

Note: Underlining of [1], figs. 0 and tabs. 4 will be deleted during pagination

All rights reserved.

No part of this publication may be translated into other languages, reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, recording, microcopying, or by any information storage and retrieval system, without permission in writing from the publisher.

Approx. printed pages:

© Copyright 2003 by S. Karger AG, Basel, Switzerland

*Running title:*

Autism – Language Impairment Loci

## Original Paper

Hum Hered

DOI: 10.1159/0000XXXXX

020

022

023

24  
25  
26  
27

# Examination of Potential Overlap in Autism and Language Loci on Chromosomes 2, 7, and 13 in Two Independent Samples Ascertained for Specific Language Impairment

n 28

029

Christopher W. Bartlett<sup>a</sup> Judy F. Flax<sup>a</sup> Mark W. Logue<sup>b</sup> Brett J. Smith<sup>d</sup>  
Veronica J. Vieland<sup>b,c</sup> Paula Tallal<sup>a</sup> Linda M. Brzustowicz<sup>a,d,e</sup>

030

031

032

033

<sup>a</sup>Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, N.J.;

<sup>b</sup>Program in Public Health Genetics, Coll Public Health, and <sup>c</sup>Department of Psychiatry, College of Medicine,

University of Iowa, Iowa City, Iowa; <sup>d</sup>Department of Genetics, Rutgers University, and <sup>e</sup>Department of Psychiatry,

University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, N.J., USA

035

036

Received: June 10, 2003

Accepted after revision: August 25, 2003

037

038

039

040

041

**KARGER**

Fax +41 61 306 12 34

E-Mail [karger@karger.ch](mailto:karger@karger.ch)

[www.karger.com](http://www.karger.com)

© 2003 S. Karger AG, Basel

0001-5652/. . . . . \$19.50/0

Accessible online at:

[www.karger.com/hhe](http://www.karger.com/hhe)

Christopher W. Bartlett

Rutgers University, Department of Genetics, Nelson Biological Laboratories, Room B314

604 Allison Rd

Piscataway, NJ 08854-8095

Tel. +1 732 445 1638, Fax +1 732 445 1636, E-Mail [bartlett@axon.rutgers.edu](mailto:bartlett@axon.rutgers.edu)

060

n 061

062

---

**Key Words**

Autism · Language impairment · Multiple data sets ·  
Heterogeneity · Linkage analysis

064

065

-066

-067

-068

-069

070

071

072

073

-074

075

-076

077

078

-079

080

081

082

083

084

-085

086

-087

088

089

090

---

**Abstract**

Specific language impairment is a neurodevelopmental disorder characterized by impairments essentially restricted to the domain of language and language learning skills. This contrasts with autism, which is a pervasive developmental disorder defined by multiple impairments in language, social reciprocity, narrow interests and/or repetitive behaviors. Genetic linkage studies and family data suggest that the two disorders may have genetic components in common. Two samples, from Canada and the US, selected for specific language impairment were genotyped at loci where such common genes are likely to reside. Significant evidence for linkage was previously observed at chromosome 13q21 in our Canadian sample (HLOD 3.56) and was confirmed in our US sample (HLOD 2.61). Using the posterior probability of linkage (PPL) to combine evidence for linkage across the two samples yielded a PPL over 92%. Two additional loci on chromosome 2 and 7 showed weak evidence for linkage. However, a marker in the cystic fibrosis transmembrane conductance regulator (7q31) showed evidence for association to SLI, confirming results from another group (O'Brien et al. 2003). Our results indicate that using samples selected for components of the autism phenotype may be a useful adjunct to autism genetics.

Copyright © 2003 S. Karger AG, Basel

094

**Introduction**

-096

097

098

099

100

101

102

-103

104

105

106

107

108

Specific language impairment (SLI) is a neurodevelopmental disorder clinically defined as a failure to acquire and/or use language normally given adequate education and a suitable environment and in the absence of mental retardation, speech-motor or sensory deficits. SLI affects approximately 7% of children entering kindergarten [1] and is associated with generally poor academic outcomes if unresolved [2–5]. SLI has a genetic component as evidenced by familial aggregation and twin studies [6–9]. Recently, two groups have performed genomewide screens to search for SLI susceptibility genes and have attained LOD scores greater than 3 at non-overlapping loci on chromosomes 13, 16 and 19 [10, 11].

118 In contrast to SLI, autism is a relatively rare disorder  
119 that presents with abnormal development of language and  
120 social responses/initiation, and is also characterized by  
121 stereotypic behavioral repertoires [12]. The severity of  
- 122 language related deficits in autism can make administra-  
- 123 tion of standardized tests such as IQ difficult if other con-  
124 founding behavioral traits are present. Autism has a  
125 genetic basis evidenced by twin studies and numerous  
126 groups have undertaken the search for susceptibility genes  
127 [13, 14].

128 The language-only phenotype that is associated with  
- 129 specific language impairment is not as severe as the lan-  
130 guage difficulties seen in autism. Children with SLI are  
131 very able to perform many tasks useful in diagnosis or  
132 research, allowing the accurate measurement of reading,  
133 language and IQ. SLI is also a common disorder, making  
134 ascertainment of extended families with multiple affected  
135 individuals a reasonable task. Several lines of evidence  
136 have suggested that there may be genetic overlap between  
137 autism and SLI.

- 138 Familial studies have repeatedly found that after ascer-  
139 taining a proband for autism, the first and second degree  
- 140 relatives are commonly observed to have cognitive defi-  
141 cits that differ from autism in severity [15–20]. Language  
142 deficits are reported in many family studies of autism,  
143 though subjects have not been expressly evaluated for  
- 144 SLI. Twin studies have shown that while the MZ concor-  
145 dance rate for a strict diagnosis of autism is only 36%, the  
- 146 co-twin commonly has cognitive deficits, usually involv-  
147 ing language delay. Using a more liberal autism spectrum  
- 148 diagnosis that includes language delay raises the MZ con-  
149 cordance rate to 82% [21].

150 Language delay is more common in persons with SLI  
- 151 than the overall population [22], and the language diffi-  
152 culties that appear in relatives of probands with autism do  
153 not co-occur with mental retardation or general cognitive  
154 impairments [23–25]. Thus, the language phenotype of  
155 co-twins and relatives of probands with autism appears  
- 156 quite similar to the phenotype of SLI. Additionally, lan-  
157 guage profiles of children with autism indicate that at  
- 158 least a portion of children with autism have language pro-  
159 files similar to that of SLI children [26]. As standardized  
160 tests are not practical for many children with autism (only  
161 about half Kjelgaard and Tager-Flusberg's [26] sample  
- 162 could be measured using all tests), it is unclear what per-  
163 centage of cases with autism at the population level would  
164 have similar language profiles to SLI.

-174 Genetic studies of autism have suggested several loca-  
-175 tions where susceptibility genes may reside including  
-176 chromosomes 2q, 7q, and 13q [27–39]. All three loci at  
-177 2q31, 7q31–32 and 13q21 have been suggested by more  
-178 than one group. Convergence of linkage results on these  
-179 chromosomal regions in independent samples suggests  
-180 their involvement in the etiology of autism.

-181 The region on chromosome 7q31–32 was implicated in  
-182 an autism linkage study of 83 sib pairs by the Internation-  
-183 al Molecular Genetic Study of Autism Consortium [27]  
-184 which remained high after adding an additional 69 sib  
-185 pairs (MLS 3.55) [28]. Several other groups have suggest-  
-186 ed linkage to the same region [29, 33, 37, 40]. This region  
-187 was initially suspected to contain a language-related au-  
-188 tism gene by coincident linkage with the SPCH1 locus,  
-189 which has since been cloned (FOXP2) [41]. However,  
-190 studies have failed to detect defects in FOXP2 that are  
-191 associated with autism [42–44].

-192 Genetic association of SLI has been examined in the  
-193 7q31–32 region using a categorical definition of language  
-194 impairment and markers located in and around FOXP2  
-195 [45]. Two markers that showed nominal significance after  
-196 correction for multiple tests further indicating the pres-  
-197 ence of a language gene not necessarily related to autism.  
-198 The significant markers are located ~5 Mb centromeric  
-199 and ~3 Mb telomeric to FOXP2, respectively. O'Brien et  
-200 al. [45] failed to find association of SLI within FOXP2  
-201 itself, as was the case in two UK SLI samples [46, 47].  
-202 Additionally, a genome scan for dyslexia in a sample of  
-203 Finnish families suggested linkage to the region with an  
-204 NPL score of 2.77, with one family showing an NPL of  
-205 4.21 [48]. Direct sequencing of six subjects with dyslexia  
-206 and three controls failed to identify coding mutations in  
-207 FOXP2. Taken together, these data suggest that FOXP2  
-208 is not involved in either autism-related language impair-  
-209 ments or more common language impairments (i.e. dys-  
-210 lexia and SLI).

-211 Using quantitative trait linkage analysis with age of  
-212 onset for first word, a suggestive QTL was localized to the  
-213 7q autism region in the Autism Genetic Resource Ex-  
-214 change family collection [49]. The quantitative trait for  
-215 age at first word generated much higher evidence for link-  
-216 age to this region than the original analysis using a qualita-  
-217 tive autism diagnostic scheme [32]. The Collaborative  
-218 Linkage Study of Autism, (CLSA) performed an addition-  
-219 al analysis on their genome scan data [31]. When they sub-  
-220 set their families by the presence or absence of phrase  
-221 speech delay (PSD), defined by concordance of both  
-222 affected sibs for onset of phrase speech >36 months, the  
-223 LOD score for markers in the 7q31–32 region increased in  
-224 the PSD group [37].

234 Chromosome 13q21 is a second region that has been  
-235 implicated in both autism and SLI through linkage analy-  
236 sis. The CLSA [31, 37] initially found a multipoint HLOD  
237 of 2.3 in this region. However, when the families were  
238 subset on the PSD criteria, the PSD group had an HLOD  
239 of 2.54 while in the non-PSD group the HLOD was 0.0.  
240 This area was also suggested in another genome scan using  
-241 a screening set of families, but the MLS went to approxi-  
242 mately zero when a second set of families was added [30].  
243 As autism is a heterogeneous disorder, simple admixture  
-244 may account for the reduced MLS as power to detect link-  
-245 age in the presence of heterogeneity can decrease as sam-  
246 ple size increases when pooling is used to combine the  
247 data [50].

248 The SLI3 locus on chromosome 13 (OMIM # 607134)  
249 [11], directly overlaps with the potential autism locus [31,  
250 37]. As part of an SLI genome scan in 5 nuclear and  
251 extended Canadian families, three phenotypes were  
252 tested for linkage under dominant and recessive modes of  
253 inheritance. The SLI3 locus was found using a reading  
254 discrepancy phenotype (single nonword reading 1 SD  
-255 below nonverbal IQ) assuming a recessive mode of inheri-  
256 tance (LOD = 3.52, genomewide empirical p value  
257 <0.05). Intriguingly, the same maker in both the SLI and  
258 autism studies yielded the maximum multipoint HLOD  
259 score (D13S800).

260 Chromosome 2 is a third location with evidence for  
261 linkage to a language/autism phenotype. The IMGSA  
262 sample yielded a multipoint MLS of 4.8 using a narrow  
-263 diagnosis phenotype [28]. This region has also been exam-  
264 ined through stratification of two independent samples  
265 based on the PSD criteria described for the CLSA (above)  
266 [36, 39]. In both cases, the evidence for linkage increased  
267 in the PSD group. In Buxbaum et al. [36], the peak linkage  
268 finding overlapped IMGSA [38], while in Shao et al.  
269 [39] the peak was ~15 cM away.

270 Taken together, these data suggest that SLI and autism  
-271 may share a genetic component. Using a sample ascer-  
-272 tained for SLI has allowed us to carefully select our pro-  
273 bands and create detailed language and reading profiles  
274 that will increase our power to detect loci that influence  
275 language acquisition. While SLI is not autism, the use of  
276 our sample may prove to be a valuable adjunct to autism  
277 research by finding linkage to a genetic component that  
-278 overlaps both disorders or by finding a language impair-  
-279 ment susceptibility gene that modulates the autism phe-  
280 notype (either additively or epistatically).

290 This study presents linkage results from two samples  
- 291 selected for SLI. The first was a fairly homogeneous sam-  
292 ple of Celtic ancestry described in detail previously as part  
- 293 of an SLI genome scan [11]. The second sample was ascer-  
294 tained in the US without strict requirements for ethnic  
- 295 homogeneity. The two samples differ in marker allele fre-  
296 quencies and may differ in disease allele frequencies or  
297 display locus heterogeneity. We therefore sought to find a  
298 statistically rigorous way to combine linkage information  
299 across these two samples.

300 A variety of methods can be used to combine linkage  
- 301 information when different types of heterogeneity are sus-  
302 pected. This paper follows the notation of Vieland et al.  
- 303 [50], which illuminates several key points about combin-  
304 ing linkage information across samples. Briefly, two data  
305 sets can be pooled for calculation of one HLOD that  
306 assumes one theta and one alpha for both samples  
307 (HLOD-P) and loses power if the genetic models differ  
308 between samples. Alternatively, the HLOD scores can be  
309 calculated separately then added across samples (HLOD-  
310 S). As the maximum HLOD cannot be less than zero, the  
311 HLOD-S cannot accumulate negative evidence and is  
312 therefore anti-conservative.

313 It is also possible to collect the linkage information  
314 from each sample in the form of a posterior probability of  
- 315 linkage (PPL). The PPL is a Bayesian method for collect-  
316 ing linkage information in a flexible statistical framework  
- 317 that allows for removal of nuisance parameters by inte-  
318 grating them out of the equation [50–52]. If the admixture  
- 319 parameter between two samples is different, then combin-  
320 ing linkage information handling admixture as a nuisance  
321 parameter would be more efficient than either simple  
- 322 pooling (HLOD-P) or summation (HLOD-S). Further-  
323 more, the ‘apparent’ mode of transmission could differ  
324 between families with the same complex disease locus  
325 based upon heterogeneity at other loci. Thus, integration  
- 326 of parameters such as disease gene frequency and the pen-  
327 etrance vector will allow the data to accumulate valid  
328 linkage information without constraining the entire data  
329 set to one or two less than ideal alternatives such as the  
330 MMLS-C [53, 54]. In order to examine these propositions  
- 331 about combining linkage data in a real data set, we calcu-  
332 lated three statistics (HLOD-P, HLOD-S, and PPL) and  
333 discuss the potential differences.

**346 Subjects and Methods***347 Families and Phenotype Assignment*

348 The second sample consisted of 22 nuclear and extended families  
349 from the United States (N = 279). In each of these families, a proband  
350 was ascertained by either clinical referral or by announcement of the  
351 study criteria at professional conferences (see below for proband  
352 behavioral criteria). Families with at least one additional affected  
- 353 family member were included; the final sample consisted of 19 Cau-  
354 casian and 3 Hispanic families. Assessment of the US sample and  
355 proband designation were the same as described in Bartlett et al. [11];  
356 the assessment included measures of language, reading, non-verbal  
- 357 intelligence as well as an oral-speech mechanism screening. Addi-  
358 tionally, hearing was assessed by positive identification of 500 Hz (at  
359 30 dB), 1000, 2000 and 4000 Hz (at 20 dB) pure tones.

360 The overall data set consisted of two independent samples. The  
361 first sample was described in Bartlett et al [11]. Briefly, 2 nuclear and  
- 362 3 extended families of Celtic ancestry living in Canada were pheno-  
363 typed with language/reading measures (n = 73 subjects). Thirteen  
364 additional subjects (86 total) had DNA available. A speech language  
365 pathologist screened families by telephone interview for a history of  
366 language impairment segregating in the family. Families with a  
367 strong family history of language impairment were scheduled for  
- 368 assessment. All subjects received a comprehensive neuropsychologi-  
369 cal battery administered by an experienced tester in their own  
370 homes.

371 US families were included in the study if at least two persons met  
372 criteria for an SLI proband. All subjects were enrolled and tested  
373 after giving informed consent that conformed to the guidelines for  
374 treatment of human subjects approved by Rutgers University.

375 The three diagnostic classifications of impairment from Bartlett  
376 et al. [11] were employed. The classifications were not mutually  
377 exclusive; an individual subject could meet the criteria for more than  
- 378 one of the following classifications. A subject was classified as Lan-  
- 379 guage Impaired if their spoken language quotient on the Test of Lan-  
380 guage Development (TOLD) was  $\leq 85$ . A subject was classified as  
381 Reading Impaired if their single nonword reading score (Word  
382 Attack) was one standard deviation below their performance IQ.  
383 Clinical Impairment criteria are described in detail in Bartlett et al.  
384 [11]. Briefly, a subject was defined as clinically impaired if they fell  
385 into one of the following three groups. First, the subject was language  
386 impaired or reading impaired. Second, the subject was not language  
387 impaired, but scored one standard deviation below the mean on  
388 three individual subtests of TOLD or scored  $\leq 85$  on the receptive  
389 language measure (Token Test). Third, the subject had a history of  
390 language difficulty for at least two years during childhood. It was not  
391 necessary to exclude any subject from analysis because of mental  
392 retardation, abnormal hearing, oral motor or structural defects.  
393 Table 1 summarizes the diagnostic overlap for the American and  
394 Canadian samples.

*396 Genotyping*

397 All family members who were willing to submit DNA samples  
398 (n = 365) were genotyped. DNA was extracted from peripheral blood  
399 samples or buccal swabs as described previously [11]. Microsatellite  
400 markers were genotyped on chromosomes 2, 7, and 13 as shown in  
401 tables 2-4 using previously described methods [55]. The marker,  
402 CFTR-TET, is on chromosome 7 as described in Gasparini et al [56]  
403 (GDB:182312). PCR primers were ordered from Research Genetics  
404 as part of the Human Map Pairs set or redesigned from the GDB  
405 locus sequence using the Primer 3 program [57].

*Statistical Analysis*

Parametric analysis was performed with FASTLINK version 4.1P programs [58, 59]. The Language Impairment, Reading Impairment, and Clinical Impairment phenotypes were each analyzed under both a dominant and a recessive mode of inheritance, for a total of six analyses. Model parameters were the same as in Bartlett et al. [11]. Heterogeneity testing with performed with HOMOG. Marker allele frequencies were estimated separately for the two samples, by allele counting using all genotyped unrelated individuals. Genetic distance between markers were taken from the Marshfield Map [60]. SimWalk2 v2.83 [61] was used for genotype mistyping analysis as well as for generation of haplotypes. Files were analyzed several times using slightly different parameters and random number seeds to ensure convergence on a stable solution. Genotypes with a mistyping probability  $>0.05$  were compared to the raw data by two independent evaluators. Ambiguous genotypes were repeated or excluded. Haplotypes were used to determine crossover events in affected individuals within 13q21. Families with LOD scores greater than 0.6 at markers D13S1317 and D13S13109 were included in the determination of the critical region.

To combine linkage information across samples, we have calculated the HLOD for each sample separately [62], the HLOD-S and HLOD-P [50], and the PPL [11, 50, 51, 63, 64]. The PPL directly measures the probability that the recombination fraction between the marker and a putative disease gene is  $<0.5$  and incorporates a prior probability of linkage of 2% [65, 66]. Nuisance parameters such as the penetrance vector, disease gene frequency and the admixture parameter,  $\alpha$ , were integrated out assuming essentially uniform prior distributions for these parameters.

Family-based association tests were performed with the Pedigree Disequilibrium Test (PDT) version 4.0 [67, 68]. The PDT is a valid test of linkage and association in general pedigrees even in the presence of population stratification and calculates two alternatives of the test statistic. The AVE PDT weights each individual family's contribution equally, regardless of pedigree size and complexity while the SUM PDT weights families proportional to these factors. For the three markers where genotypes were omitted due to ambiguity ( $n = 4$ ), the TDT-AE was run instead of the PDT to ensure the correct type-I error rate [69]. To correct Type-I error for multiple correlated phenotypes and the use of both PDT statistics, we simulated 1000 unlinked markers using SIMULATE. All individuals (excluding those without available DNA) and all pedigrees were the same as used in the actual analysis, but marker genotypes were generated without regard to affection status. Each unlinked simulated marker was analyzed with both PDT statistics three times, once with each phenotype. The resultant p values were compiled into a single distribution and compared to the results of the actual analysis.

**Results**

In the 13q21 region, ten markers were genotyped in both samples. The four markers D13S788, D13S1317, D13S800 and D13S1306 were reported previously for the Canadian sample in Bartlett et al [11]. Data for 13q21 are summarized in table 2. Under the recessive reading impairment model, both samples maximize at D13S1317 (US, 2.616; Canadian, 3.565; HLOD-P, 6.031). Combining both samples with the HLOD-S shows a global maximum of 6.181 at the same location. Visual inspection of the by family LOD scores in the US sample indicates that most of the linkage signal comes from 4 pedigrees. De-

487 spite three of these four pedigrees showing evidence for  
488 linkage throughout the 13q21 region, the HLOD's for the  
- 489 whole US sample becomes greater than 1 in only two loca-  
490 tions (D13S1309 and D13S1317). Multipoint analysis of  
491 the US sample using markers D13S1309 and D13S1317  
492 decreased the HLOD slightly to 2.380. Marker D13S1231  
493 did not show evidence for linkage in the US sample  
494 (HLOD = 0), despite close proximity to D13S1317  
n 495 (<1 cM). The polymorphism information content (PIC)  
496 for D13S1231 was only 0.605 in the US sample, one large  
497 pedigree in particular showed a reduction of 0.6 LOD  
498 units due to homozygosity in several founders.

499 The PPL shows the effect of combining the Canadian  
500 and US samples. At D13S1317 the US PPL was 0.168  
501 while the Canadian PPL was 0.542. The combined PPL  
- 502 for the reading impairment phenotype was 0.923. Haplo-  
503 typing indicated critical recombination events in affected  
504 pedigree members between D13S1303 and D13S1309 as  
505 well as between D13S800 and D13S792. The critical  
- 506 region for SLI3 is still rather large, 7 cM with a corre-  
507 sponding physical distance of 16 Mb. However, this  
508 region contains only 12 known genes.

509 Chromosome 7 yielded two HLOD-S scores greater  
510 than 1 (D7S3052, 1.579 recessive clinical impairment;  
511 D7S2426, 1.776 recessive language impairment). The  
512 various heterogeneity linkage statistics for these two loci  
513 are displayed in table 3 by marker and phenotype. The  
514 strongest evidence for linkage was at D7S2426 where the  
515 combined US and Canadian PPL was 0.087 and the  
- 516 HLOD-S was 1.776 using the language impairment phe-  
517 notype. The combined PPL for marker D7S3052 was  
518 0.017 using the clinical impairment phenotype, with a  
519 corresponding HLOD-S of 1.579. The highest PPL for  
- 520 CFTR-TET was 0.017 using the clinical impairment phe-  
521 notype though it should be noted that CFTR-TET was  
522 only biallelic in our sample with low information content  
523 for linkage (heterozygosity = 0.43). As these two markers  
524 (D7S3052 and CFTR-TET) were previously implicated to  
- 525 be involved in language by genetic association, we per-  
526 formed the PDT on all chromosome 7 markers using the  
527 three phenotypes. The only marker with  $p < 0.05$  was  
528 CFTR-TET using the reading impairment phenotype  
- 529 (SUM PDT,  $p = 0.0262$ ; AVE PDT,  $p = 0.0223$ ). Simula-  
530 tion of 1000 unlinked markers analyzed with all three  
531 phenotypes show this result to be nominally significant  
532 after correcting for multiple phenotypes ( $p < 0.045$ ), but  
533 not after correcting for multiple markers ( $p < 0.32$ ).  
534 Though the PDT is a statistically valid test of linkage and  
535 association in extended pedigrees, such as ours, the power  
536 of 27 pedigrees is small.

546 Only one chromosome 2 marker gave rise to an  
- 547 HLOD-S greater than 1 (D2S2314, 1.716 recessive clinical  
548 impairment) with a corresponding PPL of 0.051. The  
549 language impairment model also showed evidence for  
550 linkage under the same genetic model (PPL 0.051,  
551 HLOD-S = 1.601). All chromosome 2 results are reported  
552 in table 4.

## 555 Discussion

557 In this study, we have demonstrated linkage of SLI to  
558 13q21 in an independent dataset ascertained in the US,  
559 lending further support to our previous linkage data in  
560 Canadian SLI families [11]. This replication occurred  
- 561 using the same phenotype and trait model as the Cana-  
- 562 dian families. The 13q21 locus is evident in our SLI fami-  
563 lies from the US, increasing the possibility that this locus  
564 is the same locus described in the CLSA's autism families  
- 565 that were also collected in the US [37]. Further, the link-  
- 566 age results of both groups are further supported by cyto-  
567 genetic evidence from a 13q21 deletion implicated in  
568 autism [34]. The paucity of known genes in the region, if  
- 569 true, will facilitate the joint cloning efforts that are cur-  
570 rently underway.

571 Visual inspection of the pedigrees at 13q21 indicates  
572 that only a small minority of US families are providing  
573 evidence for linkage (<30%), it appears unlikely that the  
574 gene(s) on 13q21 are necessary for SLI susceptibility and  
575 may not be a general risk factor in other populations. All  
- 576 of the families that provide evidence for linkage (Cana-  
577 dian and US) are Caucasian and were selected to have  
578 more than 1 person with a language impairment. Since a  
579 meta-analysis of family studies [7] has demonstrated that  
580 nuclear families ascertained through an SLI proband have  
581 no other affected family members ~ 31% of the time, our  
582 samples are likely to be biased towards finding genes that  
583 segregate in higher density families which may not be  
584 common in singleton family units ascertained through the  
585 same or different phenotypic criteria.

- 586 Our combined Canadian and US samples failed to pro-  
587 vide conclusive linkage results on either chromosomes 2  
588 or 7. A number of different reasons could explain these  
- 589 results. Since the PPL was greater than the prior probabili-  
- 590 ty of linkage for both chromosomes, though not tremen-  
591 dously so, if genes influencing language acquisition reside  
- 592 at these locations, they could be of relatively low pene-  
593 trance with small effect size and therefore difficult to  
594 detect with linkage analysis. If these genes have epistatic  
- 595 interactions with necessary or sufficient autism suscepti-  
596 bility loci, their effects may be more pronounced in  
597 autism samples. Additionally, the loci on chromosomes 2  
- 598 and 7 could be providing weak evidence due to locus het-  
- 599 erogeneity on a large scale, or could simply be false posi-  
600 tives. Furthermore, our phenotypes are derived from

610 scores on standardized tests of language and reading,  
611 which differs from affection status based on autism or  
612 phrase speech delay. Further work and collaboration will  
613 be required to refine our understanding of the role of these  
614 loci in both disorders.

615 Our sample demonstrated nominal association of a  
616 marker in CFTR with SLI. Use of extended pedigrees for  
617 categorical association studies is still being developed and  
618 for the statistical tests available, power is not very high for  
619 small samples [67]. Despite low power, we have suggested  
620 one of the same markers for association with SLI as  
621 O'Brien et al. [45]. They advocated an as of yet undefined  
622 mutation in FOXP2 or the promoter as a likely candidate  
623 for SLI susceptibility. However, none of their markers in  
624 FOXP2 showed evidence of association and the two  
625 markers they did implicate are ~5 Mb (D7S3052) and  
626 ~3 Mb (CFTR-TET) on either side of FOXP2. As 3–5  
627 Mb is rather large in terms of linkage disequilibrium, it  
628 may be more parsimonious to assume that CFTR or  
629 immediately surrounding genes are stronger positional  
630 candidates to influence language acquisition in autism  
631 and SLI. WNT2 is adjacent to CFTR, and has also shown  
632 evidence for association to autism with the majority of the  
633 association signal attributable to the PSD group [70].

634 The results of pooling our different samples yielded  
- 635 several interesting points. For D7S2426 under the Read-  
636 ing and Language phenotypes, both samples converged on  
637 1.0 as an estimate of the admixture parameter. However,  
638 the HLOD-P showed a decrease from 1.0 to 0.25 for the  
639 reading phenotype and 0.55 for the language phenotype.  
- 640 This illustrates simulation and analytical results in nu-  
641 clear families [71, 72] and highlights the difficulty in  
642 interpreting the admixture parameter in finite samples of  
643 extended pedigrees such as ours. Despite this difficulty,  
644 the HLOD is still a powerful tool for linkage detection  
645 allowing for heterogeneity in a given single sample [50,  
646 73–75]. As expected, in all cases the HLOD-P was lower  
- 647 than the HLOD-S, yet this was seen even when the admix-  
648 ture parameter for the HLOD-P was estimated to 1.0.

649 Over the markers considered, the HLOD-P did not  
650 agree with the PPL as much as the HLOD-S. This was  
- 651 suggested by Vieland et al. [50] for simulated nuclear fam-  
- 652 ilies, and also appears to be the case for extended pedi-  
653 grees. However, there was one data point where the  
- 654 HLOD-S and PPL results might lead to different interpre-  
655 tations about the likelihood of a susceptibility locus at this  
656 location (D7S3052, clinical impairment in Table 3) which  
657 could have several possible explanations. However, the  
658 computational differences between the HLOD-S and the  
659 PPL do not require these two statistics to be in agreement.  
660 The HLOD-S has been maximized over many parameters  
- 661 (2 thetas, 2 alphas, 2 modes of inheritance, and 3 pheno-  
662 types per marker) and is hard to interpret as the expected  
663 value of the HLOD-S under the null hypothesis given our  
664 maximizations is certainly greater than zero. However,

674 the PPL is a much better tool for accumulating evidence  
675 *against* linkage compared to the HLOD-S, due to the anti-  
676 conservative nature of summing statistics that can never  
677 be less than zero (the constituent HLOD's). Though we  
-678 have, in essence, maximized the PPL by use of three dif-  
679 ferent phenotypes, the PPL has two distinct advantages  
-680 over traditional methods, first being the ability to accu-  
681 mulate negative evidence as mentioned above, and the  
682 second being reduction of the probability of observing a  
683 misleading PPL as the sample size increases [50]. We do  
684 acknowledge that maximizing over phenotypic models  
685 will tend to inflate the probability of larger scores when  
686 there is no linkage, which is of concern, however the PPL  
687 increased when an independent data set was added, which  
688 provides another indicator that linkage is quite likely.

689 O'Brien et al. [45] is the second SLI genetics study to  
690 show the utility of dichotomizing quantitative traits for  
691 linkage and association analysis. In that study, the only  
692 results significant enough to report were with categorical  
693 phenotypes. The use of quantitative analysis for either  
-694 linkage or association failed to demonstrate/suggest link-  
695 age or association. Though use of a categorical trait  
696 requires an arbitrary (in the genetic sense) threshold for  
697 affection, it appears that the information lost through  
-698 dichotomy can be made up by the inherently greater sta-  
-699 tistical power of categorical linkage in at least *some* cir-  
700 cumstances. This phenomenon seems similar in form to  
701 the simulation and analytical results of Terwilliger [76]  
-702 where it was demonstrated that using a more determinis-  
-703 tic genetic model (i.e. higher penetrances and lower dis-  
704 ease frequencies) is more robust for linkage detection in  
-705 categorical analysis, than a less deterministic (though pos-  
706 sibly more realistic) genetic model since the latter assumes  
-707 a priori that many meioses are uninformative. While cate-  
-708 gorical analysis is not a 'one size fits all' statistical tech-  
709 nique and must still be used with caution and careful  
710 thought, it appears that the boundary between when  
711 researchers should use categorical versus quantitative  
712 analysis is very unclear. While categorical analysis seems  
-713 to be useful in SLI research, it will be important to devel-  
714 op pooling techniques for quantitative traits that have  
715 equivalent properties to the PPL.

**718****Acknowledgements**

-719 We would like to thank the participating families, who contrib-  
-720 uted their time and patience to make this study possible; Anne Bas-  
-721 sett for her assistance in ascertainment of the Canadian families; Te-  
722 resa Realpe-Bonilla, Linda Hirsch and Jason Nawyn for managing  
-723 the phenotype database; Jared Hayter and Ray Zimmerman for tech-  
-724 nical assistance in the laboratory; the testers associated with the Lab-  
-725 oratory of Paula Tallal; Neda Gharani for comments on earlier ver-  
726 sions. This research was supported by grants from the March of  
727 Dimes (Support to LMB), the National Alliance for Autism Research  
728 (Support to CWB and LMB). PT, LMB and JFF were supported by  
729 NIDCD RO1 DC01654. VJV is supported by NIH MH52841.

## References

- 1 Tomblin JB, Records NL, Buckwalter P, Zhang X, Smith E, O'Brien M: Prevalence of specific language impairment in kindergarten children. *J Speech Lang Hear Res* 1997;40:1245-1260.
- 2 Stothard SE, Snowling MJ, Bishop DV, Chipchase BB, Kaplan CA: Language-impaired preschoolers: a follow-up into adolescence. *J Speech Lang Hear Res* 1998;41:407-418.
- 3 Snowling MJ, Adams JW, Bishop DV, Stothard SE: Educational attainments of school leavers with a preschool history of speech-language impairments. *Int J Lang Commun Dis* 2001;36:173-183.
- 4 Snowling M, Bishop DV, Stothard SE: Is preschool language impairment a risk factor for dyslexia in adolescence? *Child Psychol Psychiatry Allied Disciplines* 2000;41:587-600.
- 5 Catts HW, Fey ME, Tomblin JB, Zhang X: A longitudinal investigation of reading outcomes in children with language impairments. *J Speech Lang Hear Res* 2002;45:1142-1157.
- 6 Nasir J, Cohen W, Cowie H, Maclean A, Watson J, Seckl J, O'Hare A: Genetics of specific language impairment. *Prostaglandins Leukot Essent Fatty Acids* 2000;63:101-107.
- 7 Stromswold K: Genetics of spoken language disorders. *Hum Biol* 1998;70:297-324.
- 8 Stromswold K: The cognitive neuroscience of language acquisition; in Gazzaniga M (ed): *The new cognitive sciences*. Cambridge, MA, MIT Press, 2000, pp 909-932.
- 9 Stromswold K: The heritability of language: A review and metaanalysis of twin adoption and linkage studies. *Language* 2001;77:647-723.
- 10 The SLI Consortium: A genome-wide scan identifies two novel loci involved in specific language impairment. *Am J Hum Genet* 2002;70:384-398.
- 11 Bartlett CW, Flax JF, Logue MW, Vieland VJ, Bassett AS, Tallal P, Brzustowicz LM: A major susceptibility locus for specific language impairment is located on 13q21. *Am J Hum Genet* 2002;71:45-55.
- 12 Fombonne E: The epidemiology of autism: a review. *Psychol Med* 1999;29:769-786.
- 13 Badner JA, Gershon ES: Regional meta-analysis of published data supports linkage of autism with markers on chromosome 7. *Mol Psychiatry* 2002;7:56-66.
- 14 Folstein SE, Rosen-Sheidley B: Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat Rev Genet* 2001;2:943-955.
- 15 Narayan S, Moyes B, Wolff S: Family characteristics of autistic children: a further report. *J Autism Dev Disord* 1990;20:523-535.
- 16 Landa R, Folstein SE, Isaacs C: Spontaneous narrative-discourse performance of parents of autistic individuals. *J Speech Hear Res* 1991;34:1339-1345.
- 17 Landa R, Piven J, Wzorek MM, Gayle JO, Chase GA, Folstein SE: Social language use in parents of autistic individuals. *Psychol Med* 1992;22:245-254.
- 18 Bailey A, Palferman S, Heavey L, Le Couteur A: Autism: the phenotype in relatives. *J Autism Devel Disord* 1998;28:369-392.
- 19 Szatmari P, MacLean JE, Jones MB, Bryson SE, Zwaigenbaum L, Bartolucci G, Mahoney WJ, Tuff L: The familial aggregation of the lesser variant in biological and nonbiological relatives of PDD probands: a family history study. *J Child Psychol Psychiatry* 2000;41:579-586.
- 20 Pickles A, Starr E, Kazak S, Bolton P, Papanikolaou K, Bailey A, Goodman R, Rutter M: Variable expression of the autism broader phenotype: findings from extended pedigrees. *J Child Psychol Psychiatry* 2000;41:491-502.
- 21 Folstein S, Rutter M: Infantile autism: a genetic study of 21 twin pairs. *J Child Psychol Psychiatry Allied Disciplines* 1977;18:297-321.
- 22 Shriberg LD, Tomblin JB, McSweeney JL: Prevalence of speech delay in 6-year-old children and comorbidity with language impairment. *J Speech Lang Hear Res* 1999;42:1461-1481.
- 23 Fombonne E, Bolton P, Prior J, Jordan H, Rutter M: Family study of autism: Cognitive patterns and levels in parents and siblings. *J Child Psychol Psychiatry Allied Disciplines* 1997;38:667-683.
- 24 Freeman BJ, Ritvo ER, Mason-Brothers A, Pingree C, Yokota A, Jenson WR, McMahon WM, Petersen PB, Mo A, Schroth P: Psychometric assessment of first-degree relatives of 62 autistic probands in Utah. *Am J Psychiatry* 1989;146:361-364.
- 25 Szatmari P, Jones MB, Tuff L, Bartolucci G, Fisman S, Mahoney W: Lack of cognitive impairments in first-degree relatives of children with pervasive developmental disorders. *J Am Acad Child Adolesc Psychiatry* 1993;32:1264-1273.
- 26 Kjelgaard MM, Tager-Flusberg H: An investigation of language impairment in autism: Implications for genetic subgroups. *Lang Cogn Processes* 2001;16:287-308.
- 27 International Molecular Genetic Study of Autism Consortium: A full genome screen for autism with evidence for linkage to a region on chromosome 7q. International Molecular Genetic Study of Autism Consortium. *Hum Mol Genet* 1998;7:571-578.
- 28 International Molecular Genetic Study of Autism Consortium: A genome-wide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet* 2001;69:570-581.
- 29 Philippe A, Martinez M, Guilloud-Bataille M, Gillberg C, Rastam M, Sponheim E, Coleman M, Zappella M, Aschauer H, Van Maldergem L, Penet C, Feingold J, Brice A, Leboyer M, van Maldergerme L: Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. [Erratum appears in *Hum Mol Genet* 1999 Jul;8(7):1353 Note: van Maldergerme L corrected to Van Maldergem L]. *Hum Mol Genet* 1999;8:805-812.
- 30 Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J, Kalaydjieva L, McCague P, Dimiceli S, Pitts T, Nguyen L, Yang J, Harper C, Thorpe D, Vermeer S, Young H, Hebert J, et al: A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet* 1999;65:493-507.

- 31 Collaborative Linkage Study of Autism: An autosomal genomic screen for autism. *Am J Med Genet* 2001;105:609–615.
- 32 Liu J, Nyholt DR, Magnussen P, Parano E, Pavone P, Geschwind D, Lord C, Iversen P, Hoh J, Ott J, Gilliam TC, The Autism Genetic Resource Exchange C: A genomewide screen for autism susceptibility loci. *Am J Hum Genet* 2001;69:327–340.
- 33 Shao Y, Wolpert CM, Raiford KL, Menold MM, Donnelly SL, Ravan SA, Bass MP, McClain C, von Wendt L, Vance JM, Abramson RH, Wright HH, Ashley-Koch A, Gilbert JR, DeLong RG, Cuccaro ML, Pericak-Vance MA: Genomic screen and follow-up analysis for autistic disorder. *Am J Med Genet* 2002;114:99–105.
- 34 Steele MM, Al-Adeimi M, Siu VM, Fan YS: Brief report: A case of autism with interstitial deletion of chromosome 13. *J Autism Dev Disord* 2001;31:231–234.
- 35 Gallagher L, Becker K, Kearney G, Dunlop A, Stallings R, Green A, Fitzgerald M, Gill M: Brief report: A case of autism associated with del(2)(q32.1q32.2 or (q32.2q32.3). *J Autism Dev Disord* 2003;33:105–108.
- 36 Buxbaum JD, Silverman JM, Smith CJ, Kilifarski M, Reichert J, Hollander E, Lawlor BA, Fitzgerald M, Greenberg DA, Davis KL: Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity. [Erratum appears in *Am J Hum Genet* 2001 Aug;69(2):470]. *Am J Hum Genet* 2001;68:1514–1520.
- 37 Collaborative Linkage Study of Autism: Incorporating language phenotypes strengthens evidence of linkage to autism. *Am J Med Genet* 2001;105:539–547.
- 38 International Molecular Genetic Study of Autism Consortium: Further characterization of the autism susceptibility locus AUTS1 on chromosome 7q. *Hum Mol Genet* 2001;10:973–982.
- 39 Shao Y, Raiford KL, Wolpert CM, Cope HA, Ravan SA, Ashley-Koch AA, Abramson RK, Wright HH, DeLong RG, Gilbert JR, Cuccaro ML, Pericak-Vance MA: Phenotypic homogeneity provides increased support for linkage on chromosome 2 in autistic disorder. *Am J Hum Genet* 2002;70:1058–1061.
- 40 Ashley-Koch A, Wolpert CM, Menold MM, Zaeem L, Basu S, Donnelly SL, Ravan SA, Powell CM, Qumsiyeh MB, Aylsworth AS, Vance JM, Gilbert JR, Wright HH, Abramson RK, DeLong GR, Cuccaro ML, Pericak-Vance MA: Genetic studies of autistic disorder and chromosome 7. *Genomics* 1999;61:227–236.
- 41 Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP: A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 2001;413:519–523.
- 42 Gauthier J, Joobor R, Mottron L, Laurent S, Fuchs M, De Kimpe V, Rouleau GA: Mutation screening of FOXP2 in individuals diagnosed with autistic disorder. *Am J Med Genet A* 2003;118A:172–175.
- 43 Newbury DF, Bonora E, Lamb JA, Fisher SE, Lai CS, Baird G, Jannoun L, Slonims V, Stott CM, Merricks MJ, Bolton PF, Bailey AJ, Monaco AP, International Molecular Genetic Study of Autism C: FOXP2 is not a major susceptibility gene for autism or specific language impairment. *Am J Hum Genet* 2002;70:1318–1327.
- 44 Wassink TH, Piven J, Vieland VJ, Pietila J, Goedken RJ, Folstein SE, Sheffield VC: Evaluation of FOXP2 as an autism susceptibility gene. *Am J Med Genet* 2002;114:566–569.
- 45 O'Brien EK, Zhang X, Nishimura C, Tomblin JB, Murray JC: Association of specific language impairment (SLI) to the region of 7q31. *Am J Hum Genet* 2003;72:1536–1543.
- 46 Meaburn E, Dale PS, Craig IW, Plomin R: Language-impaired children: No sign of the FOXP2 mutation. *Neuroreport* 2002;13:1075–1077.
- 47 Newbury DF, Bonora E, Lamb JA, Fisher SE, Lai CSL, Baird G, Jannoun L, Slonims V, Stott CM, Merricks MJ, Bolton PF, Bailey AJ, Monaco AP: FOXP2 is not a major susceptibility gene for autism or specific language impairment. *Am J Hum Genet* 2002;70:1318–1327.
- 48 Kaminen M, Hannula-Jouppi K, Kestila M, Lahermo P, Muller K, Kaaranen M, Myllyluoma B, Voutilainen A, Lyytinen H, Nopola-Hemmi J, Kere J: A genome scan for developmental dyslexia confirms linkage to chromosome 2p11 and suggests a new locus on 7q32. *J Med Genet* 2003;40:340–345.
- 49 Alarcon M, Cantor RM, Liu J, Gilliam TC, Geschwind DH, Consortium AGRE: Evidence for a language quantitative trait locus on chromosome 7q in multiplex autism families. *Am J Hum Genet* 2002;70:60–71.
- 50 Vieland VJ, Wang K, Huang J: Power to detect linkage based on multiple sets of data in the presence of locus heterogeneity: comparative evaluation of model-based linkage methods for affected sib pair data. *Hum Hered* 2001;51:199–208.
- 51 Vieland VJ: Bayesian linkage analysis, or: how I learned to stop worrying and love the posterior probability of linkage. *Am J Hum Genet* 1998;63:947–954.
- 52 Wang K, Huang J, Vieland VJ: The consistency of the posterior probability of linkage. *Ann Hum Genet* 2000;64:533–553.
- 53 Greenberg DA, Hodge SE, Vieland VJ, Spence MA: Power, mode of inheritance, and type I error in LOD scores and affecteds-only methods: reply to Kruglyak. *Am J Hum Genet* 1998;62:202–204.
- 54 Abreu PC, Greenberg DA, Hodge SE: Direct power comparisons between simple LOD scores and NPL scores for linkage analysis in complex diseases. *Am J Hum Genet* 1999;65:847–857.
- 55 Brzustowicz LM, Honer WG, Chow EW, Hogan J, Hodgkinson K, Bassett AS: Use of a quantitative trait to map a locus associated with severity of positive symptoms in familial schizophrenia to chromosome 6p. *Am J Hum Genet* 1997;61:1388–1396.
- 56 Gasparini P, Dognini M, Bonizzato A, Pignatti PF, Morral N, Estivill X: A tetranucleotide repeat polymorphism in the cystic fibrosis gene. *Hum Genet* 1991;86:625.

17

18

- 034  
-035  
-036  
037  
038  
039  
-040  
-041  
042  
-043  
-044  
-045  
046  
-047  
048  
-049  
050  
051  
052  
053  
054  
055  
-056  
057  
058  
059  
060  
061  
062  
063  
-064  
-065  
-066  
-067  
068  
-069  
070  
071  
072  
073  
074  
075  
076  
-077  
078  
079  
080  
-081  
082  
083  
084  
-085  
-086  
087  
088  
089  
-090  
091  
092  
093  
094  
095  
-096  
097
- 57 Rozen S, Skaletsky HJ: Primer3 on the WWW for general users and for biologist programmers; in Krawetz S, Misener S (eds): *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Totowa, NJ, Humana Press, 2000, pp. 365–386.
- 58 Cottingham RW, Jr., Idury RM, Schaffer AA: Faster sequential genetic linkage computations. *Am J Hum Genet* 1993;53:252–263.
- 59 Schaffer AA, Gupta SK, Shriram K, Cottingham Jr RW: Avoiding recomputation in linkage analysis. *Hum Hered* 1994;44:225–237.
- 60 Broman KW, Murray JC, Sheffield VC, White RL, Weber JL: Comprehensive human genetic maps: Individual and sex-specific variation in recombination. *Am J Hum Genet* 1998;63:861–689.
- 61 Sobel E, Lange K: Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am J Hum Genet* 1996;58:1323–1337.
- 62 Smith CAB: Testing for heterogeneity of recombination fraction values in human genetics. *Ann Hum Genet* 1963;27:175–182.
- 63 Logue MW: Complications of an unknown genetic model in the presence of heterogeneity for linkage analysis [Ph. D. Dissertation]. Ph D thesis, The University of Iowa, Iowa City, 2001.
- 64 Logue MW, Vieland VJ, Goedken RJ, Crowe RR: Bayesian Analysis of a Previously Published Genome Screen for Panic Disorder Reveals New and Compelling Evidence for Linkage to Chromosome 7. *Am J Med Genet, Neuropsychiatr Genet*. In press.
- 65 Elston RC: An approximation for the prior probability of autosomal linkage. *Cytogenet Cell Genet* 1975;14:290–292.
- 66 Morton NE: Significance levels in complex inheritance. [Erratum appears in *Am J Hum Genet* 1998 Oct;63(4):1252]. *Am J Hum Genet* 1998;62:690–697.
- 67 Martin ER, Monks SA, Warren LL, Kaplan NL: A test for linkage and association in general pedigrees: the pedigree disequilibrium test. *Am J Hum Genet* 2000;67:146–154.
- 68 Martin ER, Bass MP, Kaplan NL: Correcting for a potential bias in the pedigree disequilibrium test. *Am J Hum Genet* 2001;68:1065–1067.
- 69 Gordon D, Heath SC, Liu X, Ott J: A transmission/disequilibrium test that allows for genotyping errors in the analysis of single-nucleotide polymorphism data. *Am J Hum Genet* 2001;69:371–380.
- 70 Wassink TH, Piven J, Vieland VJ, Huang J, Swiderski RE, Pietila J, Braun T, Beck G, Folstein SE, Haines JL, Sheffield VC: Evidence supporting WNT2 as an autism susceptibility gene. *Am J Med Genet* 2001;105:406–413.
- 71 Vieland VJ, Logue M: HLODs, trait models, and ascertainment: implications of admixture for parameter estimation and linkage detection. *Hum Hered* 2002;53:23–35.
- 72 Pal DK, Greenberg DA: Evaluating genetic heterogeneity in complex disease. *Hum Hered* 2002;53:216–226.
- 73 Huang J, Vieland VJ: Comparison of ‘model-free’ and ‘model-based’ linkage statistics in the presence of locus heterogeneity: single data set and multiple data set applications. *Hum Hered* 2001;51:217–225.
- 74 Hodge SE, Vieland VJ, Greenberg DA: HLODs remain powerful tools for detection of linkage in the presence of genetic heterogeneity. *Am J Hum Genet* 2002;70:556–559.
- 75 Greenberg DA, Abreu PC: Determining trait locus position from multipoint analysis: accuracy and power of three different statistics. *Genet Epidemiol* 2001;21:299–314.
- 76 Terwilliger JD: On the resolution and feasibility of genome scanning approaches; in Rao DC, Province MA (eds): *Genetic Dissection of Complex Traits*. New York, Academic Press, 2000, pp 351–391.

**Table 1.** Overlap between phenotypic classifications

Phenotype	n (USA)	n (Canada)
LI only	0	0
RI only	44	4
CI only	30	11
CI+LI	38	18
CI+RI	16	6
LI+RI+CI	15	7

LI = Language Impairment; RI = reading impairment; CI = clinical impairment.

Note that by definition, individuals classified as LI will also be CI but the opposite is not necessarily true. The CI only group represents individuals that were identified by self report history or low subtest scores as described previously

**Table 2.** Maximum two-point heterogeneity LOD scores (admixture) across chromosome 13 makers for the phenotypes

Marker	cM	HLOD Canada	HLOD US	HLOD-S	HLOD-P	PPL
D13S788	45.55					
Clinical		0.848 (0.55)	0.457 (1.0)	1.305	1.296 (0.65)	0.032
Reading		1.11 (1.0)	0.816 (0.4)	1.926	1.465 (0.55)	0.041
Language		0.119 (0.1)	0.294 (1.0)	0.413	0.130 (0.1)	0.016
D13S1303	47.19					
Clinical		1.185(0.55)	0.224 (1.0)	1.409	1.322 (0.6)	0.049
Reading		1.824 (1.0)	0	1.824	1.018 (0.55)	0.101
Language		0	0.096 (1.0)	0.096	0	0.015
D13S1309	50.47					
Clinical		0.687 (1.0)	0.084 (1.0)	0.771	0.704 (1.0)	0.024
Reading		3.140 (1.0)	1.576 (0.8)	4.7155	4.488 (0.85)	0.638
Language		0	0.038 (1.0)	0.038	0	0.016
D13S1317	51.03					
Clinical		0.060 (0.15)	0.215 (1.0)	0.2757	0.076 (0.7)	0.016
Reading		3.565 (1.0)	2.616 (0.8)	6.181	6.031 (0.85)	0.923
Language		0	0.257 (1.0)	0.257	0	0.015
D13S1231	51.57					
Clinical		0	0.2469 (0.55)	0.2469	0	0.013
Reading		2.618 (1.0)	0	2.618	1.676 (0.4)	0.165
Language		0	0.038 (1.0)	0.038	0	0.015
D13S279	53.17					
Clinical		1.446 (0.65)	0.408 (1.0)	1.854	1.597 (0.7)	0.016
Reading		1.769 (1.0)	0.231 (1.0)	2.000	1.223 (1.0)	0.259
Language		0.003 (0.35)	0.129 (1.0)	0.132	0.018 (0.9)	0.015
D13S800	55.32					
Clinical		0.351 (0.35)	0.128 (1.0)	0.4789	0.212 (0.65)	0.019
Reading		2.974 (1.0)	0.242 (1.0)	3.216	2.701 (0.6)	0.228
Language		0	0.076 (0.9)	0.076	0	0.014
D13S792	57.46					
Clinical		0	0	0	0	0.014
Reading		0.656 (1.0)	0.368 (0.35)	1.024	0.896 (0.75)	0.033
Language		0	0.021(1.0)	0.021	0	0.015
D13S1306	59.79					
Clinical		0.041 (0.4)	0.205 (1.0)	0.246	0.187 (0.8)	0.014
Reading		0.74 (1.0)	0.434 (0.85)	1.174	1.145 (1.0)	0.105
Language		0.013 (0.6)	0.102 (1.0)	0.115	0.099 (1.0)	0.014
D13S160	61.17					
Clinical		0.637 (0.3)	0.112 (1.0)	0.749	0.608 (0.3)	0.013
Reading		1.711 (1.0)	0.231 (1.0)	1.942	1.223 (1.0)	0.063
Language		0.288 (0.3)	0.070 (1.0)	0.358	0.266 (1.0)	0.019

HLOD scores are for each sample separately, the HLOD-S, HLOD-P and PPL represent both samples combined. Note that when the HLOD equals 0, the admixture parameter is undefined and when the admixture parameter equals 1, the HLOD is a simple homogeneity LOD score.

**Table 3.** Maximum two-point heterogeneity LOD scores (admixture) for chromosome 7 markers by phenotype; format and notation is the same as table 2

	cM	HLOD Canada	HLOD US	HLOD-S	HLOD-P	PPL
D7S501	118.9					
415 Clinical		0.000	0.484(1.0)	0.484	0.462 (1.0)	0.026
421 Reading		0.382 (1.0)	0.483 (1.0)	0.865	0.854 (1.0)	0.027
Language		0.000	0.080 (1.0)	0.080	0.023 (1.0)	0.011
D7S2456	120.61					
427 Clinical		0.000	0.852 (0.7)	0.852	0.580 (0.55)	0.037
429 Reading		0.000	0.328 (0.35)	0.328	0.181 (0.25)	0.018
435 Language		0.000	0.682 (1.0)	0.682	0.433 (1.0)	0.024
D7S1817	121.41					
447 Clinical		0.000	0.132 (0.3)	0.132	0.087 (0.2)	0.017
449 Reading		0.057 (1.0)	0.000	0.057	0.000	0.013
455 Language		0.000	0.000	0.000	0.000	0.014
D7S3052	121.411					
467 Clinical		0.081 (0.4)	1.498 (1.0)	1.579	1.221 (0.7)	0.017
469 Reading		1.085 (1.0)	0.247 (1.0)	1.332	0.670 (1.0)	0.026
475 Language		0.0	0.117 (1.0)	0.117	0.089 (1.0)	0.015
481						
CFTR-TET	124.9					
487 Clinical		0.000	0.297 (0.5)	0.297	0.116 (0.25)	0.016
489 Reading		0.254 (1.0)	0.000	0.254	0.033 (1.0)	0.017
495 Language		0.033 (1.0)	0.000	0.033	0.089 (1.0)	0.014
501						
D7S1824	149.9					
507 Clinical		0.040 (1.0)	0.689 (0.35)	0.729	0.616 (0.3)	0.025
509 Reading		0.000	0.545 (0.95)	0.545	0.441 (1.0)	0.016
515 Language		0.000	0.370 (0.4)	0.370	0.020 (1.0)	0.019
521						
D7S2426	160.09					
527 Clinical		1.129 (1.0)	0.000	1.129	0.538 (0.25)	0.059
529 Reading		0.093 (1.0)	0.019 (1.0)	0.112	0.004 (0.25)	0.018
535 Language		1.637 (1.0)	0.139 (0.3)	1.776	1.279 (0.55)	0.087
541						

**Table 4.** Maximum two-point heterogeneity LOD scores (admixture) for chromosome 2 markers; same format and notation as used in tables 2 and 3

	cM	HLOD Canada	HLOD US	HLOD-S	HLOD-P	PPL
D2S1353	164.51					
575 Clinical		0.015 (0.2)	0.000	0.015	0.000	0.016
581 Reading		0.000	0.000	0.000	0.000	0.014
587 Language		0.076 (1.0)	0.015 (0.1)	0.011	0.025 (0.10)	0.022
D2S1776	173					
589 Clinical		0.503 (1.0)	0.000	0.503	0.263 (0.25)	0.010
595 Reading		0.000	0.065 (1.0)	0.065	0.001 (0.10)	0.011
601 Language		0.085 (0.75)	0.038 (0.2)	0.123	0.000	0.012
D2S335	175.91					
607 Clinical		0.267 (1.0)	0.000	0.267	0.000	0.009
609 Reading		0.000	0.746 (0.55)	0.746	0.530 (0.40)	0.017
615 Language		0.000	0.000	0.000	0.000	0.010
D2S326	177.53					
627 Clinical		0.008 (1.0)	0.000	0.008	0.000	0.009
629 Reading		0.006 (1.0)	0.286 (1.0)	0.292	0.449 (1.0)	0.017
635 Language		0.000	0.010 (0.05)	0.010	0.000	0.011
D2S2314	182.24					
647 Clinical		1.709 (1.0)	0.007 (0.2)	1.716	0.623 (0.3)	0.051
649 Reading		0.702 (1.0)	0.635 (1.0)	1.338	1.110 (0.75)	0.036
655 Language		1.397 (1.0)	0.204 (0.2)	1.601	0.347 (1.0)	0.051
D2S1391	186.21					
667 Clinical		0.171 (0.85)	0.000	0.171	0.000	0.023
669 Reading		0.000	0.000	0.000	0.000	0.019
675 Language		0.000	0.000	0.000	0.000	0.018

