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Speech Sound Disorder Influenced by a Locus in 15q14 Region

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Abstract Despite a growing body of evidence indicating that speech sound disorder (SSD) has an underlying genetic etiology, researchers have not yet identified specific genes predisposing to this condition. The speech and language deficits associated with SSD are shared with several other disorders, including dyslexia, autism, Prader-Willi Syndrome (PWS), and Angelman's Syndrome (AS), raising the possibility of gene sharing. Furthermore, we previously demonstrated that dyslexia and SSD share genetic susceptibility loci. The present study assesses the hypothesis that SSD also shares susceptibility loci with autism and PWS. To test this hypothesis, we examined linkage between SSD phenotypes and microsatellite markers on the chromosome 15q14–21 region, which has been associated with autism, PWS/AS, and dyslexia. Using SSD as the phenotype, we replicated linkage to the 15q14 region ($P = 0.004$). Further modeling revealed that this locus influenced oral-motor function, articulation and phonological memory, and that linkage at D15S118 was potentially influenced by a parent-of-origin effect (LOD score increase from 0.97 to 2.17, $P = 0.0633$). These results suggest shared genetic determinants in this chromosomal region for SSD, autism, and PWS/AS.

Keywords Phonology · Speech · Language · Parent-of-origin · Allele-sharing

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Introduction

Speech–sound disorder (SSD) is a common communication disorder of unknown etiology with an estimated prevalence of 15.2% in children at age 3, persisting in 3.8% of children by age 6 (Shriberg et al. 1999). The disorder is characterized by deficits in articulation, phonological processing, and in the cognitive representation of language. Although the causes of SSD have yet to be elucidated, a number of studies suggest that susceptibility to SSD is genetic, as previously described (Stein et al. 2004).

Prader-Willi syndrome (PWS) and Angelman syndrome (AS), two well characterized disorders that are influenced by loci in the 15q11–13 region with different parent-of-origin effects (Dykens et al. 2004), are associated with speech disability (Lewis et al. 2002; Moncla et al. 1999). The majority of cases of PWS (70%) are the result of a deletion in the 15q11–q13 region, with the remaining cases due to maternal uniparental disomy. The deletion subtype of PWS is associated with articulation difficulties including poor oral motor skills (Cassidy et al. 1997), and lower scores in reading, visual processing, and communication (Butler et al. 2004). Although individuals with PWS present with

depressed IQs (the average full scale IQ is 65), the SSD associated with PWS is severe and not explained by IQ alone. In a study of 55 individuals with PWS (24 with the deletion subtype), ranging in age from preschool to adult, all reported a history of or current enrollment in speech therapy for a SSD (Lewis et al. 2002). The deletion subtype of AS has also been associated with a lack of speech development and lower cognitive abilities (Moncla et al. 1999). In a survey of children with AS ($N = 20$) all vocalized but only 10 acquired single words, with 15 words the maximum number acquired (Alvares and Downing 1998). Most relied on non-speech methods of communication. Factors related to the expressive delay included cognitive delay as well as poor oral motor skills. A duplication of this region has been additionally associated with a phonological awareness deficit, single word reading impairment, articulation difficulties, language impairment, dyspraxia, and motor impairment (Boyar et al. 2001). Linkage to the 15q11–q14 region has also been demonstrated for autism (reviewed in Muhle et al. 2004) (Fig. 1), another disorder involving communication deficits, including speech and prosodic disorders (Shriberg et al. 2001). Although children with autism characteristically present with social language deficits, deficits in articulation including higher rates of residual speech sound errors have been reported (Shriberg et al. 2001).

SSD are often co-morbid with language impairment (LI). The comorbidity of SSD and LI has been estimated at 6–21% for children with receptive language disorders, and 38–62% for children with expressive language disorders (Shriberg and Austin 1998). Thus, comorbid expressive disorder is 2–3 times more common in SSD than comorbid receptive disorder. Shriberg et al. (1999) reported rates of co-morbidity between SSD and LI in children with persistent speech delay of 11–15% at 6 years of age, with considerably higher rates of 40–60% reported for preschool children (Shriberg and Austin 1998). Children with co-morbid SSD and LI often present with reading disorders (RD) at school age. High rates of co-morbidity of LI with RD have also been reported (Bishop 2001). Flax et al. (2003) found that 68% of LI probands also met the criteria for RD.

Cognitive deficits shared by SSD and RD include difficulties in the establishment of phonological, semantic, and morphosyntactic representations and in cognitive processing (Stein et al. 2004). However, SSD differs from RD in two primary ways. First, whereas reading is taught directly, speech acquisition is acquired less directly through exposure to a language environment. Second, SSD can be diagnosed in children of pre-school age, whereas RD cannot be diagnosed until children have been exposed to reading instruction. Many children with SSD eventually

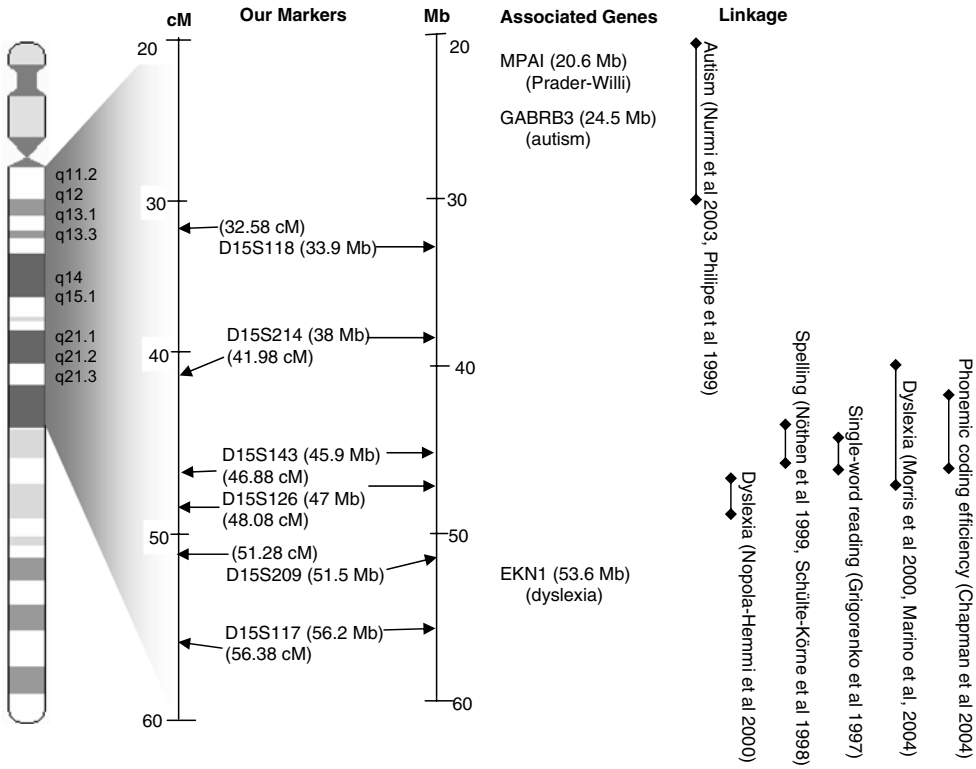

Fig. 1 Delineation of regions of linkage to PWS, AS, autism, and dyslexia for chromosome 15q11–q21. The width of the linkage interval for each study is represented by a solid line flanked by two diamonds. The reference scale for each study was determined by aligning the markers used with the UCSC physical map. Genetic (cM) and physical (Mb) distances are not to scale

develop deficits in reading, language, and spelling (Lewis et al. 2000). Pennington and colleagues have suggested that SSD predicts the development of dyslexia in individuals from high-risk families (Raitano et al. 2004). This common etiology leads to the hypothesis that the two disorders share susceptibility genes (Pennington 1997), and indeed, our previous genetic work supports this hypothesis (Stein et al. 2004).

Smith and colleagues (Smith et al. 1983) were the first to report linkage for dyslexia to chromosome 15. Other evidence suggests linkage for dyslexia to the chromosome 15q15–q21 region (DYX1; OMIM 127700) (Fig. 1; also reviewed in Fisher and DeFries 2002). Dyslexia-related endophenotypes have also been linked to this region, including single-word reading (Grigorenko et al. 1997), phonological awareness (Wigg et al. 2004), phonemic coding efficiency (Chapman et al. 2004), and word identification (Chapman et al. 2004; Wigg et al. 2004). Spelling disability has been linked to this region as well (Nöthen et al. 1999; Schulte-Körne et al. 1998; Wigg et al. 2004). However, other studies have not confirmed linkage for dyslexia or dyslexia endophenotypes to this region (Bisgaard et al. 1987; Cardon et al. 1994; Fisher et al. 2002; Rabin et al. 1993). Recently, a candidate gene in this region was identified (DYX1C1; OMIM 608706; also referred to as EKN1) and associated with dyslexia susceptibility in a Finnish population (Taipale et al. 2003). However, this association was not confirmed in a UK sample (Scerri et al. 2004) or in two Italian samples (Bellini et al. 2005; Marino et al. 2005). In a Canadian sample, dyslexia risk was associated with a different allele in EKN1 (Wigg et al. 2004). Genome scans examining linkage for specific language impairment, defined as the failure to acquire language skills in the absence of any intellectual, sensorimotor, or affective deficit, have not revealed linkage to this region of chromosome 15 (Bartlett et al. 2002; SLI Consortium 2002, 2004). Most recently, the EKN1 region has been linked to articulation and phonological memory (Smith et al. 2005).

Based on the overlapping phenotypes of SSD, PWS/AS, autism, and dyslexia, we hypothesized that loci in the chromosome 15q region that are linked to these disorders may also influence SSD susceptibility. In this study, we examined linkage of SSD phenotypes to microsatellite markers on chromosome 15q to further explore this hypothesis. EKN1 has been localized to 15q21.3 (~45.6 Mb), and the autism and PWS/AS regions lie proximal to DYX1 between 15q11–q14 (~23.2–34.0 Mb). We analyzed SSD affection as a binary trait, as well as quantitative measures of speech, language, and reading skills (i.e., quantitative endophenotypes), in families ascertained through a proband with SSD.

Methods

Study population

Children recruited as probands for the study were initially diagnosed as SSD by a speech–language pathologist. Further criteria for SSD included responses on the Goldman–Fristoe Test of Articulation (GFTA) below the 10th percentile and a minimum of four phonological process errors and severity rating of 3–4 on the Khan–Lewis Phonological Analysis Test, an additional analysis of responses to the GFTA. The GFTA is standardized for children ages 2–16 years. Children were also required to have normal hearing, as demonstrated by passing a puretone audiometric screening test; normal intelligence, defined as a performance IQ of at least 80 on the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) or Wechsler Intelligence Scale for Children, 3rd Edition (WISC-III); and a normal speech mechanism (adequate oral structures for producing speech sounds), as indicated from findings on the Total Structure subscale of the Oral and Speech Motor Control Protocol (Robbins and Klee 1987). Affected siblings of the probands met the same eligibility criteria. Affection status for parents was based on historical reports of speech–language problems or treatment. All family members provided informed consent and donated either blood or buccal samples for DNA analysis. The protocol was approved by the institutional review board of University Hospitals, Cleveland, OH. All behavioral measures utilized are cited elsewhere (Stein et al. 2004) unless otherwise noted.

Data collection

As part of a large study of familial aggregation and genetic susceptibility of SSD, a battery of age-appropriate tests was administered to the study participants to assess quantitative SSD traits. Articulation was assessed using both the GFTA and the Percentage of Consonants Correct (PCC), which assesses consonant errors in a conversational speech sample. The Nonsense Word Repetition Task (NSW) and a Multisyllabic Word Task (MSW) were given to assess phonological short-term memory and speech production in the context of nonsense words and complex

word forms, respectively. Both tasks were administered to participants aged 4–18 years.

Measures of oral motor skills included the Oral and Speech Motor Control Protocol (Robbins and Klee 1987) for children 2–6 years of age and the Fletcher Time-by-Count Test of Diadochokinetic Rate (Fletcher 1977) for older children. This test evaluates the motor function underlying speech–sound production. Both measures require the subject to rapidly repeat single (ORALMOT1) and multiple (ORALMOT2) syllable sequences. The number of syllable repetitions that the subject performs in 1 min are recorded. Normative data is available from 2 to 13 years. Three reading tests were also given to children aged 7 years and older. To assess reading decoding of real and nonsense words, we administered the Word Identification and Word Attack subtests of the Woodcock Reading Mastery Test (WRMT-ID and WRMT-AT). To measure reading comprehension, we administered the Wechsler Individual Achievement Test Reading Comprehension subtest (WIAT-RC).

Socioeconomic status (SES) was assessed through the Hollingshead Four Factory Index of Social Class (Hollingshead 1975). The Hollingshead Four Factor Index of Social Class employs the amount of formal education each parent has completed and the occupational level of each parent to compute a score for social class. The ordinal scores range for 1–5, with 1 representing the lowest social class and 5 the highest social class. Examples of occupational rankings are as follows: 1 = unskilled laborer, service worker; 2 = machine operator and semiskilled worker; 3 = skilled craftsman, clerical, or sales; 4 = medium business, minor professional, technical; 5 = major business or professional.

Molecular methods

High molecular weight DNA was isolated from an aliquot of blood with the Puregene Kit [Gentra Systems, Inc] or from buccal swabs using the BioRad InstaGene™ Matrix protocol [BioRad Laboratories, Inc.]. Microsatelite markers on chromosome 15q14–q21 (D15S118, D15S214, D15S143, D15S126, D15S209, and D15S117) were genotyped according to previously described methods (Stein et al. 2004). The genotype error rate, found by comparing replicates, was 5.2%.

Inconsistencies in the segregation of the genotypes within families were examined using MARKERINFO (S.A.G.E. v5.0). Individuals who demonstrated Mendelian inconsistencies at multiple markers that could not be resolved by retyping were treated as missing for the purpose of this analysis. The total of Mendelian inconsistencies and unresolvable genotypes represented 6.0% of the data. This error rate, along with the genotype error cited above, are likely due to poor DNA quality, since 82.74% of the missing data were found in buccal samples. After this data cleaning step, the marker informativity, in terms of Shannon information content, ranged from 0.7084 (D15S118) to 0.9480 (D15S117). Relationships were confirmed using a likelihood-based analysis of all available molecular data, implemented in RELPAIR (Epstein et al. 2000). Finally, because of the proximity of this region to those associated with PWS/AS, we examined genotypes for potential trisomy, but found no such evidence. The distances between markers were ascertained using the most recent map available (Kong et al. 2004).

Statistical analyses

Prior to statistical genetic analysis, quantitative phenotypes were stepwise adjusted for age, age^2, sex, SES, and any significant two-way interactions within a regression model. The resulting residuals were used in linkage analyses. Marker allele frequencies were estimated using maximum likelihood methods in the FREQ program of S.A.G.E. v5.0. Identity-by-descent (IBD) sharing distributions for each marker were then generated by the GENIBD (S.A.G.E. v5.0) program. Both singlepoint and multipoint IBD estimates were computed and analyzed; for brevity, only the multipoint linkage results, which are more conservative, will be described here.

We examined the chromosome 15q14–q21 region using two complementary model-free linkage analysis approaches. First, we examined linkage to the binary and quantitative traits using Haseman–Elston regression (Elston et al. 2000; Haseman and Elston 1972), which regresses the sibpair trait value on the proportion of alleles shared IBD. A variety of parameterizations, as implemented in SIBPAL, are available for modeling the sibpair trait value. We utilized the weighted sum of the squared sibpair trait difference and squared mean-corrected sibpair trait sum (w4 option). This method provides the greatest power for analysis of large samples (Shete et al. 2003). However, the power of this method in finite samples is unknown. Thus, we tried a variety of parameterizations, and the w4 consistently gave the smallest *P*-values though all analyses agreed on significance. For the analysis of binary traits, the mean was set to the population prevalence of the disorder being analyzed (3.8% for SSD (Shriberg et al. 1999), 7% for language impairment (Tomblin et al. 1997) and 5% for reading impairment (Shaywitz et al. 1990)). Empirical *P*-values

were estimated using 10,000 permutations when significance attained the α =0.05 threshold.

Second, we conducted an affected sibpair (ASP) analysis using the conditional logistic model (Goddard et al. 2001; Olson 1999), as implemented in LODPAL (S.A.G.E. v5.0). LODPAL estimates λ_i, which is the recurrence risk ratio for an affected relative pair that shares i alleles IBD (for i = 0,1, or 2). The one-parameter model adds the further constraint that λ_2=3.634λ_1–2.634, based on the minimax argument of Whittemore and Tu (1998). The effect of a covariate was assessed by estimating λ_1=exp($\beta_1 + \gamma y$), where β measures the genetic effect at the marker and y is a phenotypic covariate. In essence, the ASP model assessed the relationship between allele sharing IBD and the sibpair recurrence risk as a function of phenotypic covariates. The covariates accounted for heterogeneity between sibpairs and thus increased the sensitivity of the analysis to detect phenotypic similarity in the linked ASPs. A significant increase in the evidence for linkage, as assessed by a likelihood ratio test, indicates the covariate explains the heterogeneity between sibpairs.

Incorporation of covariates within the conditional logistic model also allowed for the inclusion of discordant and concordantly unaffected sibpairs, as well as parent-of-origin effects. A parent-of-origin effect (justified by the fact that the PWS/AS are known to be imprinted) was assessed by comparing a model partitioning allele sharing of the maternal and paternal alleles with a standard model that does not allow for maternal and paternal differences. Specifically, separate estimates of β_1 are computed for maternal and paternal alleles and models with these parameters are compared with a baseline using a likelihood ratio test. Because the parent-of-origin analysis presented here includes all sibpairs for increased power (ASPs, concordantly unaffected, and discordant), the model comparison used to assess parent-of-origin effect models has 2 df.

Results

We analyzed linkage to speech production and reading phenotypes in 151 families, 126 of which were Caucasian (Table 1). Other ethnicities included African American (16 families) and East Asian (1 family), with the remainder of mixed descent. There were 2 extended pedigrees, both of which were Caucasian. Although rates of affection were higher in males than in females, the male/female ratio did not differ by ethnicity (P = 0.67). Demographic and comorbidity data are provided in Table 1. Non-Caucasians had a significantly larger proportion of probands and siblings with language disability that the Caucasian group (P = 0.001 and P < 0.0005, respectively). The non-Caucasian group also had a greater proportion of siblings with reading disability (P = 0.002). In addition, Caucasian affected siblings tended to be younger than their non-Caucasian counterparts (P = 0.019).

Table 1 Description of families

Characteristic of sample	Caucasian families	Other ethnicities
Description of families		
Number of pedigrees	126	25
Number of individuals	563	101
Number of males/females	310/253	49/52
Number of full sibling pairs	316	42
Mean sibship size (range)	2.51 (1–7)	1.91 (1–4)
Number of half sibling pairs	10	8
Number of concordantly affected sibling pairs	99	14
Number of concordantly affected half sibling pairs	1	2
Description of individuals		
Age		
Mean age of proband (range)	6.01 (3–12)	6.26 (3–10)
Mean age of affected siblings (range)	7.25 (3–12)	8.86 (4–11)
Mean age of unaffected siblings (range	7.70 (2–17)	8.22 (3–11)
SES: Hollingshead four-factor index		
Category 1	1.7%	22.2%
Category 2	6.9%	18.5%
Category 3	18.1%	22.2%
Category 4	45.7%	22.2%
Category 5	27.6%	14.8%
Comorbidities		
Prevalence of language disability in probands	58.5%	92.0%
Prevalence of language disability in siblings	17.4%	52.6%
Prevalence of reading disability in siblings[a]	19.3%	42.1%

[a]Probands were too young at the time of enrollment to ascertain reading disability

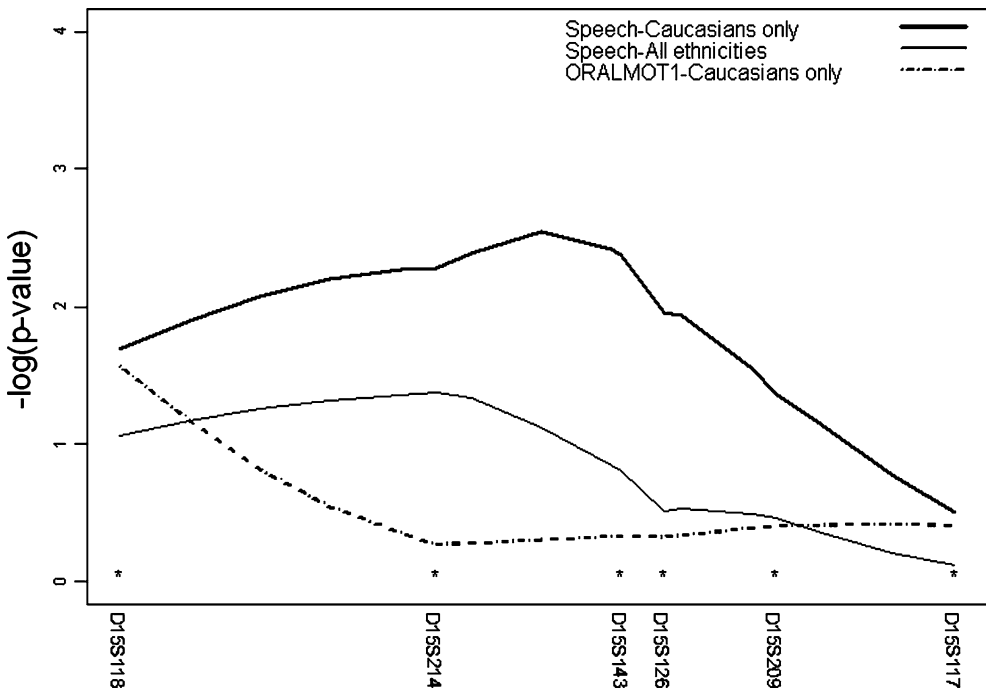

Fig. 2 Haseman–Elston linkage analysis results. Results of Haseman–Elston regression analyses for SSD in Caucasians alone and in the total sample, and for oral-motor function in Caucasians alone, plotted as $-\log_{10}$ (*P*-value) on the *Y*-axis against marker location on the *X*-axis

The results of the Haseman–Elston linkage analysis of speech affection are displayed in Fig. 2. Because there were insufficient numbers of families of other ethnicities to conduct a fully stratified analysis, we analyzed data for Caucasians only as well as for the total sample. Nominally significant evidence for linkage in the Caucasian subsample extended from marker D15S118 to D15S209, with the most significant results at D15S214 (empirical $P = 0.0072$) (Fig. 2). Analysis of the total sample revealed nominal significance at marker D15S214 ($P = 0.042$). These results suggest that the Caucasian subsample is more homogeneous than the total sample, and that there may be genetic heterogeneity in the other ethnic groups that we cannot account for given the small size of our non-Caucasian subgroup. ORALMOT1, the repetition of single syllable sounds, also demonstrated tentative linkage to D15S118 for the Caucasian subgroup (empirical $P = 0.048$). The remaining speech production and reading traits did not attain significant linkage at the $\alpha = 0.05$ level for the Caucasian subgroup (data not shown).

Results from the conditional logistic analyses are presented in Table 2. Data were again analyzed for the Caucasian subgroup as well as for the total sample, though significance was only attained in the caucasian subgroup. The inclusion of the two oral motor measures significantly increased the evidence for linkage at D15S118 and D15S214 (ORALMOT1: difference between models: $P = 0.001$ and 0.004, respectively; ORALMOT2: $P = 0.026$ and $P = 0.021$). Similarly, the inclusion of GFTA as a covariate increased the evidence for linkage at D15S118 (difference between models: $P = 0.054$) and D15S214 (difference between models: $P = 0.021$). The inclusion of NSW as a covariate also increased the LOD score at D15S118, though non-significantly (difference between models: $P = 0.133$). These results suggest that a putative locus in this region is involved in oral-motor function, articulation, and phonological memory. Inclusion of MSW and PCC did not significantly increase the evidence for linkage (data not shown). Because data on reading variables were only available for older siblings, we were unable assess the effect of these phenotypic covariates on linkage evidence.

Figure 3 displays LOD scores by chromosomal location that were obtained from analysis of parent-of-origin effects and Table 3 presents specific parameter estimates for marker D15S118. Though not significant at the $\alpha = 0.05$ level, the results suggest a parent-of-origin effect related to the linkage for SSD to the 15q14 region ($P = 0.0633$). Specifically, these results support the possibility of increased maternal allele sharing as the major contributor to the linkage signal. The fact that the parent-of-origin effect appeared to contribute only at the 15q14 end of the marker coverage, which abuts the PWS region, lends further support to this hypothesis.

Table 2 Conditional logistic models with covariates

Model	Marker	LOD score	Difference between models (*P*-value)
Baseline	D15S118	0.284	
	D15S214	0.089	
ORALMOT1	D15S118	2.595	0.001
	D15S214	1.850	0.004
ORALMOT2	D15S118	1.368	0.026
	D15S214	1.240	0.021
GFTA	D15S118	1.092	0.054
	D15S214	1.245	0.021
NSW	D15S118	0.775	0.133

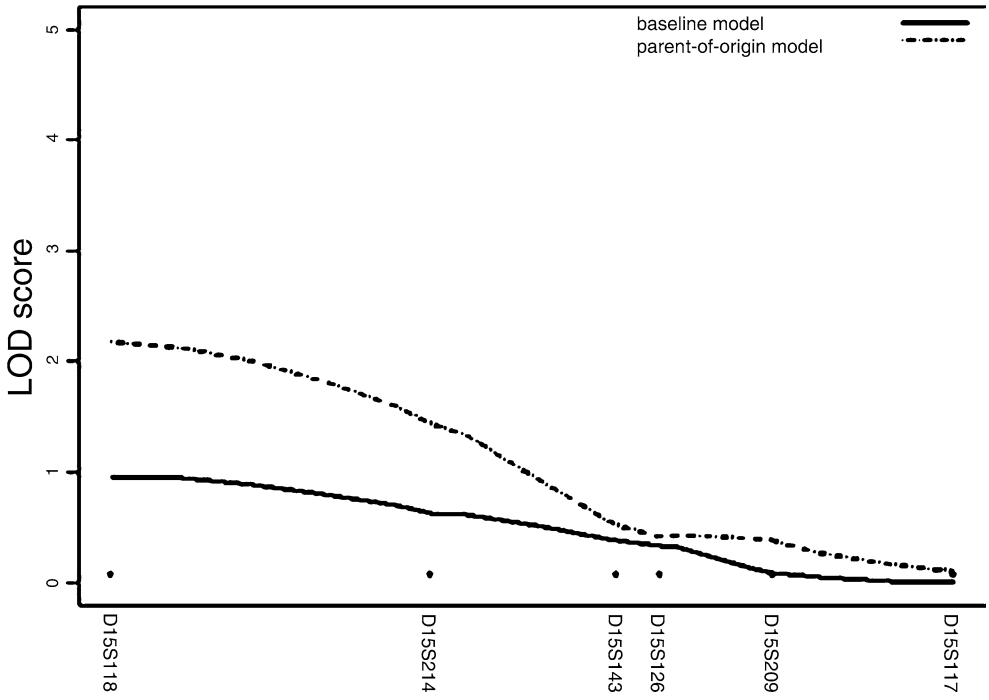

Fig. 3 Parent-of-origin analysis using conditional logistic model. Results of the conditional logistic analysis for models with and without parameters allowing for the parent-of-origin effect. LOD scores are plotted on the *Y*-axis against marker location on the *X*-axis

Table 3 Parent-of-origin analysis at marker D15S118 using all sibpairs

Model	LOD score	Beta1 m[a]	Beta1p	Difference between models (*P*-value)[b]
Baseline model	0.6304	0.1264		
Parent-of-origin model	2.1707	0.1980	0.0837	0.0633

[a]The baseline model constrains beta1 m = beta1p

[b]2 df test

Discussion

This study investigated linkage for SSD phenotypes to the region of chromosome 15 containing genes related to autism and PWS/AS (15q14), as well as to dyslexia (DYX1). We found suggestive evidence for linkage to the 15q14 region using SSD affection as a binary trait as well as ORALMOT1, but no evidence for linkage to the DYX1 region. Using a conditional logistic analysis of ASPs, we found strong evidence for linkage to D15S118 and D15S214 with the inclusion of the covariates ORALMOT1, ORALMOT2, GFTA and NSW. These covariates assess the oral-motor function (ORALMOT1, ORALMOT2), articulation (GFTA), and phonological memory (NSW) components of speech production. We also found potential evidence for a parent-of-origin effect at this locus. Together with our understanding of the speech manifestations of

PWS and autism (described below), these results suggest that a locus at or near 15q14, the region associated with PWS and autism, is also linked to SSD.

D15S118 is approximately 3 Mb distal to 15q13, which delineates the telomeric end of the PWS region (15q11–q13). Since linkage effects extend much farther than this distance, it is plausible that we are detecting the PWS/autism locus. Given the phenotypic relationship between PWS/AS and speech-related traits (Butler et al. 2004; Cassidy et al. 1997; Lewis et al. 2002; Roof et al. 2000), as well as autism (Shriberg et al. 2001), these studies suggest that a gene in the 15q11–q13 region may influence speech-related traits like SSD. Evidence of a potential parent-of-origin effect is of special relevance in this regard, as the region containing genes for PWS/AS is known to be imprinted (Dykens et al. 2004). Recently, reduced paternal transmission on 15q11.2 was associated with autism, suggesting the paternal allele is protective (Curran et al. 2005). In addition, Maestrini et al. (2000) found a maternally-inherited duplication on 15q that was associated with autism, further suggesting that our linkage results point to an autism locus rather than to DYX1. To further explore the hypothesis that a locus in this region is linked to SSD, genotyping of additional single nucleotide polymorphisms (SNPs) spanning this region is in progress. Our maximal linkage effects occur roughly 15 Mb centromeric from EKN1, which is too small a distance to definitively conclude that we are observing the effects of a different locus (Cordell 2001). However, because we did not attain even marginal significance in the DYX1 region, our findings make such a possibility unlikely. The fact that autism was not observed in the study population used to establish an association of dyslexia to the DYX1C1 region (Ylisaukko-oja et al. 2004) provides further support for distinct loci for dyslexia and autism. In fact, we hypothesize there may be additional loci in this region whose roles may be disentangled by future fine mapping and sequencing studies. Dyslexia phenotypes, moreover, have never been linked or associated to the autism/PWS/AS region.

As in previous studies (Bellini et al. 2005; Bisgaard et al. 1987; Cardon et al. 1994; Fisher et al. 2002; Marino et al. 2005; Rabin et al. 1993; Scerri et al. 2004), and a recent meta-analysis (Grigorenko 2005), we were unable to confirm linkage or association to the DYX1 region with speech or reading traits, though association may exist. Also, our linkage results differed from those of Smith et al. (2005) in both traits demonstrating linkage and in location. Though the GFTA measure attained nominally significant linkage, there is roughly 10 cM between the ends of our linked regions, and ~17 cM between our linked regions for NSW. Though we analyzed PCC, it did not attain significance, us it did in the analysis by Smith et al. (2005). Analyses in an Italian population found linkage to the DYX1 region (Marino et al. 2004), but no association to EKN1 (Marino et al. 2005), further suggesting genetic heterogeneity in this region. This suggests that DYX1C1/EKN1 may be a dyslexia susceptibility locus only in certain populations, or that it has a small effect not detectable by linkage or association. In addition to the variables described in this report, we also examined measures of vocabulary, rapid naming, verbal short-term memory, visual speed of processing, and spelling, and did not find any evidence of linkage to the 15q14–21 region (data not shown). Our lack of linkage findings for rapid naming and spelling measures may reflect differences in the measures employed by Wigg et al. (2004). Wigg et al. used the rapid digit naming subtest of the Comprehensive Test of Phonological Processing (CTOPP), whereas we employed a test assessing rapid naming of colors. The spelling metric we utilized, the Test of Written Spelling, also differed from the Wide Range Achievement Spelling test used by Wigg et al. (2004). The Test of Written Spelling distinguishes between regular and irregular spelling of words whereas the Wide Range Achievement Test does not. Spelling strategies may be different for regular words that can be sounded out than for irregular words that rely on memorization; these skills are influenced in part by phonological processing. Another important difference is that our proband group was younger, and since it is likely that a developmental trajectory affects spelling ability, our sample is not large enough to have the power to detect linkage to reading, spelling, and other quantitative measures with developmental trajectories. Our linkage results of spelling are difficult to compare to those of the German study group's, because they utilized a binary trait definition for spelling deficiency, and because of the difference between the German and English languages (Nöthen et al. 1999; Schulte-Körne et al. 1998).

Our hypothesis of multiple linked loci controlling cognitive abilities on this region of chromosome 15 bears striking similarity to the published genetic analyses of chromosome 7. Mutations in FOXP2 on chromosome 7 have been shown to be involved with apraxia/dyspraxia of speech (MacDermot et al. 2005). As noted by a recent review by Wassink and colleagues, there are several candidate regions on 7q, each yielding inconsistent results (Wassink et al. 2004). A number of studies, particularly those that analyzed language delay in autistic patients, found linkage to the region between 104 and 125 cM (Barrett et al. 1999;

Bradford et al. 2001; IMGSAC 2001), and a recent study suggested a polymorphism in FOXP2 was associated with autism (Li et al. 2005), though this was not the polymorphism associated with speech impairment. Other studies analyzing autism only have found linkage to a region between 135 and 150 cM (Alarcón et al. 2002; Ashley-Koch et al. 1999; IMGSAC 1998, 2001), including a meta-analysis refining the location to 142 cM (Badner and Gershon 2002), suggesting there are two separate loci on chromosome 7q—one controlling speech and/or language, another influencing autism. This possibility of genetic heterogeneity and multiple linked loci on chromosomes 7 and 15 warrants further research.

In sum, we have detected linkage between speech-sound phenotypes and chromosome 15q14, which has been linked to autism and PWS/AS. We did not replicate linkage to DYX1C1/EKN1, though this may be a result of the young age of our sample and/or differences in language studied. Fine mapping studies and association analyses are needed to refine these linkage results.

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