Quantitative Trait Locus for Reading Disability on Chromosome 6

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thoxycarbonyl (Fmoc) protecting group chemistry and characterized by electrospray-ionization mass spectrometry after reversed-phase high-performance liquid chromatography (HPLC) purification before and after the spin-labeling reaction.
5. Trimerization of the peptide was verified by size-exclusion HPLC at pH 4.8 and 0°C. The thermal unfolding profile monitored by EPR was nearly superimposable on the profile monitored by the circular dichroism signal at 222 nm. The transition midpoint, M_T, was 35°C for both spin-labeled peptides, which is 10°C higher than M_T of the 36-residue peptide in (*2*). This higher thermal stability might be provided by the four additional residues at the COOH-terminus.
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8. The expected τ_R for the trimer with a mass of 15 kD is approximately 7 to 8 ns.
9. The isotropic nitrogen hyperfine coupling constant for the methanethiosulfonate spin label in water, A_N, is 16.4 G. For the same spin label on L40H64C in the membrane-associate state, A_N = 15.1 G, and on residues facing the bilayer interior on the bacteriorhodopsin, A_N = 15.0 G [C. Altenbach, T. Marti, H. G. Khorana, W. L. Hubbell, *Science* **248**, 1088 (1990)].
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12. If a net positive charge is assumed to develop on the peptide at low pH, the increased affinity for negatively charged membranes can be accounted for by the accumulation of the positively charged peptide in the membrane surface layer. Membrane interactions would be enhanced by the Boltzmann factor, $\exp[-\mathbf{z}\Psi]$, where $\mathbf{z}$ is the effective valence of the peptide and Ψ is the surface potential.
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19. Vesicles extruded through polycarbonate filters with a pore size of 1000 Å were used for all measurements.
20. Below pH 5.0 the EPR spectrum was independent of the peptide-to-lipid ratio above 500, indicating that the membrane-inserted state is not affected by the lipid-to-peptide ratio.
21. The membrane-inserted fraction was approximated by the ratio of the concentration of membrane-inserted species (for example, see the broad component in Fig. 3B) to the total peptide concentration estimated by spectral subtraction analysis.
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23. Supported by the start-up fund from the University of California at Berkeley and by Lawrence Berkeley Laboratory grant 3668-19.

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Quantitative Trait Locus for Reading Disability on Chromosome 6

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Interval mapping of data from two independent samples of sib pairs, at least one member of whom was reading disabled, revealed evidence for a quantitative trait locus (QTL) on chromosome 6. Results obtained from analyses of reading performance from 114 sib pairs genotyped for DNA markers localized the QTL to 6p21.3. Analyses of corresponding data from an independent sample of 50 dizygotic twin pairs provided evidence for linkage to the same region. In combination, the replicate samples yielded a χ^2 value of 16.73 (P = 0.0002). Examination of twin and kindred siblings with more extreme deficits in reading performance yielded even stronger evidence for a QTL (χ^2 = 27.35, $P < 0.00001$). The position of the QTL was narrowly defined with a 100:1 confidence interval to a 2-centimorgan region within the human leukocyte antigen complex.

Reading disability (RD), or dyslexia, is a major social, educational, and mental health problem. In spite of average intelligence and adequate educational opportunities, 5 to 10% of schoolchildren have substantial reading deficits (*1*). Clear evidence for familial transmission has existed for almost a century, and results of recent twin and family studies have shown a substantial genetic component to the disorder (*2*), with heritable variation estimated at 50 to 70% (*3*). Mapping QTLs for RD would facilitate identification of the functional genes that cause the disorder and improve risk estimation and early diagnosis.

Several findings indicating possible linkages for RD have been reported. In a study of nine three-generation families selected for a history of specific RD, we previously obtained evidence for a possible linkage on chromosome 15 (*4*). Recently, evidence of linkage has been reported for markers in the Rh region of chromosome 1 (*5*) and with a translocation between 1p and 2q (*6*). Further research with our kindreds has yielded evidence for a linkage on chromosome 6 in the human leukocyte antigen (HLA) region, but not for linkage on chromosome 15 (*5*, *7*). The present report describes results with more informative markers on chromosome 6 in these kindreds and a replication in an independent sample of dizygotic (DZ) twins.

The HLA region was targeted for this study because of a possible association between dyslexia and autoimmune disorders (*8*). Results of previous studies have suggested that rates of autoimmune diseases are elevated in relatives of dyslexic probands and that the incidence of dyslexia is increased in relatives of probands having autoimmune illnesses (*9*). Although the causal basis of the association is unknown, the evidence for association from these independent studies points to the HLA complex as a candidate region.

Our kindred sibling sample comprises 358 individuals from 19 families who were chosen from a variety of sources, including clinics and private schools specializing in RD. Selection criteria included an extended family history of specific RD, as diagnosed by reading performance at least 2 years below expected grade level and in a pattern consistent with autosomal dominant inheritance (*10*). The twin sample comprises 50 families drawn from the Colorado twin study of RD (*3*). The twins range in age from 8 to 20 years (mean, 12.16 years). Twin pairs in which at least one member had a positive school history of reading problems were objectively and systematically selected through cooperating school districts. Individuals were administered a battery of psychometric tests including the Peabody individual achievement tests (PIAT) and the WISC-R intelligence test (*11*). Subjects with verbal or performance IQ of at least 90 were diagnosed on the basis of a composite discriminant score. Discriminant weights for PIAT reading recognition, reading comprehension, and spelling were computed from an independent sample of RD and control nontwin children in order to produce a continuous measure of RD with known psychometric properties. A comparable measure was constructed from the psychometric data obtained on the kindred sample. We refer to this measure as the discriminant score for reading performance.

One advantage of using DZ twins for linkage analysis is that they provide a perfect control for the effects of age. In the kindreds, which span three generations,

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subjects are not only of different generations but, within sibships, they are of different ages. Compensation for RD in older subjects renders diagnosis difficult. Another advantage of using twin pairs is a broader sampling of families, which increases the informativeness of the markers. Thus, data from a smaller number of DZ pairs may have more power than a greater number of sibships drawn from a few families with greater age variation.

In the original kindred study, four markers on chromosome 6 were genotyped: *BF* (properdin factor B, a serum protein), *GLO* (glyoxylase 1, an erythrocyte enzyme), and *pTHH157* and *2C5* (restriction fragment length polymorphisms, RFLPs). They are all located on the short arm of chromosome 6 in bands 6p21.31-p21.1 in the region of the HLA loci. Unfortunately, these markers are not very informative, with heterozygosity values (H) < 0.30. Subsequently, we used polymerase chain reaction (PCR) to obtain more informative DNA markers in the same region for the kindred sibships and replicated the genotyping in the independent twin sample. Five markers having $H \geq 0.60$ were typed: *D6S89*, *D6S109*, *D6S105*, *TNFB*, and *D6S87* (Table 1). The marker *TNFB* is closest to *BF* (separated by 0.8 cM) and is also located within the HLA complex. We used these five markers in a two-point interval mapping procedure (*12*) to analyze the discriminant scores for RD in both the kindred and the twin samples.

Our method is based on the sib-pair approach of Haseman and Elston (*13*), but was extended to accommodate interval mapping (*14*). Conventional sib-pair analysis (the Haseman-Elston method) involves squaring the difference between the phenotypic scores of a pair of sibs (Y), and then regressing Y onto an estimate of the proportion of alleles that sib pairs share identical by descent ($\hat{\pi}$) at a marker locus:

$$Y = \alpha + \beta\hat{\pi} \quad (1)$$

The value for β tests for variation associated with the marker locus (*13*). The extension to interval mapping involves the use of a pair of $\hat{\pi}$ values for adjacent markers to estimate π_q, the proportion of alleles shared identical by descent for the putative QTL located somewhere between the markers (*15*). The value of $\hat{\pi}_q$ depends only on the values of $\hat{\pi}$ for the two flanking markers and the assumed location of the QTL. By regressing on a range of $\hat{\pi}_q$ values, the QTL may be located at the position that provides the best statistical fit of the model to the data. This method also provides increased statistical power over the conventional sib-pair method (*12*).

In place of the statistical model employed in conventional sib-pair analysis, we used two extensions of the regression model of DeFries and Fulker (*16*). One method involves regression of the score for one sib onto the score for the second sib, the esti-

Table 1. Marker information. Information for this table was retrieved from Genome Data Base, Johns Hopkins University. Citations for the markers are as follows. *D6S89*: M. Litt, *Nucleic Acids Res.* **18**, 4301 (1990); *D6S109*: L. P. Ranum, *ibid.*, p. 1171; *D6S105*: J. L. Weber, *ibid.* **19**, 968 (1991); *TNFB* (tumor necrosis factor–β): S. A. Nedospasova *et al.*, *J. Immunol.* **147**, 1053 (1991); *D6S87*: J. L. Weber, *Nucleic Acids Res.* **18**, 4636 (1990). Map distances for *D6S89*, *D6S109*, and *D6S105* were taken from map *C6M22* [M. Chen, *Science* **258**, 67 (1992)]; for *TNFB*, from *C6M22* and *C6M55* (R. D. Campbell, personal communication) to GDB; and for *D6S87*, from *C6M43* [K. Buetow, Cooperative Human Linkage Center skeletal map, personal communication to GDB].

Marker	Location	Number of alleles	H	cM	Kindred sibs		Twin sibs	
					n	H	n	H
D6S89	6p23	13	0.90	0.0	98	0.75	46	0.83
D6S109	6p22.3-p22.2	9	0.79	6.5	114	0.73	48	0.79
D6S105	6p22-p21.3	10	0.79	22.3	98	0.72	44	0.81
TNFB	6p21.3	13	0.86	24.3	114	0.73	50	0.79
D6S87	6q22.3-q23.1	9	0.60	106.1	114	0.61	50	0.74

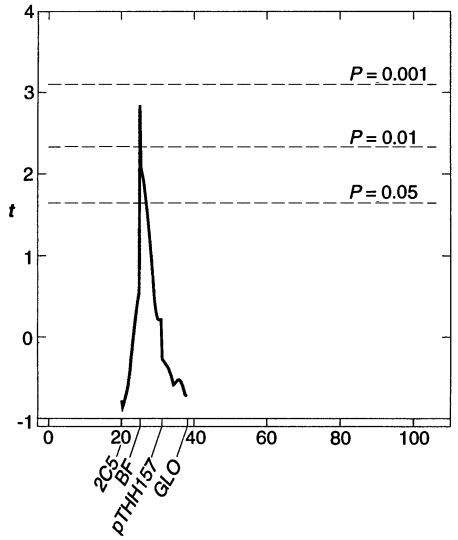

Fig. 1. Interval mapping results for four markers on chromosome 6 in kindred sibships. The polymorphism information content was 0.27 for *2C5*, 0.28 for *BF*, 0.37 for *pTHH157*, and 0.34 for *GLO*. The 19 families in the study contain 126 sib pairs for which marker data were available for this analysis. For each pair of flanking markers, the distance between markers was divided into 20 intervals, $\hat{\pi}_q$ estimates were computed for each interval, and regression equations were fitted to obtain the t statistics plotted.

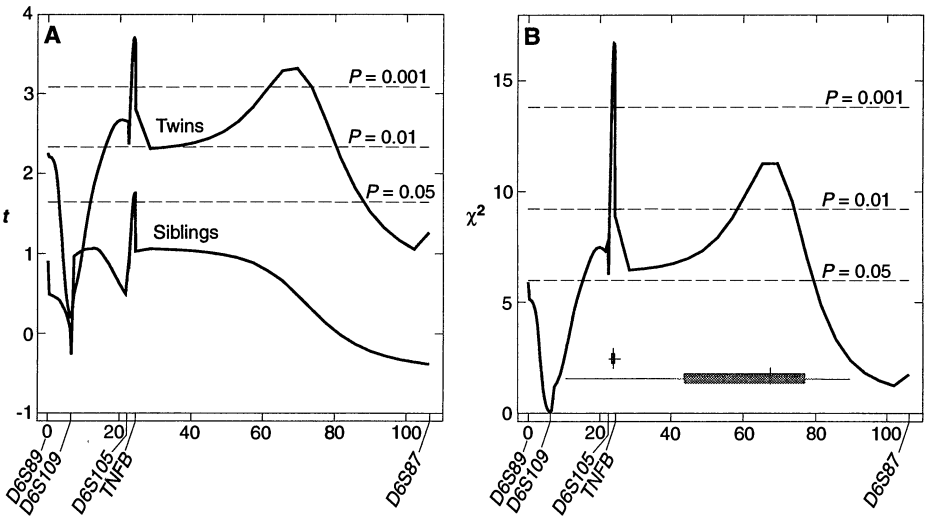

Fig. 2. Interval mapping results for five DNA markers on chromosome 6 in kindred siblings and DZ twins. (**A**) t statistic for each group. The predicted QTL is situated at position 24.2 cM in the kindred siblings and at 23.8 cM in the twins. Sample sizes and properties of the markers are given in Table 1. (**B**) Approximate χ^2 statistics for the combined samples (*22*) were computed by summing the squared t values for siblings and twins in (A). The maximal peak corresponds to a QTL 0.4 cM distal to the *TNFB* marker. The solid bars represent the one-lod support interval for the primary and secondary peaks; the odds ratios decrease by a factor of 10 within these regions. The lines extending from the solid bars indicate two-lod support intervals, representing decreases of 100 in the odds-ratio (*26*). The two-lod support interval for the combined sample is fully contained within the *D6S105-TNFB* interval. The corresponding support interval for the secondary peak between *TNFB* and *D6S87* overlaps with that of the putative QTL between *D6S105* and *TNFB*, suggesting that the secondary peak is a "ghost image" (*27*) of the major peak.

mate of π, and the product of the second sib's score with the estimate of π (*17*). If we assume only additive gene action, the model is

$$C = B_0 + B_1P + B_2\hat{\pi} + B_3P\hat{\pi} \quad (2)$$

where C is the phenotype of the first sib and P is the phenotype of the second sib. In this model, the test for linkage is provided by B_3. The statistical procedure involves double entry of data and a corresponding adjustment of the t value for B_3 (*18*). A further refinement is to include the effects of dominance in the model (*19*), when the regression becomes

$$C = B_0 + B_1P + B_2(\hat{\pi} - 0.5) + B_3P(\hat{\pi} - 0.5) + B_4[\text{abs}(\hat{\pi} - 0.5) - 0.25] + B_5P[\text{abs}(\hat{\pi} - 0.5) - 0.25] \quad (3)$$

The coefficient B_3 provides the overall test for linkage, and B_5 detects linkage to a QTL with nonadditive gene action. The multiple regression analysis has better statistical properties than the conventional sib-pair approach, particularly when used with small samples and in the presence of possible outliers. In common with other sib-pair methods, it is unnecessary to make restrictive assumptions concerning an appropriate genetic model that most other linkage methods require. This is important when searching for QTLs in complex traits where the mode of transmission is often unknown.

The second method is applicable to the analysis of samples selected for extreme phenotypes. It is based on the idea that, under linkage with a QTL, co-twins of the selected probands should differentially regress back toward the mean of the unselected population according to the proportion of alleles shared with the proband (*16*, *17*). A general test for linkage may be obtained from the B_2 coefficient when the following model is fit to the data:

$$C = B_0 + B_1P + B_2\hat{\pi} \quad (4)$$

With nonadditive gene action, the selection model is

$$C = B_0 + B_1P + B_2(\hat{\pi} - 0.5) + B_3[\text{abs}(\hat{\pi} - 0.5) - 0.25] \quad (5)$$

in which B_3 provides the test for nonadditive effects. With intense selection (for example, 5% or less of the normal distribution), the selected sample approach offers as much as a 10-fold increase in statistical power to detect a QTL over conventional sib-pair methods (*19*), and the power is further increased when it is used in the context of interval mapping (*20*).

Conventional sib-pair analysis of the discriminant measure of reading performance in the original kindred sibships involving the four markers *2C5*, *BF*, *pTHH157*, and *GLO* indicated a possible QTL in the *BF* region (*7*). Results of an interval mapping analysis (model 3) of the same four loci are shown in Fig. 1, where there is a sharp peak at the *BF* marker (position 25.1 cM) that is within the HLA complex (t = 2.84, P = 0.0027). There is no evidence of a QTL at any other location.

Results of interval mapping using the five more-informative markers are shown in Fig. 2A. For the sibling sample, there is a sharp peak located at 6p21.3 between markers *D6S105* and *TNFB* (t = 1.75, P = 0.04). For the twins, there is a more pronounced peak between the same two markers (t = 3.69, P = 0.0003), only 0.4 cM away from that of the kindred siblings. There are other secondary peaks that reach levels of statistical significance, but the confidence intervals fail to distinguish them from the major peak.

The significant results in Fig. 2A correspond to broad heritability in our model. For the twin sample, the parameter relating specifically to nonadditive gene action (dominant or recessive) was also highly significant (B_5,t = 2.78; P = 0.004). In the kindred sample, this parameter failed to reach significance (t = 0.90, P = 0.19), probably reflecting the reduced power of the test for nonadditivity over that for total heritability in this sample. The finding in the twins indicates a departure from additivity and suggests that the putative QTL has either a recessive or dominant mode of expression. Dominant transmission was also

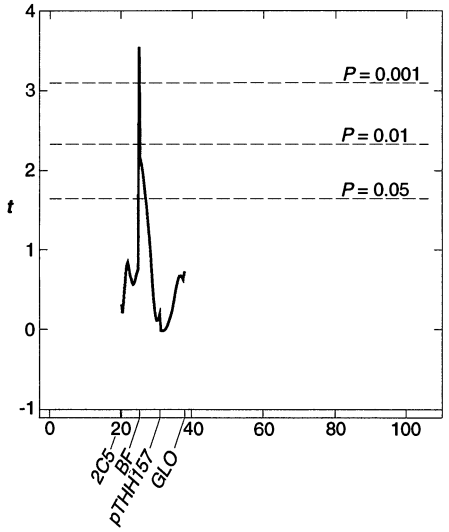

Fig. 3. Interval mapping results for original serum and RFLP markers in selected kindred sibships. Index cases, or probands, were designated on the basis of discriminant scores 2 SDs or more below the mean of the unselected population. Eighty-eight members of the 126 sib pairs met these criteria.

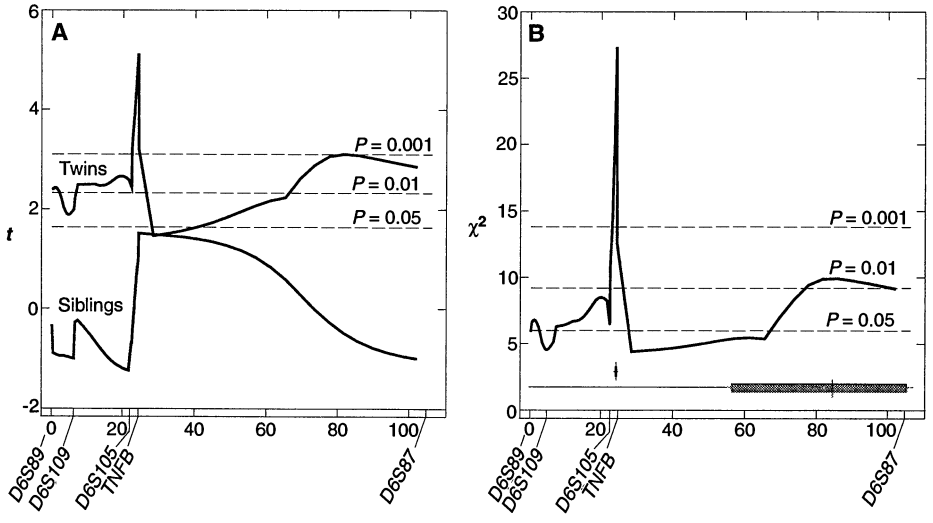

Fig. 4. Interval mapping results for DNA markers in selected samples of kindred siblings and DZ twins. Probands were designated on the basis of the initial sampling selection criteria and of discriminant scores 2 SDs or more below the mean of the unselected population. Twenty-six members of the 50 twin pairs and 80 of the kindred siblings met these criteria. (**A**) For the twins, the putative QTL is positioned 0.1 cM distal to marker *TNFB*. For the siblings, the maximal peak is situated directly on *TNFB* (position 24.3 cM). For the twin sample, the statistical significance thresholds are slightly higher than those shown in the horizontal lines because of the reduced sample size after selection (critical t = 1.70, 2.47, 3.45, for P = 0.05, 0.01, and 0.001, respectively). (**B**) Approximate χ^2 statistics for the combined samples under selection. The maximal peak corresponds to a QTL 0.1 cM distal to *TNFB*. As in the analysis of unselected twins and siblings, the two-lod support interval for the combined sample is fully contained within the *D6S105-TNFB* interval. In these data the three-lod support interval also is contained within this interval. The secondary peak between *TNFB* and *D6S87* is greatly diminished with selection, and the two-lod support interval spans the entire region of chromosome 6 encompassed by all markers.

suggested in our segregation analysis of data from the Colorado family reading study (*21*).

The evidence for linkage is further enhanced when the results are combined for the independent samples (Fig. 2B) (*22*). The peak between *D6S105* and *TNFB* provides a χ^2 value of 16.73 ($P = 0.0002$), the location support interval is extremely narrow, and the remaining peak between *TNFB* and *D6S87* appears to be a ghost image of the main peak because its support interval extends into the position of the primary peak.

Analysis of data from individuals with more extreme deficits in reading performance provides even stronger evidence for a QTL in this region. Individuals in the kindred and twin families having discriminant scores 2 SDs or more below the mean of the unselected population were designated as probands and analyzed with the selected sample regression procedure (model 5). The original markers in the kindred siblings indicated a single peak at the *BF* marker with a *t* value of 3.55 ($P = 0.0003$) (Fig. 3). This peak is located at the same position as in the unselected analysis, but with a greater level of statistical significance. Analyses of the more informative DNA markers in the kindred and twin samples indicate a QTL in the same region, with maximal peaks between markers *D6S105* and *TNFB* (Fig. 4A) (twin: $t = 5.12$, $P < 0.00001$; kindred: $t = 1.52$, $P = 0.066$). The combined kindred and twin samples reinforce this finding, providing even stronger support for a QTL in this region ($\chi^2_2 = 27.35$, $P < 0.00001$). The peak in this interval is very narrowly defined, with a 1000:1 location support interval contained within the 2-cM distance separating these two markers (*23*) (Fig. 4B).

Results of the three QTL analyses described in this report are highly consistent (*24*). Interval analysis of the *BF* and associated markers in the sibling sample suggested a possible QTL in the HLA region. The analysis of the more informative markers that were subsequently obtained on the same subjects confirmed the initial finding. Although scores from the same subjects were included in the two analyses, a new pair of markers (*D6S105* and *TNFB*) very close to *BF* also yielded significant results. The third analysis is a true replication, involving data from an independent sample of DZ twin pairs. Thus, the combined results of these three analyses provide compelling evidence for a QTL in the HLA region that influences RD (*25*).

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15. For flanking markers located at positions L_1 and L_2 and a putative QTL located at L_q, denote the recombination fractions between L_1 and L_q, L_q and L_2, and L_1 and L_2 as θ_1, θ_2, and θ_{12}, respectively. The proportions of alleles corresponding to L_1 and L_2, π_1 and π_2, are used to estimate the average number of alleles shared at the putative trait locus: $\hat{\pi}_q = \alpha + \beta_{\pi 1}\hat{\pi}_1 + \beta_{\pi 2}\hat{\pi}_2$, with solutions for the regression coefficients given as
$$\hat{\beta}_{\hat{\pi}1} = [(1 - 2\theta_1)^2 - (1 - 2\theta_2)^2(1 - 2\theta_{12})]/[1 - (1 - 2\theta_{12})^4]$$
$$\hat{\beta}_{\hat{\pi}2} = [(1 - 2\theta_2)^2 - (1 - 2\theta_1)^2(1 - 2\theta_{12})]/[1 - (1 - 2\theta_{12})^4]$$
and
$$\hat{\alpha} = (1 - \hat{\beta}_{\hat{\pi}1} - \hat{\beta}_{\hat{\pi}2})/2.$$
The region between L_1 and L_2 is divided into N intervals with $\lambda_1 = k\lambda_{12}/N$, and $\lambda_2 = \lambda_{12} - \lambda_1$ for $k = 0, 1, \ldots, N$, and θ_1 and θ_2 are determined with Haldane's mapping function $\theta = 0.5[1-\exp(-2\lambda)]$, where λ_1, λ_2, and λ_{12} are map distances corresponding to the recombination fractions. Sib-pair regressions are then fitted for each of the $N + 1$ intervals (*12*).
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18. In unselected samples, one sibling is arbitrarily designated a proband and the other a cosib, and then pairs are double-entered in opposite categories. The procedure does not affect the estimated regression coefficients, but the standard errors of the estimates must be rescaled by the square root of 2 in order to obtain unbiased *t* statistics (*16, 17*).
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21. B. F. Pennington *et al.*, *J. Am. Med. Assoc.* **266**, 1527 (1991).
22. In large samples *t* values are asymptotically distributed as normal deviates, and squared deviates, Z^2, follow a χ^2 distribution with 1 degree of freedom (df). For the two independent samples, $Z_1^2 + Z_2^2$ is approximately distributed as χ^2 with 2 df.
23. When data from selected samples are subjected to multiple regression analysis, information pertaining to both gene action and effect size can also be obtained. With regard to gene action, the relation between cosib means and $\hat{\pi}$ will differ for dominant and recessive QTLs. If a rare dominant QTL results in lower scores, the average scores of cosibs with a $\hat{\pi}$ of 0.5 will be intermediate to those with a $\hat{\pi}$ of 1.0 or 0.0. In contrast, if homozygosity for a rare recessive QTL results in lower performance, cosibs with a $\hat{\pi}$ of 0.5 or 0.0 will both manifest a similar regression to the mean. Data from the twin sample included in the present study revealed a pattern of increasing cosib means as a function of decreasing $\hat{\pi}$, thereby indicating either dominant or additive gene action for the putative QTL. The effect size of a QTL (a) may be defined as half the difference between the phenotypes of two homozygous genotypes, for example, *AA* and *aa*. When Eq. 5 is fitted to data from selected sibling pairs, B_2 provides an estimate of *2a*. The average effect size estimated from analyses of the DNA markers in the sibling and twin samples is 0.86 ± 0.18, suggesting that the putative QTL decreases reading performance by over 1.5 SDs, on average.
24. Although the gender ratio (males/females) in clinic and referred samples of RD children is often 3:1 or greater, the ratio in the present twin sample is only 1.96:1, and the linkage results obtained from the twin and kindred samples do not differ as a function of gender. When the regression models were extended to include main effects and interactions involving gender (*17*), no significant gender effects or interactions were obtained in either sample. Because of the multifactorial etiology of dyslexia and the possibility of specific subtypes, it is possible that several major loci contribute to RD. Although the primary objective of this study was to test for a QTL on chromosome 6, we have also genotyped the two samples for a number of additional DNA markers covering most of chromosomes 12 (9 markers from 12p13.3 to 12q24.33) and 15 (20 markers, 15cen to 15qter), and the Rh region of chromosome 1. Interval mapping analyses of these markers yielded no evidence for linkage at any location that replicated in the two samples. We also found no evidence for allelic association with any of our markers, including those within the HLA complex.
25. Results of a recent association study provide tentative evidence for a QTL in this region that may influence IQ [R. Plomin *et al.*, *Behav. Genet.* **24**, 107 (1994)]. Because dyslexia is associated with somewhat lower IQ, this could mean that the QTL we have located contributes to low IQ. Alternatively, it is possible that their marker is associated with verbal ability rather than general cognitive ability. To address this issue, we reanalyzed our twin data using measures of verbal, performance, and full-scale IQ in place of discriminant reading scores. There was no indication of a QTL for any measure of IQ at any location on chromosome 6. This outcome suggests that the QTL identified in the present study relates to specific RD and not to low IQ.
26. Support intervals define the boundaries for unit changes in lod scores (logarithm of the likelihood ratio for linkage). Lander and Botstein (*14*) have shown that lod scores are asymptotically distributed as $1/2(\log_{10}e)Z^2$, where Z is a normal deviate. For the independent samples in the present application, the lod transformation is $1/2(\log_{10}e)(t_1^2 + t_2^2)$.
27. C. S. Haley and S. A. Knott, *Heredity* **69**, 315 (1992).

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