genet* **63**, 279–282.

Siok, W.T., Perfetti, C.A., Jin, Z. & Tan, L.H. (2004) Biological abnormality of impaired reading is constrained by culture. *Nature* **431**, 71–76.

Smith, S.D., Kimberling, W.J., Pennington, B.F. & Lubs, H.A. (1983) Specific reading disability-identification of an inherited form through linkage analysis. *Science* **219**, 1345.

Smith, S.D., Fain, P.R., Ing, P.S. & Lubs, H.A. (1986) Genetic hetrogeneity in specific reading disability. *Am J Hum Genet* **39**, A169.

Smith, S.D., Pennington, B.F. Kimberling, W.J. & Paul, S.I. (1990) Familial dyslexia: use of genetic linkage data to define subtypes. *J Am Acad Child Adolesc Psychiatry* **29**, 204–213.

Smith, S.D., Kimberling, W.J. & Pennington, B.F. (1991) Screening for multiple genes in developmental dyslexia. *Read Writ Interdiscip J* **3**, 285–298.

Smith, S.D., Pennington, B.F., Boada, R. & Shriberg, L.D. (2005) Linkage of speech sound disorder to reading disability loci. *J Child Psychol Psychiatry* **46**, 1057–1066.

Spielman, R.S., McGinnis, R.E. & Ewens, W.J. (1993) Transmission test for linkage disequilibrium: the insulin gene region and insulin–dependent diabetes mellitus (IDDM). *Am J Hum Genet* **52**, 506–516.

Steen, K.V. & Lange, C. (2005) PBAT: a comprehensive software package for genome-wide association analysis of complex family–based studies. *Hum Genomics* **2**, 1–3.

Taipale, M., Kaminen, N., Nopola-Hemmi, J., Haltia, T., Myllyluoma, B., Lyytinen, H., Muller, K., Kaaranen, M., Lindsberg, P.J., Hannula–Jouppi, K. & Kere, J. (2003) A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. *Proc Natl Acad Sci USA* **100**, 11553–11558.

Tzenova, J., Kaplan, B.J., Petryshen, T.L. & Field, L.L. (2004) Confirmation of a dyslexia susceptibility locus on chromosome 1p34-p36 in a set of 100 Canadian families. *Am J Med Genet B Neuropsychiatr Genet* **15**, 117–124.

Wechsler, D. (1981) *Examiner's Manual. Wechsler Intelligence Scale for Children, Revised*. Psychological Corp., New York.

Wigg, K.G., Couto, J.M., Feng, Y., Anderson, B., Cate-Carter, T.D., Macciardi, F., Tannock, R., Lovett, M.W., Humphries, T.W. & Barr, C.L. (2004) Support for EKN1 as the susceptibility locus for dyslexia on 15q21. *Mol Psychiatry* **12**, 1111–1121.

Willcutt, E.G., Pennington, B.F. & De Fries, J.C. (2000) Twin studies of the etiology of comorbidity between reading disability and attention-deficit/hyperactivity disorder. *Am J Med Genet* **96**, 293–301.

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