Replication of *CNTNAP2* association with nonword repetition and support for *FOXP2* association with timed reading and motor activities in a dyslexia family sample

Beate Peter · Wendy H. Raskind · Mark Matsushita ·
Mark Lisowski · Tiffany Vu · Virginia W. Berninger ·
Ellen M. Wijsman · Zoran Brkanac

Abstract Two functionally related genes, *FOXP2* and *CNTNAP2,* influence language abilities in families with rare syndromic and common nonsyndromic forms of impaired language, respectively. We investigated whether these genes are associated with component phenotypes of dyslexia and measures of sequential motor ability. Quantitative transmission disequilibrium testing (QTDT) and linear association modeling were used to evaluate associations with measures of phonological memory (nonword repetition, NWR), expressive language (sentence repetition), reading (real word reading efficiency, RWRE; word attack, WATT), and timed sequential motor activities (rapid alternating place of articulation, RAPA; finger succession in the dominant hand, FS-D) in 188 family trios with a child with dyslexia. Consistent with a prior study of language impairment, QTDT in dyslexia showed evidence of *CNTNAP2* single nucleotide polymorphism (SNP) association with NWR. For *FOXP2*, we provide the first evidence for SNP association with component phenotypes of dyslexia, specifically NWR and RWRE but not WATT. In addition, *FOXP2* SNP associations with both RAPA and FS-D were observed. Our results confirm the role of *CNTNAP2* in NWR in a dyslexia sample and motivate new questions about the effects of *FOXP2* in neurodevelopmental disorders.

Keywords Written language · Spoken language · Modality-specific motor sequencing · Quantitative transmission disequilibrium · Linear modeling

B. Peter (✉)
Department of Speech and Hearing Sciences,
University of Washington,
P.O. Box 354875, Seattle, WA 98195, USA
e-mail: bvpeter@u.washington.edu

B. Peter · W. H. Raskind · M. Matsushita · M. Lisowski · T. Vu ·
E. M. Wijsman
Department of Medicine, University of Washington,
P.O. Box 354875, Seattle, WA 98195, USA

V. W. Berninger
Department of Educational Psychology,
University of Washington,
P.O. Box 354875, Seattle, WA 98195, USA

E. M. Wijsman
Department of Biostatistics, University of Washington,
P.O. Box 354875, Seattle, WA 98195, USA

W. H. Raskind · Z. Brkanac
Department of Psychiatry and Behavioral Sciences,
University of Washington,
P.O. Box 354875, Seattle, WA 98195, USA

Introduction

CNTNAP2 is a gene on chromosome (chr) 7 that encodes a protein belonging to the neurexin family. Mutations in *CNTNAP2* cause an autosomal recessive syndrome with mental retardation and seizures (Strauss et al. 2006), and the gene has also been implicated in autism (Alarcon et al. 2008; Arking et al. 2008; Bakkaloglu et al. 2008) and language impairment (Vernes et al. 2008). Language impairment is a disorder affecting comprehension and formulation of spoken language in the absence of hearing impairment, neurological impairment, or psychiatric disturbance (Tomblin et al. 1997; Rice et al. 2009). Difficulty with nonsense-word repetition (NWR), a task that requires reproducing a pronounceable but meaningless word in response to a spoken model, is a robust endophenotype of language impairment (Newbury et al. 2005). The role of *CNTNAP2* in language impairment was

recently investigated with quantitative disequilibrium testing (QTDT) in 184 families with common forms of the disorder. Results showed that *CNTNAP2* was significantly associated with NWR. *CNTNAP2* was also associated with composite measures of expressive and receptive language ability, but to a much smaller extent than with NWR (Vernes et al. 2008). Thus, *CNTNAP2* may be associated with processes underlying phonologic memory and other developmental disabilities, which include but are not restricted to language impairment.

The rationale to study the effects of *CNTNAP2* in language impairment was based on the discovery that this gene is regulated by another gene, *FOXP2,* also located on chr 7 (Vernes et al. 2008). Mutations in *FOXP2* have been shown to cause severe but rare syndromic deficits in language comprehension and expression, concomitant with apraxic speech and orofacial praxis deficits including simultaneous and sequential movements (Alcock et al. 2000; Lai et al. 2001; MacDermot et al. 2005). *FOXP2* sequence or structural changes, however, have not been identified in common nonsyndromic forms of language impairment (Newbury et al. 2002). In a family sample with language impairment (Rice et al. 2009), neither linkage nor association analyses provided evidence for *FOXP2* influences on NWR. Similarly, even though *FOXP2* mutations cause severe syndromic speech difficulties; they are seen very rarely in common nonsyndromic forms of speech sound disorder (MacDermot et al. 2005), defined as difficulty developing speech that can be readily understood, due to speech sound omissions, distortions, or substitutions, in the absence of known causes (Pennington and Bishop 2009). *FOXP2*, hence, appears to be necessary but not sufficient for the development of typical language and speech abilities.

In animal studies, *FOXP2* has been found to play an important role in a variety of behaviors related to vocalization and motor sequencing. For instance, songbirds require upregulation of FOXP2 in a crucial basal ganglia region during the song-learning phase (Teramitsu et al. 2004). In zebra finches with FOXP2 knocked down, the song imitations are incomplete and inaccurate (Haesler et al. 2007). In mice with an induced *FOXP2* mutation analogous to that causing speech and language impairments in humans, homozygotes showed severe cerebellar growth reductions and heterozygotes had deficits in motor learning as measured by rapid motor sequencing on running wheels, along with impaired and abnormal plasticity in striatal and cerebellar circuits (Groszer et al. 2008). These findings suggest that FOXP2 might play a role in sequential motor ability across motor systems. It is unknown whether its transcriptional target *CNTNAP2* influences motor processes.

Dyslexia is a specific disability that interferes with the acquisition of written language at the word level, characterized by deficits in accurate and/or fluent word recognition, decoding, and spelling in the presence of grossly

normal oral language skills (Lyon et al. 2003; Shaywitz and Shaywitz 2003). Difficulty with phonological processing is one of the hallmarks of dyslexia. Multiple groups, including ours, have observed that NWR performance is frequently impaired in children and adults with this disorder (Kamhi and Catts 1986; Hulslander et al. 2004; Ramus et al. 2003; Szenkovits and Ramus 2005; Brkanac et al. 2008). Because NWR deficit is a central characteristic of language impairment, it could be argued that the NWR deficits observed in dyslexia reflects concurrent language problems. However, NWR probes a number of cognitive component abilities including phonological awareness, which is a core requisite for reading. Furthermore, the dyslexia family subject set from which our sample was drawn specifically excluded comorbid low language ability (Berninger et al. 2001a, 2006). Our previous aggregation study in this sample showed that NWR was among the traits with the strongest evidence for a genetic basis (Raskind et al. 2000). The fact that NWR deficits persist into adulthood facilitates investigation of NWR in family-based dyslexia genetics studies. In our genome-wide linkage scan for NWR in a family sample with dyslexia (Brkanac et al. 2008), a locus on chr 7q at 190.9 cM approached but did not quite reach status as a region of interest. The variance component maximum LOD signal maximized approximately 23 and 57 cM from *CNTNAP2* and *FOXP2*, respectively. It is thus unlikely that either gene contributed to that signal.

Another type of deficit, difficulty with sequential finger movements, characterizes dyslexia and other neurodevelopmental disorders (Denckla 1973; Wolff et al. 1983). Finger motor sequencing was also shown to predict writing problems in an unreferred sample of typically developing children (Berninger and Rutberg 1992). Thus, there appears to be an underlying association between literacy skill and sequential finger movements.

As recently reviewed (Pennington and Bishop 2009; Peterson et al. 2007; Lewis et al. 2006), many children who are diagnosed with language impairment and/or speech sound disorder in early childhood show difficulty learning to read and spell once they enter school (Catts et al. 2002; Tomblin et al. 2000). Problems with oral language, speech, and reading can occur in isolation or co-occur in the same child, although not all observed reading problems are well captured by the dyslexia definition provided by the International Dyslexia Association (Lyon et al. 2003) that is focused on impairments in word-level reading and spelling skills.

The presence of comorbidity and shared impairments on some component traits in dyslexia, language impairment, and speech sound disorder, along with the functional relationship between *CNTNAP2* and *FOXP2,* provided a rationale to investigate the possibility of *CNTNAP2* and *FOXP2* associations with performance on measures of

NWR, written language, and sequential motor abilities in families ascertained through a child with dyslexia. Specifically, we investigated (a) whether *CNTNAP2* and/or *FOXP2* are associated with NWR, sentence repetition (SR), and measures of reading in a family sample with dyslexia, which would replicate findings from the family study of language impairment (Vernes et al. 2008) and extend them to dyslexia; and (b) whether *CNTNAP2* and/or *FOXP2* variants are associated with motor sequencing abilities not only in oral but also in finger tasks, which might imply a more systemic involvement in motor systems beyond the oral system. Based on the finding that language deficits are associated with *FOXP2* in rare cases and *CNTNAP* in common cases, our expectation for the dyslexia sample was that *CNTNAP2* variants would be more likely than *FOXP2* variants to show associations with phenotypes related to spoken and written language as well as sequential motor tasks.

Methods

Participants

Data for this study were collected by the University of Washington Learning Disabilities Center (UWLDC) and with the approval of the University of Washington's Human Subjects Institutional Review Board. The participant recruitment process and the phenotyping and genotyping procedures have been described in detail elsewhere (Berninger et al. 2006; Raskind et al. 2000). Each participating family was identified through a proband child in grades 1 through 9 who met dual criteria of discrepancy from the Verbal Comprehension Index (prorated verbal IQ, VIQ) and low achievement (below the population mean and typically well below) for dyslexia in accuracy or rate of orally reading pseudowords, real words, or passages, or spelling and/or for dysgraphia in automatic alphabet letter writing or spelling. The VIQ-reading discrepancy criterion is not sufficient for a clinical diagnosis of dyslexia because identification of hallmark phenotype impairments is also required (Berninger et al. 2001b), but IQ-discrepant reading deficits are more likely to provide significant heritability estimates, compared to reading deficits without such a discrepancy (Olson et al. 1999). The probands' average word reading and decoding skills on nationally normed measures were more than .67 *SD* below the population mean and ranged from 1.5 to 1.8 *SD* below their VIQ. On average, probands met inclusionary criteria on 6.0 of 10 measures of reading and spelling and on 4.1 of 6 measures of writing (Berninger et al. 2006). To participate in the study, the proband was required to have a VIQ of at least 90 on the Wechsler Intelligence Scale for Children-3rd Edition (Wechsler 2008), to exclude children with a higher probability of having an undiagnosed neuro-logic or developmental disorder. Children with neurological or psychiatric disorders or other medical conditions, or preschool history of significant difficulty in learning language or other developmental milestones, which could have impaired their reading or writing for reasons other than dyslexia or dysgraphia were excluded, except for attention deficit/hyperactivity disorder. The protocol for the UWLDC study included measures of verbal reasoning/comprehension, reading and writing and phenotypes known to be associated with dyslexia or dysgraphia organized in a working memory architecture (Berninger et al. 2006). In addition, blood samples were collected from participants for DNA analyses. For the present study, 188 family trios from separate families of predominantly Caucasian ethnic background were selected. Per parent report, the probands were of the following ethnic backgrounds: 92.0% Caucasian, 2.1% Asian, 1.1% African American, 1.1% Native American, .5% Hispanic, and 3.2% other or unknown. These trios were the same as those reported in a previous study on associations of dyslexia to the DYX1 locus and DYX1 candidate genes (Brkanac et al. 2007). Tests were administered by graduate student research assistants. All were from an APA-accredited and NASP-approved school psychology program and had completed assessment courses. Many had school psychology certification and were trained and closely supervised by the family genetics clinical coordinator, a licensed or license-eligible psychologist, and the PI of the clinical core who is a licensed psychologist.

Phenotypes

Six phenotypes (Table 1) were tested for SNP association with *CNTNAP2* and/or *FOXP2* polymorphisms. To evaluate the role of NWR, we selected the Nonword Repetition subtest from the Comprehensive Test of Phonological Processing (Wagner et al. 1999). In this task, participants hear a pronounceable but meaningless pseudoword and imitate it once. This task requires storage and processing of the phonological information in short-term memory. SR, also referred to as sentence imitation, is considered a sensitive measure of expressive language ability and difficulty with this task is one of the psycholinguistic markers of language impairment (Conti-Ramsden et al. 2001). It is one of the subtests contributing to the composite measure of expressive language reported in the language impairment study by Vernes et al. (2008). The task requires reproduction of sentences verbatim in response to a given model, once for each sentence. For the purposes of this study, the Sentence Memory subtest from the Stanford-Binet Intelligence Scale (Thorndike et al. 1986) was selected as a measure of SR. The score reflects the ability to reproduce sentences of increasing length and complexity. Performance in this task depends on memory factors in the context of lexical meaning and syntactic frameworks and it

Table 1 Descriptive statistics for proband and parent groups

Age and Phenotype	Population mean (SD)	N Probands	Proband mean (SD)	N Parents	Parent mean (SD)
Age (months)	N/A	188	129 (22)	376	525 (59)
NWR	10 (3)	103	7.97 (1.73)	202	7.09 (1.81)
SR	50 (8)	102	50.88 (8.72)	198	47.78 (7.99)
RWRE	0 (1)	188	−0.69 (1.18)	372	2.14 (1.00)
WATT	100 (15)	188	85.31 (12.11)	371	101.45 (10.88)
RAPA	0 (1)	103	−0.30 (1.50)	199	−0.14 (1.23)
FS-D	0 (1)	101	0.09 (1.06)	200	0.72 (0.75)

Norms for RWRE are based on a prepublication version of the TOWRE (Thorndike et al. 1986) and norms for RAPA and FS-D are based on UWLDC measures (Catts et al. 2002). Because the assessment battery was modified during the 10 years in which the participants were evaluated, not all participants were tested for all the measures

offers an interesting theoretical contrast with memory for nonwords as captured with NWR, where lexical meaning or syntactic frameworks are absent.

To evaluate associations with reading ability, we selected two complementary measures of reading. Rate of real word reading, here termed real word reading efficiency (RWRE), was evaluated with a subtest of the prepublication version of the Test of Word Reading Efficiency (TOWRE) (Torgesen et al. 1999), on which participants orally read as many real words as possible within 45 s. The published version of this subtest is titled Sight Word Efficiency. Accuracy of nonword reading was evaluated with the Word Attack subtest (WATT) of the Woodcock Reading Mastery Test–Revised (Woodcock 1987) on which participants orally read pronounceable but meaningless nonwords from a list under untimed conditions. A number of previous studies including ours (Berninger et al. 2006; Raskind et al. 2005) confirmed that both of these measures detect core impairments in individuals with dyslexia and have a strong genetic component.

Motor sequencing during a speech-like activity was evaluated with a motor speech task requiring rapid repetition of the syllable sequence "pataka" ten times (Fletcher 1978). This measure assesses rapid alternating place of articulation (RAPA) of the three stop consonants (bilabial, alveolar, velar) in this sequence. Performance on this measure (total time for all the repetitions) contributed uniquely to oral passage reading in the children (rate) and adults (accuracy) with dyslexia in a phenotyping study (Berninger et al. 2008a). An analogous measure of motor sequencing for a hand task is the finger succession-dominant hand (FS-D) task (Berninger and Rutberg 1992). FS-D involves timed, sequential finger maneuvers, requiring that participants perform five repetitions of sequential finger to thumb touches with index to middle finger to ring finger, and then pinky. The score in the current study is the total time required for the dominant hand. Because the data were collected under two slightly

different protocols, measures for NWR, SR, RAPA, and FS-D were not always available for participants who had completed RWRE and WATT (see Ns in Table 1). Norms for RAPA and FS-D were based on UWLDC measures (Berninger et al. 2006) and norms for RWRE, on a prepublication version of the test. Table 1 summarizes descriptive statistics for each phenotype in the two groups. Several differences between the proband and parent cohorts are noted. The probands had low reading scores whereas the parents had average WATT scores and above-average RWRE scores. The probands achieved FS-D scores near the population mean whereas the parents achieved higher scores. These differences may in part result from the fact that the probands but not the parents were selected for low reading ability. Another consideration is the fact that the test instruments were normed on child and young adult samples, and it is possible that performance on timed measures of reading and finger sequencing increases in adults beyond the highest available norm age. Norming samples covered age 5 through 24 years for NWR, age 3 through 23 for SR, age 6 through 24 for RWRE, grades K through 12 for WATT, grades K through 6 for FS-D, and age 6 through 13 for RAPA. Table 2 shows power to detect biased transmission and association for each phenotype and lists the minimum genetic effects under each of three modes of inheritance, given the sample size, 80% power, p values of 0.05, and two-sided tests.

Genotyping

Of the nine *CNTNAP2* SNPs with the strongest statistical associations in the language impairment family study (Vernes et al. 2008), five were in complete linkage disequilibrium with neighboring SNPs in this sample. Thus, only rs851715, rs2710102, rs1723623, and rs4431523, all located between exons 13 and 14, were retained for further analysis. In addition, six highly informative SNPs were selected as tags for the following genomic environments of

Table 2 Minimum genetic effects in variable units and z scores (power=0.8, p=0.05, two-sided test) to detect biased transmission and association in the given sample

Phenotype	Minimum genetic effect for QTDT			Minimum genetic effect for LAM		
	Additive	Dominant	Recessive	Additive	Dominant	Recessive
NWR	1.6 (0.53)	2.2 (0.73)	2.4 (0.80)	0.8 (0.27)	1.0 (0.33)	1.7 (0.57)
SR	4.3 (0.54)	5.8 (0.73)	6.5 (0.81)	2.0 (0.25)	2.6 (0.33)	3.0 (0.38)
RWRE	0.4 (0.40)	0.6 (0.60)	0.7 (0.70)	0.2 (0.20)	0.3 (0.30)	0.4 (0.40)
WATT	6.1 (0.41)	8.3 (0.55)	9.1 (0.61)	2.9 (0.19)	4.0 (0.27)	4.0 (0.27)
RAPA	0.6 (0.60)	0.8 (0.80)	0.8 (0.80)	0.3 (0.30)	0.4 (0.40)	0.4 (0.40)
FS-D	0.6 (0.60)	0.8 (0.80)	0.9 (0.90)	0.3 (0.30)	0.4 (0.40)	0.4 (0.40)

the *FOXP2* gene (Fig. 1): rs2035980, rs923875: promoter region and CpG island upstream from exon 1; rs12533005: transcription start site; rs10230558: polyQ region between exons 7 and 8; rs7782412, rs936146: zinc finger region. Two forkhead box SNPs, rs12154391 and rs12705970, were found to be in high linkage disequilibrium with one SNP in the zinc finger region and were excluded from the analysis.

In both the proband and parent cohorts, SNP genotypes showed two blocks of correlated SNPs for each of the two selected genes. The following *FOXP2* SNPs were mutually significantly correlated: rs12533005, rs10230558, rs7782412, and rs936146. The absolute magnitude of the correlation coefficients in neighboring SNPs ranged from .34 to .63 in the probands and from .35 to .61 in the parents (all p values <.0001). This indicates that the two SNPs near the promoter and CPG island were neither in linkage disequilibrium with each other nor with the remaining SNPs in this gene, whereas the SNPs located near the transcription start site, PolyQ region, zinc finger region, and, by inference, the forkhead box region, were significantly correlated as linear pairs. The four selected *CNTNAP2* SNPs were significantly correlated, with absolute correlation coefficients between neighboring SNPs ranging from .64 to .83 in the probands and from .70 to .81 in the parents (all p values <.0001). This pattern is not surprising, given that these SNPs are all located between exons 13 and 14.

All SNPs were in Hardy–Weinberg equilibrium, as calculated in the parent cohort only and using an exact test of the probability of observing the number of heterozygotes conditional on the number of minor allele copies, as

implemented in PEDSTATS (Wigginton and Abecasis 2005). Table 3 lists the SNPs, their physical positions (dbSNP130, NCBI Build 36), minor allele frequencies in the parent cohort, and p values from Hardy–Weinberg equilibrium testing in the parent cohort.

Following the *TaqMan Genotyping Master Mix Protocol* for a 10-μL reaction, PCR amplification and allele identification were carried out with an ABI 7500 Real-Time PCR system and ABI Sequence Detection Software Version 1.31. The reaction mixture contained 5 μL of TaqMan Genotyping Mix, 2.5 μL of water, 0.5 μL of an AB Validated TaqMan SNP Genotyping Assay probe mixture, and 10 ng of DNA. Enzyme activation occurred at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing and extension at 60°C for 1 min. PEDSTATS (Wigginton and Abecasis 2005) was used to check for genotyping errors and pedigree inconsistencies.

Statistical analysis

To explore associations among the selected traits, pairwise correlations were calculated. Because of the familial relationships among the participants, these calculations were carried out separately in the parents and in the probands. A Bonferroni correction was used to correct for 15 comparisons in two groups, arriving at testwise significance level of alpha=.0017.

For the association analyses, relevant covariates for each phenotype variable were explicitly determined using linear regression modeling. Age and sex have previously been found to affect many of the variables in the family
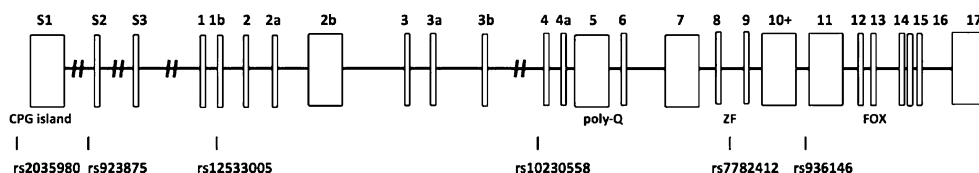

Fig. 1 Exon architecture of *FOXP2* (not drawn to scale) and position of selected SNPs. *ZF* zinc finger. Exons longer than 200 bp are represented with wide rectangles. Structure adapted from recent studies of *FOXP2* elements (Bruce and Margolis 2002; Schroeder and Myers 2008)

Table 3 Summary of selected *FOXP2* and *CNTNAP2* SNPs

Gene	Marker, AB ID	Physical position (dbSNP130)	Genetic environment	SNP alleles (Major/Minor)	Minor allele frequency	Hardy–Weinberg test p value
FOXP2	rs2035980, hCV1189578	113495736	Promoter, CPG Island	T/C	0.3630	0.2215
	rs923875, hCV1115584	113522272	Promoter, CPG Island	A/C	0.4348	0.4624
	rs12533005, hCV220195	113843291	Transcription Start Site	G/C	0.4535	0.0963
	rs10230558, hCV1604741	114032985	PolyQ Region	A/T	0.4880	0.6047
	rs7782412, hCV1604757	114077651	Zinc Finger	T/C	0.4420	0.5996
	rs936146, hCV7598355	114081641	Zinc Finger	G/C	0.4144	0.1657
CNTNAP2	rs851715, hCV2669966	147157839	Betw. Exons 13 and 14	A/G	0.3413	1.0000
	rs2710102, hCV11430714	147205323	Betw. Exons 13 and 14	C/T	0.4827	0.9181
	rs17236239, hCV34470590	147213238	Betw. Exons 13 and 14	A/G	0.3493	0.8202
	rs4431523, hCV32080083	147228099	Betw. Exons 13 and 14	T/C	0.3293	0.8150

participant set from which this sample was drawn, despite age adjustments in the normed test instruments and were, therefore, included as covariates in our previous studies (Brkanac et al. 2008; Raskind et al. 2000; Chapman et al. 2004). For this reason, age in months, sex, and an interaction between age and sex were evaluated for significant contributions to the score distributions of each of the selected phenotype variables, separately in the probands and parents. For the proband cohort, age was identified as a minor but statistically significant covariate for RWRE and RAPA. For the parent cohort, sex was identified as a covariate for NWR, SR, RWRE, and WATT, and age was also a minor but statistically significant covariate for NWR and SR. All measures had been preadjusted for age but not sex. One plausible reason for the need to add age as a covariate is that test norms, as mentioned, were based on child or young adult data and standard scores for adults were typically based on the highest normed age.

Because the data were collected at different time points under slightly different phenotyping protocols, not all tasks were completed by all families. In general, presence or absence of data in a given variable affected all members of a trio equally, because the phenotype was not assessed due to protocol differences. Trios with missing data for all three members were dropped from the statistical analysis. In rare cases, data were not available for a parent. For instance, in four trios, NWR data were not available for one of the parents. Missing parental phenotype data have no effect on QTDT, and in the linear association models, these missing datapoints were dropped.

To replicate the design in the language impairment study of *CNTNAP2* (Vernes et al. 2008), preferential SNP allele transmission for the selected phenotypes was evaluated with a family-based QTDT as implemented in MERLIN (Abecasis et al. 2002), selecting the Abecasis orthogonal model. This model depends only on within-family variance and a linear term describing parental mating type. The following alternative hypotheses are specified in terms of mating type (Ewens et al. 2008):

For aa X Aa parental mating type : $Y = \mu + \beta w + E$;
For Aa X Aa parental mating type : $Y = \mu + \alpha + \beta w + E$;
For AA X Aa parental mating type : $Y = \mu + 2\alpha + \beta w + E$.

where

Y is the quantitative phenotype
μ is the population mean
α is the linear term describing the expected mean offspring phenotype based on the parental genotypes and an additive model for the number of risk alleles
W denotes the number of excess risk alleles in a child
E is a random residual term with mean zero and variance σ^2 and the null hypothesis being tested is $\beta = 0$.

Because this model is based on the genotypes of parents and offspring but the phenotypes of only the offspring, covariates were selected based on their relevance to the probands only. Consequently, RWRE and RAPA were adjusted for participant age. Parental phenotypes were explicitly excluded from the analysis. We generated empirical p values for the within-family component w of the association, defined as excess transmission of the allele arbitrarily coded as "1" (Abecasis et al. 2000), as

implemented in MERLIN (Abecasis et al. 2002). In this permutation framework, each within-family component *w* is randomly replaced with itself or with its mirror image, −*w*, with equal probability (Abecasis et al. 2000).

Because our sample consisted predominantly of families of Caucasian descent as per parent report, we were able to add more powerful analysis tools based on linear association modeling (LAM) that were not restricted by the presence of population stratification. LAM is based on simple linear regression of phenotypes on genotypes and has the advantage of greater statistical power to detect association, compared to QTDT, because genotypes and phenotypes of all participants can be considered, whereas QTDT disregards all double homozygous parental mating types and all parental phenotypes. Associations were evaluated using PLINK version 1.06 (Purcell et al. 2007). In these procedures, covariates with relevance for each of the two cohorts and for each phenotype were incorporated. When LAM was run on the genotypes and phenotypes from the entire sample, the procedure was corrected for family structure by permuting parental phenotypes (Purcell et al. 2005), as implemented in PLINK. To further investigate differences between the two cohorts, LAM was conducted in the proband and parent cohort separately. All empirical *p* values in the QTDT and association testing were based on 100,000 permutations.

Given the lack of independence among the selected phenotypic traits and also among the SNPs, as documented with significant correlations, Bonferroni adjustments are unnecessarily stringent. Therefore, empirical significance was determined using Monte-Carlo global assessments of significance (McIntyre et al. 2000), as implemented in MERLIN (Abecasis et al. 2002), for an experimentwise alpha of.05. To evaluate the role of *CNTNAP2* in the six phenotypes, a single test *p* value of.0027 was required (.0014 for each cohort separately). To evaluate the role of *FOXP2* in these traits, a single test *p* value of .0015 was required (.0008 for each cohort separately). To further evaluate the distribution of the *p* values and their conformity to the expected distribution under the null hypothesis, they were compared to a distribution of –log10 *p* values expected under the null hypothesis of no association, using a QQ plot. The Monte-Carlo assessment of significance takes the lack of independence among the genotypes into account but not the correlations among the phenotypes, justifying the use of a QQ plot as an additional indication of significance.

Results

Pairwise correlation calculations among the six selected traits (Table 4) showed several statistically significant

correlations of moderate magnitude. In the probands as a group, four of the 15 pairwise correlations were statistically significant. In the parent group, which consisted of twice as many participants as the proband group, nine of the 15 pairwise correlations were statistically significant. All correlations except one were of the same sign in the parents and children, even if they were not statistically significant. Overall, ten of the 13 significant correlations involved measures of reading ability. In the probands, neither RAPA nor FS-D were correlated with each other or any of the reading, nonword, or sentence repetition tasks, but in the parents, RAPA was significantly correlated with FS-D. It was also correlated with RWRE, which is consistent with previous findings (Berninger et al. 2008a). Also in the parents, FS-D was correlated with WATT, a different reading skill shown to be related to poor spelling (Garcia et al. in press), the persistent impairment in adults with a history of dyslexia (Berninger et al. 2008a).

Empirical *p* values from all association tests with values <.05 are listed in Table 5. One *CNTNAP2* SNP, rs2710102, was associated with NWR at *p*=.0174, as calculated with QTDT. This association was nominally significant. Although it did not reach the adjusted alpha threshold of.0027, it exceeded expectation under the null hypothesis as seen in the QQ plot (Fig. 2). No other *CNTNAP2* association with a *p* value <.05 was found in any of the selected phenotypes.

Regarding the role of *FOXP2* in NWR and SR, QTDT and LAM resulted in several NWR associations. NWR was associated with rs7758412 at *p*=.0174 as tested with QTDT. LAM showed an NWR association with rs936146 in the proband cohort at *p*=.0067. These results met nominal and QQ criteria for significance. No *FOXP2* SNP showed an association with SR that met nominal, QQ, or adjusted alpha criteria for significance.

One measure of reading, RWRE, was associated with *FOXP2* SNPs. QTDT showed a nominally significant association with rs7782412. LAM within the entire sample confirmed this association with *p*=.0086. LAM within the cohorts separately found strong evidence of this association in the proband cohort at *p*=.0012. Within the parent cohort, rs923875 was associated with RWRE at 0=.0046. All RWRE SNP associations calculated with LAM met nominal and QQ criteria for significance. No associations with *p* values <.05 were found for WATT.

Both measures of timed sequential movement were associated with *FOXP2* SNPs. Within the parent cohort, LAM showed that rs12533005 was associated with RAPA at *p*=.0061, which met nominal and QQ criteria for significance, and a neighboring SNP, rs10230558, was nominally significantly associated with RAPA (*p*=.0498). QTDT showed a nominally significant association with FS-D for rs778412 and LAM within the parent cohort showed

Table 4 Pairwise correlation coefficients (and corresponding nominal asymptotic *p* values) for the selected traits

	NWR	SR	RWRE	WATT	RAPA	FS-D
NWR		**0.3626**	**0.2516**	**0.3662**	0.1559	0.1336
		(<0.0001)	**(0.0003)**	**(<0.0001)**	(0.0283)	(0.0607)
SR	0.2058		**0.2428**	**0.3830**	0.1165	−0.0282
	(0.0380)		**(0.0006)**	**(<0.0001)**	(0.1040)	(0.6946)
RWRE	0.2723	**0.3110**		**0.5079**	**0.2305**	0.1922
	(0.0054)	**(0.0015)**		**(<0.0001)**	**(0.0011)**	(0.0064)
WATT	**0.3322**	**0.5374**	**0.5022**		0.2171	**0.2344**
	(0.0006)	**(<0.0001)**	**(<0.0001)**		(0.0021)	**(0.0008)**
RAPA	0.1001	0.1770	0.1710	0.2858		**0.3407**
	(0.3142)	(0.0751)	(0.0841)	(0.0034)		**(<0.0001)**
FS-D	0.1781	0.1283	0.2396	0.1375	0.1433	
	(0.0748)	(0.2033)	(0.0158)	(0.1704)	(0.1529)	

Data from the parents are listed in the upper right triangle and data from the probands, in the left lower triangle. Statistical significance is indicated by bold font

a nominally significant association with FS-D for a neighboring SNP, rs936146. LAM showed associations with FS-D for rs923875 in the entire sample at $p=.0211$ and within the parent cohort at $p=.0035$. A neighboring SNP, rs12533005, was also associated with RS-D in the parent cohort at $p=.0223$. All three of these associations met nominal and QQ criteria for significance.

Discussion

The purpose of this study was to evaluate associations of polymorphisms in *CNTNAP2* and *FOXP2* with multiple traits in a sample of 188 family trios with dyslexia. We investigated whether *CNTNAP2* and/or *FOXP2* variants are associated with NWR, SR, reading, and sequential oral and finger movements in dyslexia, using QTDT and LAM.

Regarding *CNTNAP2* associations with the selected phenotypes, the QTDT analysis identified a NWR associa-

tion with one SNP, rs2710102, which was also shown to be associated with variations in NWR in the language impairment sample reported by Vernes et al. (2008). This result confirms the findings in the language impairment sample and extends them to a sample with dyslexia, implying that NWR in both disorder types may be influenced by the same genetic mechanism. In the present study, only one SNP showed this association and with lower statistical significance, compared to the language impairment study. There are several possible explanations for this discrepancy. It is possible that the strength of association increases with increasing severity of disability; the probands in the language impairment sample (Vernes et al. 2008) were more severely impaired (average—1 SD) than the probands in our study (average—.75 SD). In addition, NWR data were not available from all participants in the present study, which lowered the power to detect association. There were also methodological differences. Our study included one trio per family whereas the language impairment study included siblings, whether affected or not.

Table 5 Association results

Gene	Task	Marker	Risk Allele	QTDT	LAM (Parental Phenotypes Permuted)	LAM (Probands Only)	LAM (Parents Only)
CNTNAP2	NWR	rs2710102	Minor	*0.0174*			
FOXP2	NWR	rs7782412	Major	*0.0174*			
		rs936146	Minor			*0.0067*	
	RWRE	rs923875	Major				*0.0046*
		rs7782412	Major	0.042	*0.0086*	*0.0012*	
	RAPA	rs12533005	Major				*0.0061*
		rs10230558	Major				0.0498
	FS-D	rs923875	Major		*0.0211*		*0.0035*
		rs12533005	Major				*0.0223*
		rs7782412	Major	0.0456			
		rs936146	Major				0.0253

Nominal empirical *p* values <.05 are reported; bold italicized type indicates significance based on QQ evidence
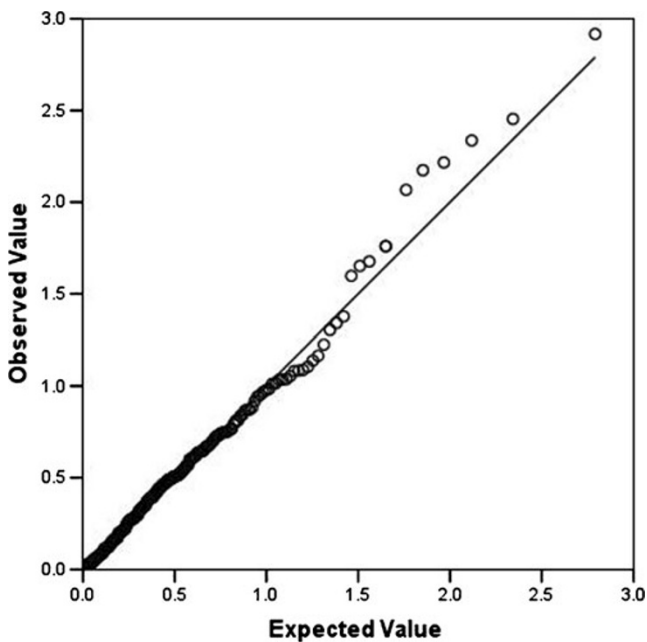
Fig. 2 QQ plot of quantiles from observed -10log values of the empirical *p* values compared to expected values under the null hypothesis

This larger and more broadly based sample may have provided greater variability in the data, conceivably increasing the power. Furthermore, the studies used different sets of nonwords for stimuli. Future studies should replicate this experiment in a larger dyslexia sample.

The hypothesis regarding an association between SR and *CNTNAP2* polymorphisms was not supported by the results, despite adequate power to detect association in this sample. The expectation to find this association was based on the Vernes et al. (2008) finding that a composite measure of expressive language was associated with one of the *CNTNAP2* SNPs. One possible reason for the absence of this association in our dyslexia sample is our use of a narrower phenotype, sentence memory, that is analogous to one of the subtests incorporated in the composite measure in the language impairment study. The association reported by Vernes et al. (2008) for the composite language measure was seen in only one of the SNPs and of much smaller magnitude than that for NWR. The fact that the present study showed one nominally significant *CNTNAP2* association with NWR but none with SR is consistent with this pattern.

The expectation to find *FOXP2* SNP associations with measures of spoken and written language and motor sequencing was based on the functional relationships between this gene and *CNTNAP2*, which is known to influence component traits in nonsydromic forms of language impairment. Because the dyslexia sample contained nonsyndromic forms of that disorder, we expected to find

primarily *CNTNAP2* SNP associations but unexpectedly found *FOXP2* SNP associations for several of the phenotypes. For instance, we found a nominal *FOXP2* SNP association with NWR, whereas in a previous study of language impairment, no evidence for *FOXP2* SNP associations with NWR or composite measures of expressive and receptive language was found (Newbury et al. 2002).

This study provides supportive evidence for the hypothesis that *FOXP2* influences performance on RWRE, a measure of reading that incorporates an element of rapid motor ability in the speech production domain. The task is to orally read words on a list under timed conditions and higher scores indicate a more rapid rate of reading the words. This task, however, also requires coordination of phonological, orthographic, and morphological codes and semantic representations (Berninger et al. 2008b). In contrast, no association was found for WATT, a reading task that also requires coordination of phonological, orthographic, and morphological codes but without access to semantic representation (Berninger et al. 2008b). This task is not timed and thus is less likely to tax the rate of motor output in the speech production system.

Our results provide suggestive evidence for the hypothesis that *FOXP2* variants are associated with timed sequential finger-to-thumb tapping. Linear association testing in the parent group showed three SNPs that were associated with FS-D. One of them, rs12533005, was also associated with RAPA. Given what is known about impaired oral praxis in individuals with *FOXP2* mutations, we expected to find an association with the RAPA measure. A *FOXP2* SNP association with motor sequencing in a finger task is consistent with a more general influence on motor systems beyond the oral and verbal systems. *FOXP2* associations with measures of movement sequencing across motor systems are consistent with the finding that mice with induced *FOXP2* mutations showed cerebellar growth reductions and gross motor impairments (Groszer et al. 2008). These associations are also consistent with the finding that, in individuals with *FOXP2* mutations, gray matter density was reduced in the cerebellum and the caudate nucleus, both of which are crucial for motor control (Belton et al. 2003).

To integrate the findings from *FOXP2* into a coherent picture, gene regions and cohort characteristics should be considered. Regarding gene regions, it is possible that the region surrounding the promoter and transcription start site harbors a variant that influences motor sequencing ability shared by RWRE, RAPA, and FS-D, all of which were collected under timed conditions and high scores require not only accurate but also rapid performance. RAPA and FS-D sample similar motor skills in that they require sequential movements in repeated sets but they differ in that RAPA taxes the motor speech system whereas FS-D involves finger movements in the grapho-motor system. Reading real words from a list as fast as possible involves

rapid sequential articulation of single words in addition to components of reading such as written word recognition. In this context, it is noteworthy that associations between FS-D and measures of writing have been documented, as FS-D brain activation was found to be significantly predicted by behavioral measures of word spelling in a sample that includes children with and without dysgraphia (Richards et al. 2009). By contrast, the zinc finger region and, because of its strong linkage disequilibrium with the probed SNPs downstream, perhaps also the forkhead box region may harbor a variant that influences the more linguistic aspects of reading ability. In the zinc finger region, association signals were seen in rs7782412 for NWR and RWRE and in rs936146 for NWR.

Regarding the associations in the two cohorts separately, one obvious difference, as mentioned, is the fact that the parent cohort consisted of twice as many participants as the proband cohort, which implies greater power to detect association. Furthermore, it is possible that the adults expressed variability in the more motoric aspects of word reading efficiency whereas the probands expressed variability in the more linguistic aspects of word reading efficiency. Adults have often reached their biological limits of motor speeds and their scores are more likely to represent a biological substrate of genes that are not exhibiting developmentally related changes in expression. In children, motor and other performance is still undergoing maturation and may vary due to other developmental changes that reflect the involvement of additional genes, for example those that affect myelination rates. Consistent with this hypothesis is our observation that FS-D and RAPA were significantly correlated in the parents but not in the probands. An increasing trajectory for motor speed in children, leveling off in adults and decreasing in older adults in direct parallel with myelin disintegration, has been described in the finger tapping literature (Gualtieri and Johnson 2006; Bartzokis et al. 2010; Prigatano 2007). Children may be more likely than adults to express the linguistic aspects of reading as a true representation of their biological limits. The children in this study all had dyslexia and the variability in their scores may be more tightly linked to the biological cause of their reading disability. Not all adults had dyslexia and the variability in their scores may represent other more randomly varying factors. We reiterate that the tests used in this study were normed largely on children and standard scores for adults are less reliable.

Together, these results provide support for a *CNTNAP2* SNP association with NWR in family trios with dyslexia, a sample not previously evaluated for this association. This study also provides suggestive evidence that *FOXP2* influences NWR, rapid oral reading, and measures of rapid motor sequencing ability beyond the oral motor and motor speech domain to also include finger movements. The findings motivate new research regarding the role of *FOXP2* in motor systems besides speech-related and hand movements, not only in samples with reading impairments but also with speech and language impairments, and also regarding differential *CNTNAP2* and *FOXP2* effects on various aspects of reading ability, including phonemic awareness, working memory, spelling, and handwriting.

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