

# Linkage of speech sound disorder to reading disability loci

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**Background:** Speech sound disorder (SSD) is a common childhood disorder characterized by developmentally inappropriate errors in speech production that greatly reduce intelligibility. SSD has been found to be associated with later reading disability (RD), and there is also evidence for both a cognitive and etiological overlap between the two disorders. The present study tested whether SSD is linked to replicated risk loci for RD. **Method:** One hundred and eleven probands with SSD and their 76 siblings were tested with measures of speech, phonological memory (Nonword Repetition – NWR), and phonological awareness and genotyped for linkage markers on chromosomes 1p36, 6p22, and 15q21. Both single point and multipoint linkage were tested with multiple methods. **Results:** The speech and NWR phenotypes were linked to the RD loci on chromosomes 6 and 15, with suggestive results for the RD locus on chromosome 1. **Conclusions:** It now appears that several RD loci are pleiotropic for SSD, extending the findings of Stein et al. (2004) for the RD locus on Chromosome 3. **Keywords:** Behavioral genetics, comorbidity, genetic, reading disorder, speech disorder.

The overall goal of the present study was to test whether speech sound disorder (SSD) is linked to risk loci for reading disability (RD), specifically those on chromosomes 1p36, 6p22, and 15q21 (see Fisher & DeFries, 2002 for a review). SSD is a common childhood disorder that affects as many as 16% of children at age 3 (Shriberg, 2002) and about 4% of children at age 6 (Shriberg, Tomblin, & McSweeney, 1999). It is defined by developmentally inappropriate errors in speech production that reduce intelligibility, and it is distinct from stuttering or mutism. In the past, SSD has been called *articulation* disorder and, more recently, *phonological* disorder. We prefer the SSD nomenclature because it recognizes that this disability has antecedents in both articulatory (sensorimotor) and phonological (cognitive-linguistic) domains. This term has been used recently in the classification of speech disorders from a genetic standpoint in the Online Mendelian Inheritance in Man (2005; #608445). As issues of genetic etiology and phenotypic overlap are resolved, the terminology may be refined further.

The rationale for hypothesizing a genetic overlap between a written language disorder, RD, and a spoken language disorder, SSD, derives from converging evidence of an association between the two disorders at three levels of analysis: symptom, cognitive, and etiological. We will next briefly review this evidence and then describe the current study.

## Symptom overlap

It is well documented that children with early speech/language problems are at increased risk for later literacy problems (Aram, Ekelman, & Nation, 1984;

Bishop & Adams, 1990; Catts, Fey, Tomblin, & Zhang, 2002; Hall & Tomblin, 1978; Magnusson & Naucler, 1990; Rutter & Mahwood, 1991; Scarborough & Dobrich, 1990; Snowling, Bishop, & Stothard, 2000; Shriberg & Kwiatkowski, 1988; Snowling & Stackhouse, 1983; Tomblin, Freese, & Records, 1992) and that individuals with literacy problems retrospectively report increased rates of earlier speech and language problems. Moreover, the latter association is not limited to retrospective reports, as young children selected for family risk for dyslexia (RD) and followed prospectively also have higher rates of preschool speech and language problems than controls (Pennington & Lefly, 2001; Gallagher, Frith, & Snowling, 2000; Lyytinen et al., 2002; Scarborough, 1990). One problem, however, is that these previous studies have rarely distinguished SSD from specific language impairment (SLI), which is defined by deficits in semantics and syntax. So, it is less clear which subtypes (or components) of SSD, per se, presage which kinds of later literacy problems.

## Cognitive overlap

The large majority of children with problems in printed word recognition (i.e., dyslexia or RD) have deficits on measures of phonological processing, both explicit (i.e., phoneme awareness) and implicit (i.e., phonological memory and rapid serial naming). There is also accumulating evidence that many children with speech and language difficulties have phonological processing problems, such as deficits on measures of phoneme awareness and phonological memory (Bishop, North, & Donlan, 1995; Bird & Bishop, 1992; Bird, Bishop, & Freeman, 1995; Clarke-Klein & Hod-

son, 1995; Edwards & Lahey, 1998; Kamhi, Catts, Mauer, Apel, & Gentry, 1988; Leonard, 1982; Lewis & Freebairn, 1992; Montgomery, 1995.)

These previous studies, however, have not resolved two key issues: 1) which phonological processing deficits are specific to SSD (apart from comorbid SLI) and 2) what is similar and what is distinct in the profiles of phonological processing deficits in SSD and RD? We began to address these issues in another study using the present sample, in which we tested how preliteracy skills in SSD varied across four subgroups, defined by crossing persistence of SSD and presence of language impairment (LI) (Raitano, Pennington, Tunick, Boada, & Shriberg, 2004). We found main effects for both persistence and LI, such that each factor was associated with worse performance on phonological awareness (PA) and other preliteracy measures. Even SSD children with normalized speech without LI were significantly worse than controls on PA. These results suggest a fairly extensive cognitive overlap between RD and SSD, apart from comorbid SLI, since PA deficits are a hallmark of RD. Yet there still may be phonological processing or other problems that distinguish SSD from RD. Although the constructs of phoneme awareness, phonological memory, and other phonological processing skills overlap theoretically (they all depend on phonological representations) as well as empirically (measures of these constructs are all moderately correlated), they nevertheless have distinct cognitive components and likely *independent* phenotypic and genetic covariance with SSD as compared to RD.

### *Etiological overlap*

Support for a shared etiology for SSD and RD has been provided by Lewis and colleagues (Lewis, 1992; Lewis, 1990; Lewis, Ekelman, & Aram, 1989), who found that SSD and RD are co-familial. In the current study sample, we have also found that SSD and RD are co-familial and that they co-segregate (Tunick, Boada, Raitano, Shriberg, & Pennington, submitted). We also found that SSD and RD are coheritable as well (Tunick & Pennington, 2002).

A partly shared genetic etiology would explain the documented symptom and cognitive overlap between SSD and RD, and would predict that some of the risk loci already identified for RD will also be risk loci for SSD, which is the main hypothesis of the current study. Several replicated risk loci or QTLs for RD have been identified, on chromosomes 1p, 2p, 3p-q, 6p, 15q, and 18p. Of these, the loci on 1p36 (gene symbol DYX8), 6p22.2 (DYX3), and 15q21 (DYX1) have been replicated by multiple independent studies which specifically targeted these regions and involved many families (Fisher & DeFries, 2002). Thus, these regions are likely to have higher gene frequencies and/or greater penetrances, making them excellent candidates for study of pleiotropic effects.

In an independent study, Stein and colleagues found linkage of SSD to the RD locus on chromosome 3 (Stein et al., 2004). They tested several related phenotypes, including SSD itself (as measured by the Percentage of Consonants Correct-Revised, discussed later), phonological memory (as measured by a nonword repetition test), phonological awareness, and reading. All of these phenotypes were linked to the RD risk locus on chromosome 3, indicating that this locus affects phonological development and contributes to the comorbidity between SSD and RD.

In sum, there is converging evidence from the symptom, cognitive, and etiological levels for an association between RD and SSD. A parsimonious hypothesis to explain this robust association between the two disorders is that they share at least some of their genetic risk factors. Support for this hypothesis has already been provided by the results of Stein et al. (2004), who found that the RD risk locus on chromosome 3 is pleiotropic for SSD. The present study extends the Stein et al. (2004) results by testing whether SSD is also linked to other well-replicated RD loci on chromosomes 1,6, and 15.

## Methods

### *Subjects*

The probands were 111 kindergarten children (age range 5–6 years,  $M = 68.9$  mos,  $SD = 8.1$ ) with a history of SSD ascertained through public and private schools and advertisements in the greater metropolitan area of Denver, Colorado. Some children were ascertained via special education personnel in the 4 area school districts. Parents of these children were sent a letter requesting permission for the school personnel to provide the names of the children to our laboratory. Another subset of children was identified by having the school district send out a letter describing our study to all parents of kindergarten children. Interested parents called our neuropsychology laboratory directly. Lastly, some children were also recruited via radio advertisements broadcast in the Denver area. Once families were ascertained via one of these methods, they all completed a phone interview to ensure that probands met the inclusionary and exclusionary criteria. Probands in the study had to have been previously evaluated for SSD and must have received speech/language therapy for this disorder. They had to live in monolingual English-speaking homes, and could not have any medical or genetic conditions which could be contributory, such as prematurity, birth complications, mental retardation, autism spectrum disorder, sensorineural hearing loss, craniofacial anomaly (e.g., cleft palate), or any other known or acquired neurologic condition. Siblings ( $N = 76$ ) between 5–8 years old ( $M = 86.5$  months,  $SD = 17.3$ ) had to meet the same criteria but were included regardless of their speech history. All probands and siblings were subsequently tested in our laboratory using an extensive battery of standardized and experimental tasks assessing auditory and speech perception, speech production, language, non-verbal ability,

and preliteracy measures. These are detailed in Raitano et al. (2004).

Genetic data were available from a total of 86 sib pairs from 65 families, although the exact number of sib pairs varied across phenotypes due to some missing data. The ethnic distribution (79% Caucasian) of study families was representative of Metro Denver and they were predominantly middle class (Mother's education:  $M = 15.7$  yrs.,  $SD = 2.4$ ; Father's education:  $M = 15.6$  yrs.,  $SD = 2.5$ ). Parents of all subjects gave consent and children gave assent under the approval of IRBs from the University of Denver and the University of Nebraska Medical Center. These subjects are described in more detail in Raitano et al. (2004).

### Behavioral measures

Parents were asked to fill out a detailed medical history for each of the children in the study to assess possible exclusionary factors more completely. A pure-tone hearing screening was conducted at the beginning of the first testing session to assure that the children had thresholds equal to or lower than 25 dBHL for .5, 1, 2, and 4 kHz frequencies bilaterally. An Orofacial Screening Examination was used to confirm adequate structure and function of the peripheral speech mechanism. Nonverbal intelligence (NVIQ) was assessed using the Matrices and Pattern Construction tests of the Differential Ability Scales (DAS; Elliot, 1990). Standard scores for these two subtests were averaged and subjects with a mean score less than 70 were excluded from the study. The NVIQ for both probands ( $M = 103.60$ ,  $SD = 11.6$ ) and siblings ( $M = 108.1$ ,  $SD = 10.9$ ) were solidly in the Average range.

Seven phenotypes were used in the linkage analyses, including five measures of speech development and two spoken language measures of phonological skill (nonword repetition and phonological awareness), which had previously been found to be associated with both SSD and RD, as discussed earlier. The first measure of speech development was a dichotomous diagnosis of affected status based on whether the child had a positive clinical history of SSD. The second measure of speech development was the Goldman-Fristoe Test of Articulation (GFTA; Goldman & Fristoe, 1986), a single word elicitation procedure where all production errors (e.g., omissions, substitutions, and distortions) are counted regardless of whether or not the error is developmentally appropriate. The total error score was converted to a standard score based on age norms, and used as a quantitative trait in the linkage analyses.

The remaining speech phenotypes were based on a blind transcription of a 50-utterance conversational speech sample subsequently analyzed using the Speech Disorders Classification System (SDCS; Shriberg, 1993; Shriberg, Allen, McSweeney, & Wilson, 2001). Unlike the GFTA, which penalizes a child for developmentally appropriate errors, the SDCS phenotypes are based only on developmentally inappropriate speech errors (e.g., omissions and substitutions not appropriate for the child's age). One phenotype was the age- and sex-standardized Percentage of Consonants Correct-Revised score (zPCC-R), and the other two were composite scores based on a model that discriminated children with persistent speech delay from children with typical

speech development. The model was derived from a separate sample of 759 children in 6 age groups (ages 3–8) by first performing exploratory univariate logistic regression with 120 variables, followed by selection of the 17 variables that showed the best sensitivity, specificity, and diagnostic accuracy. These 17 variables were then applied in multivariable logistic regression with Forward Selection with Switching, which reduced the number of variables down to 7, all of which contributed significantly to the prediction of the speech diagnosis. The form of the model was

$$\log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1x_1 + \beta_2x_2 + \dots + \beta_kx_k$$

where  $p$  was the probability that children were speech disordered, and  $x_1, x_2, \dots, x_k$  were the predictor variables. Two transformed measures of  $p$  were used as phenotypes: Speech1, the log odds ( $\log(p/(1-p))$ ), and Speech2, the probability estimate ( $1/(1 + e^{-\log(p/(1-p))})$ ).

The two final phenotypes were quantitative spoken language measures of phonologic skills. The first was a composite measure of phonological awareness (PA), comprised of the Bird and Bishop (1992) rhyme judgment task, and the Elision, Blending Words, and Sound Matching subtests of the Comprehensive Test of Phonological Processing (Wagner, Torgesen, & Rashotte, 1999). The raw scores from these four measures were averaged, age-corrected, and converted to a z-score to yield a PA composite score.

The second phonologic skill phenotype was Nonword Repetition (NWR), which measures the ability to repeat nonsense words varying in syllabic length. The version used was that of Dollaghan and Campbell (1998). This version, unlike more commonly used NWR measures, used nonword stimuli selected to reduce syllable lexicality, phoneme predictability, and articulatory difficulty confounds. Stimuli were presented via headphones and subjects' productions recorded. The proportion of phonemes correctly repeated was computed and then age-regressed for use as the NWR phenotype.

We were concerned that the NWR and PA phenotypes were potentially confounded with speech development itself, since NWR and most PA measures require speech production. However, our sample of SSD probands still performed significantly worse than our control sample (see Raitano et al., 2004) when this potential confound was removed. We re-scored the NWR, giving subjects credit for errors on phonemes that they consistently misarticulated on the GFTA. Even with this correction, probands were significantly worse than controls [(SSD:  $M$  total % phonemes correct = 72.1,  $SD = 11.4$ ; Controls:  $M$  total % phonemes correct = 84.2,  $SD = 7.2$ ;  $t(44) = -6.24$ ,  $p < .001$ ]. In addition, one of our PA measures (Rhyme Judgment) did not require speech production, yet the SSD group ( $M = 8.49$ ,  $SD = 3.4$ ) still performed worse than controls ( $M = 11.66$ ,  $SD = 2.4$ ) on this measure [ $t(102.1) = -6.50$ ,  $p < .001$ ]. So, the phonological deficit in this SSD sample is not restricted to output phonology.

### Genotyping

DNA was extracted from buccal brushes obtained from all participating children and their available biological parents. Markers from dyslexia candidate regions on

chromosomes 1p36, 6p22.2, and 15q21 were typed using fluorescently labeled primers on an automated ABI 3700 DNA sequencer (Applied Biosystems, Foster City, CA). The markers and their positions are shown in Table 1. The order of markers was determined from the published genome map NCBI MapViewer ([http://www.ncbi.nlm.nih.gov/mapview/map\\_search.cgi?taxid=9606](http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606)), and genetic distances were based on the deCODE map, also available at the NCBI site. This map was selected over the Marshfield map, since it is based on more meioses, but if a marker was not placed on the deCODE map, the position was extrapolated based on the physical distance and the relative distances between flanking markers on the Marshfield map. Heterozygosity was calculated from the study population using the Genetic Analysis System (GAS) v. 2 software (A. Young, Oxford University, 1993–1995). Allele calling was done by ABI Genotyper v. 3.7 software, and inheritance checking was done with GAS. The count recs feature in GENEHUNTER 2.1\_r5b (Kruglyak et al., 1996) and the error feature in MERLIN (Abecasis, Cherny, Cookson, & Cardon, 2002) were also used to detect errors in map placement or genotyping.

### Linkage analysis

The mode of inheritance for SSD is unknown, but like RD, it is assumed to be a nonmendelian quantitative trait. Accordingly, nonparametric linkage analysis methods were used. A variety of methods are available for these analyses, but several are not appropriate for

our selected sample. For example, variance components methods are powerful, but assume multivariate normality of the probability distributions for the phenotype values (Feingold, 2001, 2002; Fisher & DeFries, 2002). Regression based sib-pair methods are robust to sample selection, and several approaches have been shown to be as powerful as variance components methods under certain conditions, such as the revised Haseman–Elston method, the DeFries–Fulker method (DeFries & Fulker, 1985; Cardon, Fulker, & Cherny, 1995) and MERLIN-REGRESS (Sham, Purcell, Cherny, & Abecasis, 2002). However, the latter derives its strength from the inclusion of the mean, variance, and heritability of the phenotypes from an unselected population, and these parameters are not available for our research variables. Of the regression methods, we selected the DeFries–Fulker Augmented analysis as our benchmark analysis since it has been used in our previous studies with RD (Gayán et al., 1999; Davis et al., 2001; Knopik et al., 2002; Deffenbacher et al., 2004). The DeFries–Fulker test is based on the regression to the mean of a sibling's phenotype score relative to a proband's score and their identity by descent (IBD) at a specific marker locus; that is, if a QLT is linked to the marker, higher IBD will result in a decreased difference (regression to the mean) between the siblings (Fulker et al., 1991). An interval analysis method is used for multipoint analysis, taking information from flanking markers to calculate IBD values for intervening locations (Cardon & Fulker, 1994). The SAS macro QMS2 (Lessem, Cherny, & Lessem, 2001)

**Table 1** Genotyping markers: Determination of map position from short arm terminus

Marker	Map position (cM)	Reference map (kb)	deCODE map (cM)	Marshfield map (cM)	Heterozygosity (observed)
Chromosome 1p36					
D1S199	0	19702	37.48	45.33	0.86
D1S2843	1.008	20256		46.61	0.69
D1S478	2.52	21345	40.00	48.53	0.84
D1S2698	5.29	23014	42.77	52.70	0.58
D1S2620	5.41	23609	42.89	52.70	0.59
D1S470	10.88	29828	48.36	57.83	0.73
Chromosome 6p22					
D6S1605	0	16416	35.70	31.01	0.78
D6S274	1.50	16854	37.20	32.62	0.79
D6S1567	2.71	17553	38.41	33.43	0.83
D6S1588	9.16	22153	44.86	38.24	0.73
D6S1663	10.36	22711	46.06	40.14	0.72
D6S1554	13.16	24952	48.86	42.27	0.75
D6S1571	13.56	25072	49.26	42.98	0.69
D6S105	15.05	27879	50.89	44.41	0.83
D6S306	15.10	28034	50.89	44.41	0.58
D6S258	15.15	29129		44.41	0.74
D6S1683	15.20	29261	50.89	44.41	0.71
D6S273	15.61	31792	51.31	44.96	0.75
D6S1666	15.92	32722		45.50	0.73
D6S291	19.81	36373	55.51	49.50	0.75
Chromosome 15q21					
D15S1044	0	37456		39.72	0.74
D15S994	0.49	38369		40.25	0.70
D15S143	5.80	45691	46.31	45.62	0.68
D15S1028	7.31	46784	47.82	45.62	0.87
D15S978	7.43	47062		45.62	0.70
D15S1017	9.43	49769	49.94	45.62	0.69
D15S1029	13.43	53750		47.85	0.61
D15S117	15.95	56267	56.46	51.21	0.86

implements the D-F Augmented multipoint analysis, but a reliable single point method is not available. Since multipoint analyses are dependent upon accuracy of the genetic map, the results were corroborated with a comparable single point linkage analysis using the revised Haseman–Elston approach in the S.A.G.E. 4.5 program SIBPAL (2003) using the mean-corrected cross-product of the phenotypes for each pair of sibs.

Since each methodology has its strengths and weaknesses (see Fisher & DeFries, 2002 for a summary), we also used analyses contained in GENEHUNTER 2.1\_r5b (Kruglyak, Daly, Reeve-Daly, & Lander, 1996) for verification. These are also multipoint methods, but use a maximum likelihood approach for estimation of IBD, taking into account information across all markers in the region. The 'NPL' analysis was used for the dichotomous diagnosis phenotype, and the 'nonparametric' analysis option was used for quantitative traits. The GENEHUNTER2 nonparametric test was originally implemented in the MAPMAKER/SIBS program (Kruglyak & Lander, 1995), and is a truly nonparametric test, in that it does not require any assumptions about the distribution characteristics of the phenotype. It is also a multipoint test, taking into account the information of all of the markers in the region simultaneously to calculate the IBD values. The test is based on the Wilcoxon rank-sum test, in which the sib pairs are ranked by the degree of phenotypic difference and a test statistic is derived as a function of the rank and the IBD at a given locus. The variance of the test statistic is used in the computation of a Z-score. Calculation of the NPL score (*nonparametric linkage*; Kruglyak et al., 1996) uses a more traditional linkage approach rather than a rank-sum test. This procedure builds on the multipoint method of calculation of IBD scores in MAPMAKER/SIBS by not only using the complete marker information, but also using complete pedigree information for the estimation of inheritance vectors for each marker. This information is combined with the phenotype information to determine if the inheritance vector at a marker locus reflects the segregation of a causal gene. (See Nyholt, 2002 for further discussion of the GENEHUNTER2 package.)

We did not correct for multiple analyses since the phenotypes are highly correlated (see Table 3 below) and the markers are tightly linked. In these circumstances, a Bonferroni correction would be too conservative, and an appropriate correction does not exist (e.g., Francks et al., 2004).

Since the regions we are investigating have been previously reported as possible regions of linkage to RD

in multiple data sets, we have adapted the criteria for acceptance of linkage proposed by Thomson (1994) which takes into account replication of linkage in multiple data sets, e.g., 1) weak linkage or association ( $p < .05$ ) obtained in at least 3 independent data sets or 2) moderate ( $p < .01$ ) obtained in at least 2 data sets or 3) strong linkage or association ( $p < .001$ ) in one, or in the overall, data set. We realize, however, that this assumes that SSD is a pleiotropic effect of the RD locus. To obtain an estimate of the power of our sample to detect linkage using a regression-based method, we used the DESPAIR program within the SAGE 3.1 package ([http://darwin.cwru.edu/sage/older\\_despair.php](http://darwin.cwru.edu/sage/older_despair.php)). This approach considers the effect of genetic heterogeneity, which is extremely likely with this disorder. The sibling relative risk,  $\lambda$ , is used as a measure of the genetic contribution to a trait in this analysis. Using available population data for incidence and sibling recurrence risk, we estimated  $\lambda$  at 10.5. Similarly,  $\lambda$  for RD can be estimated at 5. Therefore, we examined a range of  $\lambda$  from 5–12, with 50% of families linked to a given locus. With these assumptions, the number of sib pairs required to detect linkage at a criteria of  $p = .05$  ranged from 58–97. For a criterion of  $p = .01$ , 94–157 sib pairs were required. Since our analyses include 82 sib pairs, we should have sufficient power to replicate previous linkages.

## Results

### Subjects

The means and standard deviations for the quantitative phenotypes are presented in Table 2, with affected status determined using the diagnostic criteria described above. There were 26 males and 25 females in the unaffected group, and 57 males and 28 females in the affected group, reflecting the higher male:female sex ratio in clinically ascertained populations. In all, both phenotypes and genotypes were available from 61 families with 82 sib pairs.

Several of the phenotypes were highly correlated, as seen in Table 3. Note that for 3 of the phenotypes, higher scores were associated with poorer performance, accounting for some of the negative correlations between measures. Measures of articulation (zPCC-R, Speech1 and Speech2) were highly correlated, but tended to have low correlations with the phonologic measure PA, while the NWR and GFTA

**Table 2** Characteristics of the phenotypic measures

Phenotype	Unaffected				Affected				Total population			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
PA	49	0.013	1.003	4.685	90	-0.127	0.972	4.575	140	-0.077	0.978	4.750
NWR	35	0.183	0.773	3.679	82	-0.292	0.915	4.605	118	-0.140	0.900	4.605
GFTA <sup>1</sup>	49	-0.318	0.818	3.732	90	0.458	0.765	3.357	140	0.175	0.869	3.903
zPCC-R	48	0.733	0.960	5.790	89	-0.549	2.290	15.050	138	-0.085	2.022	15.310
Speech1 <sup>1</sup>	48	-1.192	0.783	4.315	90	0.050	2.407	17.065	143	-0.385	2.043	17.065
Speech2 <sup>1</sup>	48	0.253	0.140	0.777	90	0.448	0.299	0.935	143	0.381	0.266	0.935

<sup>1</sup>Higher scores indicate poorer performance on these tests.

**Table 3** Correlations between quantitative phenotypic measures

	PA	NWRE	GFTA	zPCC-R	SPEECH1	SPEECH2
PA	1					
NWR	0.400	1				
GFTA <sup>1</sup>	-0.307	-0.485	1			
zPCC-R	0.100	0.508	-0.570	1		
SPEECH1 <sup>1</sup>	-0.089	-0.517	0.550	-0.905	1	
SPEECH2 <sup>1</sup>	-0.108	-0.412	0.630	-0.773	0.862	1

<sup>1</sup>Higher scores on these measures indicate poorer performance.

phenotypes showed more moderate correlations with all of the other phenotypes.

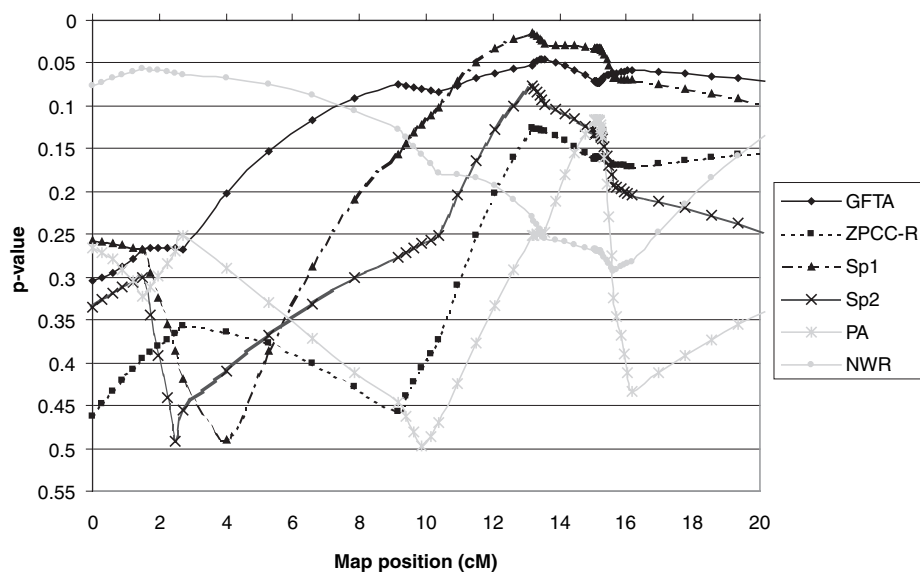
### Chromosome 1

Linkage analyses of chromosome 1 markers did not reach statistical significance with the GENEHUNTER or DeFries–Fulker Augmented methods, although one phenotype, GFTA, showed significance values approaching .065 in the region between D1S2698 and D1S26920. Single point analysis with SIBPAL corroborated this, with  $p = .053$  at D1S2620. Grigorenko et al. (2001) found evidence for linkage of RD across this entire region, with possible peaks at D1S199 and D1S470 when linkage to chromosome 6 was also taken into account, whereas Tzenova, Kaplan, Petryshen, and Field (2004) found linkage of RD around D1S507, which is distal to D1S199 and not covered in this study. Thus, while these results are suggestive of a genetic relationship between RD and SSD, a larger sample size and additional markers would be needed to detect linkage and determine the correspondence to an RD locus.

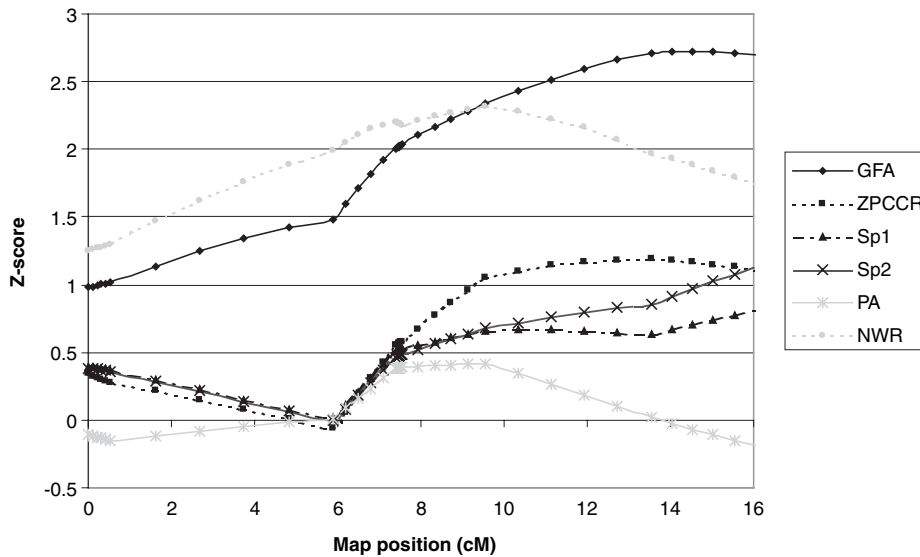
### Chromosome 6

The GENEHUNTER analyses were not significant with chromosome 6 markers, but two phenotypes, GFTA and Speech1, showed weakly significant results with the DeFries–Fulker Augmented analysis (Figure 1). In this figure, the significance level of the analysis is plotted against the map position along the chromosome.

Both phenotypes gave peaks in the region between markers D6S1554 and D6S1571, with  $p = .046$  for GFTA and  $p = .015$  for Speech1. Single point analysis placed the peak of linkage for GFTA slightly distally at D6S1588 ( $p = .044$ ), but did not detect linkage with Speech1. Interestingly, single point analysis detected linkage across the entire region with the diagnosis phenotype with a peak significance of  $p = .0006$  at D6S1571. Since the DeFries–Fulker Augmented method is designed for quantitative traits, this phenotype was not analyzed by that method. The region of highest significance for these three phenotypes corresponds to the small region of linkage and association seen with RD between



**Figure 1** Linkage of chromosome 6 markers and SSD phenotypes using DeFries–Fulker Augmented analysis. The significance ( $p$ ) of linkage is graphed against the chromosomal position of the markers on 6p22.2, with the  $p$  terminus to the left and the centromere to the right. Two phenotypes, GFTA and Speech1, reached significance levels indicative of replication of linkage to the reading disability locus on chromosome 6p22.2. All of the phenotypes except NWR appear to peak between markers D6S1554 (13.16 cM) and D6S1571 (13.56 cM). This corresponds to the highest region of linkage for reading disability observed in three independent studies



**Figure 2** Linkage of chromosome 15 markers and SSD phenotypes using GENEHUNTER2 nonparametric quantitative analysis. In this representation of linkage on chromosome 15q, the centromere is to the left and the q terminus is to the right. The NWR and GFTA measures both give Z scores over 2. The DYX1C1 locus (EKN1 gene) is 300 kb centromeric to D15S1029, which is placed at 13.43 cM

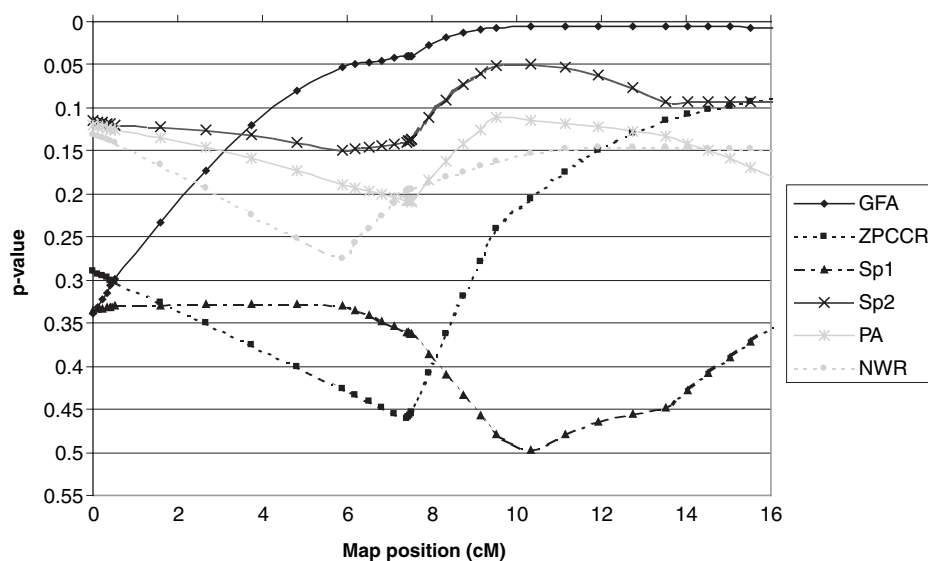
markers D6S1588 and D6S1571 by Deffenbacher et al. (2004) and corroborated by Francks et al. (2004) and Cope et al. (2005).

**Chromosome 15**

In contrast to the findings in the other chromosomal regions, the GENEHUNTER nonparametric analysis produced Z scores over 2 for the NWR and GFTA measures, and scores over 1 for the zPCC-R measure (Figure 2). The significant regions for all three phenotypes overlapped, with maximum Z scores of

2.310 for NWR between D15S1017 and D15S1029, 2.719 for GFTA between D15S1029 and D15S117, and 1.189 for zPCC-R very close to D15S1029.

The GFTA phenotype also showed significant linkage with the DeFries-Fulker Augmented analysis, with a peak of significance of  $p = .0053$ , also between D15S1017 and D15S1029 (Figure 3). This was verified by single point analysis, although the peaks were slightly distal ( $p = .011$  at D15S1029 and  $p = .006$  D15S117). The only other phenotype which reached weakly significant levels was Speech2,  $p = .049$  between D15S1017 and D15S1029.



**Figure 3** Linkage of chromosome 15 markers to SSD phenotypes using the DeFries-Fulker Augmented analysis. The DeFries-Fulker method verifies the linkage of GFTA to this region of chromosome 15, and also shows suggestive linkage of the Speech2 phenotype. As with the GENEHUNTER analysis, the region of linkage overlaps the DYX1C1 locus

The region of linkage for these phenotypes corresponds to the gene EKN1, which has been proposed as a candidate for RD (DYX1C1) (Taipale et al., 2003; Wigg et al., 2004). On the physical genomic map, this gene is located around 53.5 Mb from the p terminus, and the marker D15S1029 has been placed at 53.8 Mb (UCSC Human Genome Browser: <http://genome.ucsc.edu/cgi-bin/hgTracks?hgsid=37953916&hgt.out2=+3x+&position=chr15%3A53683735-53817336>).

## Discussion

The goal of this study was to test the hypothesis that SSD is linked to replicated RD loci, a hypothesis based on converging evidence of an association between SSD and RD at the symptom, cognitive, and etiological levels of analysis. We found evidence for replication of linkage of SSD phenotypes to RD candidate regions on chromosome 6p22 and 15p21, with suggestive results for chromosome 1p36. This was seen most consistently with the GFTA phenotype. Along with the results of Stein et al. (2004), our results indicate linkage of both SSD and RD in at least 3 chromosomal regions, suggesting that this subset of genes influences both disorders. The possibility of separate but linked genes cannot be ruled out, although it seems unlikely that such groups of genes would be present in all of these regions. A more parsimonious explanation is that RD and speech sound disorder share neurologic functions that are disrupted by genes in these regions.

If these findings are true, this study also supports the reported linkage of RD to EKN1 on chromosome 15. The strength of the linkage in this study could even suggest that SSD, particularly as measured by the GFTA phenotype, is a more salient phenotype than RD. As has been found with RD, however, it is perilous to characterize a locus as influencing one component phenotype more than another when the phenotypes are highly correlated (Pennington, 1997; Fisher et al., 1999; Gayán et al., 1999).

Although this study is similar to that of Stein et al. (2004) in the definition of SSD and the associated phenotypes (NWR and PA), there are two differences. First, the number of sib pairs in the current study is less than half the sample of 200 studied by Stein et al. (2004). Even with a relatively small sample of sib pairs, we found evidence for linkage, a result which suggests fairly strong effects. It will be important to replicate these results in larger samples and in other labs. The second difference concerns the age range of the subjects, which was 3–16 years ( $M = 7.35$ ) in Stein et al. (2004) and 5–8 years in the current study. None of our probands and only some of their siblings had begun formal reading instruction.

These results suggest the hypothesis that both SSD and dyslexia have a phonologic basis, and that

the core deficit for the speech phenotype is primarily phonologic rather than articulatory-motoric. To test this, children with SSD would need to be followed to determine if they do have a higher risk for RD, and children with RD should be tested thoroughly for residual articulatory defects. Further studies will also be needed to identify the genes involved and to determine genetic and/or environmental factors that may result in more symptoms of SSD in some families and RD in others. Finally, it is noteworthy that these RD/SSD regions have *not* been implicated in a related disorder which also shows comorbidity with SSD and RD, specific language impairment (SLI Consortium, 2002). This suggests that there are additional genetic influences on the development of speech and language disorders.

## Acknowledgements

This work was supported by a grant from NIH (MH38820). We thank Nancy Raitano, Rachel Tunnick, and Christa Hutaff for help with data collection on this project, and Karen Deffenbacher, Judy Kenyon, and Denise Hoover for their assistance in data management. We also thank Lauren McGrath and an anonymous reviewer for very helpful comments on the manuscript. An earlier version of this study was presented at The American Society for Human Genetics meeting in 2003.

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