

small seamounts and ridges, interspersed with sills in the east, reflects an eastward change of some combination of tensional crack distribution and the volume rate of magma generation: either the thermal anomaly beneath the tension cracks diminished or the cracks themselves narrowed. The two effects may be combined.

The eastward sequence may also represent a time sequence, formed progressively as the Pacific plate moved northwestward. We have as yet only two indications of age along the Cross-grain: middle Miocene (14 Myr) sills/flows at 138° W, and possible late Eocene (36–40 Myr) for certain Line Islands Cross Trend features^{18–20}. This age difference along the cross-grain is ~25 Myr and the distance is ~2,000 km, giving an average rate, assuming progression, of ~8 cm yr⁻¹, a figure close to the progression rate of the Hawaiian chain. Several ridges in the Line Islands Cross Trend appear to end westward in an elbow, congruent with the elbow junction between the Emperor and Hawaiian chains, also late Eocene in age²¹.

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Evidence for a genetic aetiology in reading disability of twins

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Reading disability (dyslexia) is a major social, educational, and mental health problem. Although estimates of prevalence vary, up to 10–15% of school-age children have severe reading deficits in spite of average intelligence and adequate educational opportunity¹. That reading disability may have a constitutional basis has long been recognized², and results of twin and family studies suggest that one or more of its forms may be heritable^{3,4}; however, definitive evidence for a genetic aetiology has not been reported. Establishing a heritable basis for reading disability could suggest possible causes, give improved risk estimates, facilitate early diagnosis, and provide validity tests for ostensible subtypes. In this report, we apply a recently developed multiple regression analysis^{5,6} to data collected from a sample of 64 pairs of identical twins and 55 pairs of fraternal twins, in which at least one member of the pair is reading disabled, and present evidence for a significant genetic aetiology.

In twin studies of pathology, a comparison of concordance rates for a particular disorder in identical (MZ) and fraternal (DZ) twins provides a test for genetic aetiology. When index

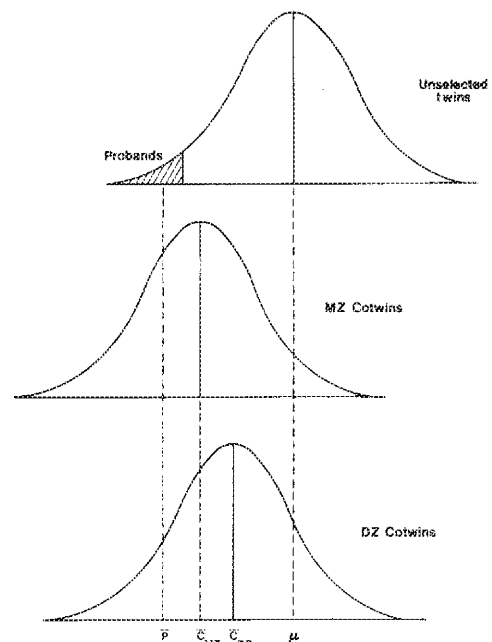


Fig. 1 Hypothetical distributions for reading performance of an unselected sample of twins, and of the identical (MZ) and fraternal (DZ) cotwins of probands with a reading disability. The differential regression of the MZ and DZ cotwin means toward the mean of the unselected population (μ) provides a test of genetic aetiology.

cases are chosen because of their extreme scores on some continuous variable (such as reading ability), a more appropriate test is to compare the means of the cotwins of the MZ and DZ probands. For example, when probands have been identified by low test scores (see Fig. 1), the scores of both MZ and DZ cotwins will regress toward the mean of the unselected population. But, to the extent that the condition is heritable, this regression toward the mean should differ for MZ and DZ cotwins. Because the coefficient of relationship for MZ twins is one, whereas that for DZ twins is one-half, scores of DZ cotwins should regress more toward the mean of the unselected population than do those of MZ cotwins. Thus, if the means for MZ and DZ probands are equal, a simple *t*-test of the difference between the means for MZ and DZ cotwins would be a test for genetic aetiology. Fitting the following multiple regression model to the twin data, however, provides a more general and statistically more powerful test:

$$C = B_1P + B_2R + A$$

where *C* is a cotwin's predicted score, *P* is the proband's score, *R* is the coefficient of relationship, and *A* is the regression constant. *B*₁, the partial regression of cotwin's score on proband's score, is a measure of average twin resemblance. *B*₂, the partial regression of cotwin's score on the coefficient of relationship, equals twice the difference between the means for MZ and DZ cotwins after covariance adjustment for the difference between MZ and DZ probands. Thus, the significance of *B*₂ provides a direct test for genetic aetiology. Moreover, the ratio of *B*₂ to the difference between the means for probands and for the unselected population gives a measure of the extent to which this difference is due to heritable influences⁵.

As a part of the Colorado Reading Project⁷, an extensive psychometric test battery, which includes 11 subtests from the Wechsler Intelligence Scale for Children-Revised (WISC-R, excluding Mazes) (ref. 8) or the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (ref. 9), is being administered to MZ and DZ twin pairs, with at least one member of each pair reading disabled, and to a comparison group of control twins, who are normal readers. To minimize the possibility of ascertainment

Table 1 Mean discriminant scores and standardized test scores of 64 identical (MZ) and 55 fraternal (DZ) reading-disabled probands and their cotwins expressed as deviations from control means

Measure	Identical		Fraternal		B_1	B_2
	Proband	Cotwin	Proband	Cotwin		
Discriminant	-2.75	-1.98	-2.79	-1.63	$0.77 \pm 0.08^*$	$-0.77 \pm 0.27^*$
Reading Recognition	-2.30	-1.65	-2.39	-1.32	$0.67 \pm 0.08^*$	$-0.79 \pm 0.28^*$
Reading Comprehension	-1.89	-1.36	-1.76	-1.10	$0.50 \pm 0.10^*$	-0.38 ± 0.30
Spelling	-1.90	-1.29	-1.89	-1.02	$0.50 \pm 0.09^*$	$-0.53 \pm 0.30^*$
Coding	-0.64	-0.63	-0.97	-0.78	$0.61 \pm 0.09^*$	-0.12 ± 0.31
Colorado Perceptual Speed	-1.23	-1.06	-1.24	-1.04	$0.53 \pm 0.09^*$	-0.05 ± 0.27
Digit Span	-0.89	-0.71	-1.14	-0.52	$0.51 \pm 0.10^*$	$-0.62 \pm 0.33^*$

B_1 is the partial regression of cotwin's score on proband's score, a measure of average MZ and DZ twin pair resemblance. B_2 is the partial regression of cotwin's score on the coefficient of relationship and equals twice the difference between the means of MZ and DZ cotwins after covariance adjustment for the difference between probands. The sample of reading-disabled twin pairs includes 39 female MZ, 25 male MZ, 26 female DZ, and 29 male DZ. Although there are significant gender differences, with males typically scoring lower than females, the average scores of MZ and DZ probands do not differ significantly and gender differences do not differ as a function of zygosity. When an augmented regression model that included gender as a dummy variable was fitted to these data, there was no evidence for differential genetic aetiology as a function of gender.

* $P < 0.05$, one-tailed test.

bias, the twins' school records are reviewed for reading problems, such as low reading-achievement scores, referral to resource rooms because of poor reading ability, or reports by school psychologists. A discriminant function score is computed for each member of the pair based upon three measures of reading performance (Reading Recognition, Reading Comprehension, and Spelling subtests of the Peabody Individual Achievement Test¹⁰), two subtests from the WISC-R or WAIS-R (Coding-B and Digit Span), and the Colorado Perceptual Speed Test¹¹. Discriminant weights were estimated from an analysis of independent data obtained from a sample of 140 reading-disabled and 140 control non-twin children in which the rate for correct classification was ~95%. To be included in our sample, at least one member of each prospective reading-disabled twin pair must be classified as affected by the discriminant analysis, and the twin with the lower discriminant score is designated as the proband. In addition, probands must have a minimum IQ of 90 (on either the Verbal or Performance scale of the WISC-R or the WAIS-R), show no evidence of neurological, emotional, or behavioural problems, and have no uncorrected visual or auditory acuity deficits. Zygosity is determined with 95% accuracy using the Nichols and Bilbro¹² questionnaire and confirmed if necessary by blood test. Results described in the present report are based upon analyses of data from 119 twin pairs (mean age 12.7 years).

The average discriminant scores and standardized test scores of the MZ and DZ probands and their cotwins are presented in Table 1. Because probands were selected on the basis of the discriminant score, the fit of the basic regression model to data for that measure is appropriate as a test of genetic aetiology. The average discriminant scores of MZ and DZ probands are -2.75 and -2.79, respectively, over 2.5 standard deviations below the mean for matched control twins. The means for their cotwins are -1.98 and -1.63. Thus, the scores of the DZ cotwins have regressed 1.16 standard deviation units on the average toward the control mean, whereas those of the MZ cotwins have regressed only 0.77 standard deviation units. The highly significant B_2 estimate ($P = 0.003$, one tailed) is a function of this differential regression of MZ and DZ cotwin scores, and provides evidence for the heritable nature of reading disability.

The pattern of findings with respect to each of the individual tests is also consistent. The difference between the means for DZ probands and their cotwins exceeds that between MZ probands and their cotwins for each measure, and corresponding estimates of B_2 are significant for Reading Recognition ($P = 0.003$), Spelling ($P = 0.04$), and Digit Span ($P = 0.03$). It can also be seen from Table 1 that the average resemblance for members of MZ and DZ twin pairs (i.e., B_1) ranges from 0.50 to 0.77, each of which is significant.

Because the discriminant score and individual measures listed in Table 1 are correlated with general mental ability, an extended model that included full-scale IQ as a covariate was also fitted to the data. In general, results of this analysis are congruent with those from the fit of the basic regression model to the same data set. The B_2 estimates again are significant for the discriminant score, reading recognition, spelling and digit span, with associated P values of 0.003, 0.003, 0.04, and 0.05, respectively. Thus, the significance of the B_2 estimates presented in Table 1 is not attributable to the greater similarity in general mental ability of MZ compared with DZ twin pairs.

Although a genetic aetiology for reading disability is inferred from these results, it does not completely account for observed group differences between probands and controls. An estimate of the extent to which the group difference is heritable (h_g^2) could be obtained by dividing the estimate of B_2 by the difference between the proband mean and that of the unselected population. However, the unselected population mean is unknown because both the reading-disabled and control samples were selected on the basis of reading performance. Nevertheless, an approximate estimate of the unselected population mean was obtained from a weighted average of the control and proband means (95% and 5%, respectively) and then used to estimate h_g^2 . The resulting estimate for the discriminant score is 0.29 ± 0.10 , suggesting that about 30% of the reading deficit in probands arises from heritable factors. Thus, environmental differences contribute to the observed group differences. Furthermore, these results suggest that the performance deficits of reading-disabled children are not due solely to a fully penetrant major gene, though one or more of its forms could be inherited in a simple Mendelian manner⁴.

The flexibility of the regression analysis of selected twin data used here would permit testing for differential genetic aetiology by simultaneous analysis of data from probands of ostensibly different subtypes. Thus, if reading disability is a heterogeneous disorder¹³, such analysis could provide evidence for subtype validity. Moreover, by fitting an augmented model to the data, the extent to which individual differences in the unselected population are due to heritable influences (h^2) and to shared environmental influences (c^2) could be estimated⁵. h_g^2 and h^2 could differ in magnitude as a result of the aetiology of the deficit in probands differing from that of individual variations within the normal range. For example, deviant scores of probands could arise from environmental insult, whereas differences among individuals within the normal range may be multifactorial in origin. Thus, a comparison of h_g^2 and h^2 estimated from the same data set would provide a test of the hypothesis that probands merely represent the lower end of a normal distribution of individual differences¹⁴. These hypotheses will be tested in

the Colorado Reading Project when a larger sample of reading-disabled twins becomes available.

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Two forms of transforming growth factor- β distinguished by multipotential haematopoietic progenitor cells

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Type- β transforming growth factors (TGF- β s) are polypeptides that act hormonally to control proliferation and differentiation of many cell types^{1,2}. Two distinct homodimeric TGF- β polypeptides, TGF- β 1 and TGF- β 2 have been identified which show ~70% amino-acid sequence similarity^{3,4}. Despite their structural differences, TGF- β 1 and TGF- β 2 are equally potent at inhibiting epithelial cell proliferation and adipogenic differentiation³. The recent immunohistochemical localization of high levels of TGF- β in the bone marrow and haematopoietic progenitors of the fetal liver⁵ has raised the possibility that TGF- β s might be involved in the regulation of haematopoiesis. Here we show that TGF- β 1, but not TGF- β 2, is a potent inhibitor of haematopoietic progenitor cell proliferation. TGF- β 1 inhibited colony formation by murine factor-dependent haematopoietic progenitor cells in response to interleukin-3 (IL-3) or granulocyte-macrophage colony stimulating factor (GM-CSF), as well as colony formation by marrow progenitor cells responding to CSF-1 (M-CSF). The progenitor cell lines examined were ~100-fold more sensitive to TGF- β 1 than TGF- β 2, and displayed type-I TGF- β receptors with affinity ~20-fold higher for TGF- β 1 than TGF- β 2. These results identify TGF- β 1 as a novel regulator of haematopoiesis that acts through type-I TGF- β receptors to modulate proliferation of progenitor cells in response to haematopoietic growth factors.

Murine IL-3 (multi-CSF) supports the proliferation of multipotential progenitor cells as well as granulocyte, macrophage, erythroid, megakaryocyte and mast cells⁶. The effect of TGF- β s on haematopoietic progenitor cell proliferation was studied using both fresh haematopoietic cells and IL-3-dependent haematopoietic cell lines^{7,8}. The B6SutA cell line forms multilineage granulocyte-macrophage erythroid-mast cell colonies as well as macroscopic haemoglobinized erythroid bursts in response to IL-3 and erythropoietin in semisolid medium (Fig.

1). Picomolar TGF- β 1 markedly (~70%) inhibited multilineage as well as haemoglobinized colony formation by B6SutA cells (Fig. 1). A significant but less extensive inhibition of colony formation by TGF- β 1 was observed in 32D-C13 cells, an IL-3-dependent bilineage progenitor cell line which differentiates to mast cells in response to IL-3, or granulocytes in response to G-CSF (refs 7 and 9). In contrast, TGF- β 2 at concentrations up to 1 nM did not affect colony formation by either cell line (Fig. 1) even though it acts at picomolar concentrations on other mouse cell types³.

The formation of colonies by freshly isolated bone marrow cells in response to IL-3, GM-CSF (which stimulates production of granulocytes and macrophages)¹⁰, and CSF-1 (which preferentially supports the proliferation and development of cells of the monocyte/macrophage lineage)¹¹ was inhibited 37±1%, 21±2% and 10±2% by 0.1 nM TGF- β 1, respectively (not shown). To reduce the possibility that marrow stromal cells or other accessory cells interfered with the effects of TGF- β 1, the experiments were repeated with non-adherent haematopoietic progenitors from murine long-term bone marrow cultures (LTBMCs)¹². TGF- β 1 inhibited strongly the formation of colonies induced by IL-3 or GM-CSF and to a lesser extent by L-cell conditioned medium¹¹ used as a source of CSF-1 (Fig. 1). Again, TGF- β 2 was ineffective in regulating colony formation by haematopoietic progenitors from LTBMCs (Fig. 1). TGF- β radioreceptor assays¹³ of culture supernatants showed no significant difference between the rates of degradation of TGF- β 1 and TGF- β 2 which were 20-25% per day when the factors were added at 0.5 nM to suspension cultures (10⁶ cells ml⁻¹) of B6SutA cells. TGF- β 1 added three days after plating was still capable of inhibiting colony formation (Fig. 1), but cells eventually became refractory to TGF- β 1 as no inhibition of further colony formation was observed when TGF- β 1 was added on day 7 (data not shown).

The effect of TGF- β on the proliferative response of B6SutA and 32D-C13 cells to IL-3 was also examined in suspension cultures. TGF- β 1 induced a marked decrease in thymidine incorporation into macromolecules in both cell lines (Fig. 2). In contrast, proliferation of B6Sut-C127 cells, an IL-3-independent B6SutA-derived clone, was not affected by TGF- β 1 (Fig. 2) even though these cells have apparently normal TGF- β receptors. As in the inhibition of haematopoietic colony formation, TGF- β 2 was much less potent than TGF- β 1 in exerting an antiproliferative response on B6SutA cells and 32D-C13 cells in suspension culture. In this assay, TGF- β 2 exhibited at most ~1% of the potency of TGF- β 1 (Fig. 2). These results suggest that the TGF- β s control proliferation of the haematopoietic cells examined by interaction with receptors that can discriminate between TGF- β 1 and TGF- β 2.

Two of the three types of cell surface receptors for TGF- β identified in many cells by affinity labelling are glycoproteins of relative molecular mass (M_r) ~53,000 (53 K) (type-I receptors) and ~73 K (type-II receptors), and both have higher affinity for TGF- β 1 than for TGF- β 2 (refs 3 and 14). The type-III TGF- β receptor is a large (~300 K) glycoprotein¹⁵ which has an affinity for TGF- β 1 similar to that for TGF- β 2 (ref. 3). Occupancy of type-III receptors with TGF- β 1 or TGF- β 2 has been implicated in the mediation of certain responses to TGF- β s, including inhibition of epithelial cell proliferation, inhibition of adipogenesis, and induction of cell adhesion protein expression, because TGF- β 1 and TGF- β 2 are equally potent at eliciting these responses³. Despite the high affinity of type I and type II receptors for TGF- β 1, no biological actions had previously been ascribed to them.

Analysis¹⁶ of ¹²⁵I-labelled TGF- β 1 binding to the haematopoietic cell lines indicated the presence of a single class of high-affinity binding sites (240 sites per cell with dissociation constant K_d =9 pM in B6SutA; 110 sites per cell with K_d =19 pM in B6Sut-C127; 270 sites per cell with K_d =16 pM in 32D-C13) (not shown). Affinity labelling of B6SutA cells by