Letters to the Editor

Am. J. Hum. Genet. 63:267–269, 1998

A Severely Affected Male Born into a Rett Syndrome Kindred Supports X-Linked Inheritance and Allows Extension of the Exclusion Map

To the Editor:

In its classic form, Rett syndrome (RTT [MIM 312750]) is a childhood neurodevelopmental disorder that has been convincingly described only in females. Therefore, femaleness has been considered a diagnostic criterion (Holm 1985). Although the disorder is usually sporadic, rare familial recurrences have supported the hypothesis of a dominant X-linked mutation and have been extremely valuable in defining the candidate regions on the X chromosome (Archidiacono et al. 1991; Ellison et al. 1992; Schanen et al. 1997). Recently, two male children with severe encephalopathies were born to putative mutant-gene carriers in families with recurrent RTT (Schanen et al. 1998). One of them is the son of the obligate carrier in family 3 of our recent report (fig. 1*A*) (Schanen et al. 1997). He has phenotypic features that are associated with RTT, including acquired microcephaly, profound developmental delay, hypotonia, seizures, respiratory irregularities, constipation, and growth retardation. Since extensive testing did not identify an alternative etiology for his neurological problems, we hypothesize that he expresses an inherited RTT mutation for which he would have an a priori risk of 50%.

Under this assumption, we have extended the genotypic analysis of this family that had previously allowed us to exclude the RTT locus from DXS1053, in Xp22.2, through DXS1222, in Xq22.3 (Schanen et al. 1997). After parental consent to an institutional review board–approved protocol was given, a blood sample was obtained from III-2 and was used for extraction of DNA and for establishment of a lymphoblastoid cell line. Microsatellite-marker typing was performed as described elsewhere, by means of commercially available primer pairs (Research Genetics) (Schanen et al. 1997). Several new markers were added, to better refine the sites of meiotic recombination.

Thirty-six X-linked microsatellite markers were typed in the male proband and his family (fig. 1*B*) (Dib et al. 1996; Nagaraja et al. 1997). Comparison of the haplotypes for the three affected individuals allows extension of the previously excluded region. On the short arm, III-1 and III-2 are discordant for maternal alleles, from DXS7104 through DXS996, the most distal informative marker in Xp22.32. Not excluded is an ~5–6-Mb region flanked by DXS1053, in Xp22.2, and DXS7104, in Xp22.31, a region that contains two loci at which the three probands are concordant, and DXS1224, which was uninformative.

As indicated by the broken line in figure 1*B*, the RTT locus was previously excluded from DXS1053, on the short arm, to DXS1222, on the long arm (Ellison et al. 1992; Schanen et al. 1997). Therefore, we expanded the number of markers tested in the nonexcluded region of Xq. Genotyping of the affected male III-2 revealed discordant inheritance of maternal alleles for III-1 and III-2, from DXS990 (Xq21.33) through DXS425 in Xq25. For markers distal to DXS425, there was concordant inheritance of grandmaternal alleles, for all three probands. These results extend the exclusion map by ~20 Mb on Xq. Note that the three probands are concordant for the grandpaternal allele at DXS983 (Xq12). If one considers the possibility that the mutation arose on the X chromosome from I-1, then the data from this family exclude the RTT locus from the entire X chromosome, except for this region on the proximal long arm; however, this latter region has been excluded by studies of other families (Ellison et al. 1992; Schanen et al. 1997). Furthermore, the skewed X-inactivation pattern in the very mildly affected transmitting female (II-2) strongly implicates a grandmaternal origin of the mutant gene (Schanen et al. 1997). Because the X-inactivation pattern from I-2 was found to be random in both blood and skin fibroblasts, she was considered to have germ-line mosaicism for the RTT mutation. Thus, the genotypic data from II-5 cannot be used reliably for exclusion of the RTT locus.

Identification of a male, who is severely affected with a neonatal encephalopathy, in a family with recurrent classical RTT strengthens the hypothesis that RTT is caused by an X-linked gene. Although RTT has long been thought of as a male-lethal X-linked disorder, this case and similar cases born in RTT sibships (Brown 1997; Schanen et al. 1998) suggest that males who carry

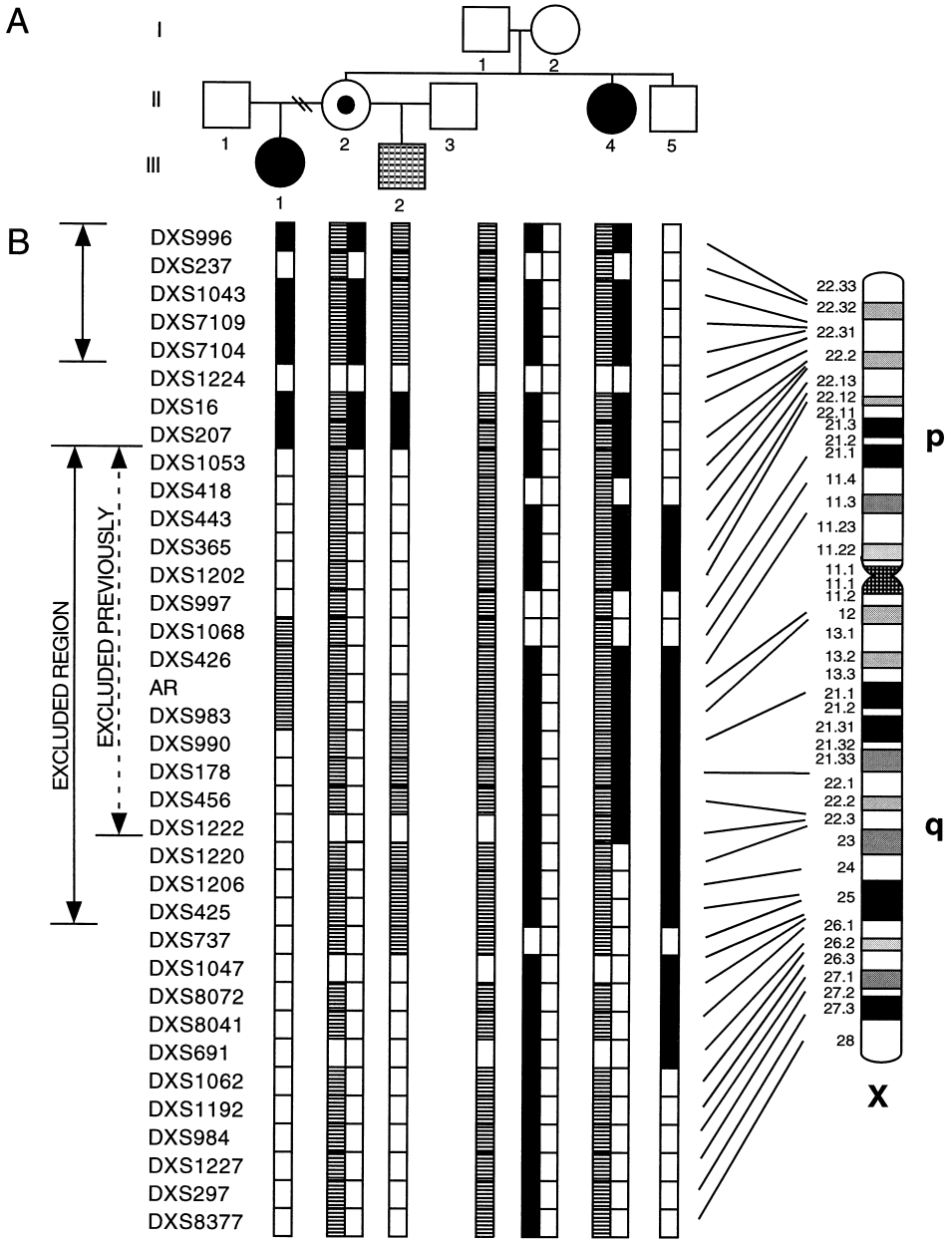

Figure 1 Exclusion mapping of X-chromosome markers. *A,* Pedigree for the RTT kindred. Probands with classic RTT are denoted by blackened symbols; and the severely affected male is designated by a cross-hatched symbol. *B,* Genotyping results for 36 microsatellite markers. The alleles of I-1 are indicated by hatched squares, when they are distinguishable from both alleles of I-2, and by open squares, when they are identical to an allele of I-2. For III-1, only the maternal haplotype is shown, as deduced from studies of II-1 (data not shown). The broken line defines the region excluded by previous studies; and the unbroken lines indicate the regions newly excluded on the basis of the data reported in the present study. Approximate chromosomal band positions of the markers are indicated (X-chromosome ideogram is from Francke 1994; data are from Nagaraja et al. 1997).

a RTT mutation may survive. The identification of such cases in sibships with diagnosed RTT females requires a carrier mother who either is a germ-line mosaic or has a favorably skewed X-inactivation pattern. These instances are rare. The majority of RTT females are sporadic—that is, are due to de novo germ-line mutations. Since oocytes carrying such a mutation are equally likely to be fertilized by a Y-bearing sperm, males with RTT mutations should arise sporadically at a frequency two-thirds that of RTT females, if mutation rates were equal in males and females. Although the possibility that most of these conceptuses will die in utero cannot be excluded,

it is of interest that the case discussed here (III-2) was judged to be normal at birth, was sent home, and suffered an apneic event at 5 d of age (Schanen et al. 1998).

Therefore, the search for the RTT gene receives a further stimulus from the prospect of its use not only for diagnostic testing of young females who exhibit symptoms suggestive of RTT but also for investigation of unexplained neonatal death or infantile apnea and failure to thrive in males. The genotyping data reported here narrow the unexcluded regions of the X chromosome and focus the gene search to a small interval on Xp and the distal long arm.

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Electronic-Database Information

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for RTT [312750])

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Alternative Interpretation of Reported Paracentric Inversion

To the Editor:

In the recent article in the *Journal,* entitled "Molecular Analysis of Deletion (17)(p11.2p11.2) in a Family Segregating a 17p Paracentric Inversion: Implications for Carriers of Paracentric Inversions," Yang et al. (1997) describe a patient with an interstitial deletion of the short arm of chromosome 17, del(17)(p11.2p11.2). The father of the patient carried a chromosome rearrangement of 17p, which was interpreted as a paracentric inversion, inv(17)(p11.2p13.3). The deletion was considered to arise from an unequal crossing-over event associated with the formation of an inversion loop at meiosis.

An alternative cytogenetic explanation for the father's karyotype is a direct or inverted intrachromosomal insertion of a region from 17p11.2 to 17p13.1, into band p13.3 of the short arm of chromosome 17—that is, ins(17)(p13.3p11.2p13.1) or ins(17)(p13.3p13.1p11.2). Pairing at meiosis, with recombination within the insertion, can result in either deletion of the inserted segment or duplication of the inverted segment (see Gardner and Sutherland 1996). Therefore, an intrachromosomal insertion is a logical explanation for the del(17) observed in the patient reported by Yang et al. This is compatible with the observed banding pattern of the father's rearranged chromosome 17 and does not require any unusual mechanism of "unequal crossing-over" to generate the observed chromosome abnormality. Therefore, this case does not provide evidence for a risk of viable chromosome abnormalities being generated from a parental paracentric inversion.

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Reply to Callen

To the Editor:

In our recent article in the *Journal* (Yang et al. 1997), we showed that an interstitial deletion of 17p11.2 had arisen after meiotic recombination in a carrier of an apparently balanced paracentric inversion (PAI; with breakpoints at 17p11.2 and 17p13.3). Considering all the cytogenetic and molecular evidence, especially the facts that (*a*) the breakpoints of the proband's interstitial deletion "flanked" the proximal breakpoint of the paternal PAI (the proximal Smith-Magenis syndrome (SMS) markers were deleted in spite of not being inverted), (*b*) some markers involved in the PAI were not deleted (the *PMP22* locus), and (*c*) the position of the recombination in paternal meiosis was mapped within the immediate vicinity of the resulting deletion, we proposed a model of unequal crossing-over at the base of an inversion loop.

In response to our article, Callen has raised an interesting point. He proposes an alternate explanation, wherein pairing at meiosis, followed by recombination between an *insertion*-bearing and the normal chromosome 17 homologue could result in the interstitial chromosomal deletion observed in the proband. We agree that a within-arm direct or inverted *insertion* is an important differential diagnosis in cases of suspected paracentric inversions, given the significantly enhanced risk of chromosomal imbalance associated with the former. However, although within-arm insertions (direct or inverted) can result in deletion or duplication of the inserted sequence (Gardner and Sutherland 1996), they cannot result in a concurrent deletion of non*inserted* sequences (proximal SMS markers) and sparing of *inserted* sequences (*PMP22* markers).

Taken together, the data seem to favor our hypothesis of an unequal crossing-over at meiosis, as proposed in our article. However, it should be noted that we have yet to formally exclude Callen's proposal—or even the possibility that the deletion arose de novo as a result of a slightly more proximal (unequal) recombination in 17p11.2.

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Anticipation in Familial Hodgkin Lymphoma

To the Editor:

Anticipation in childhood malignancy has been described by several investigators (Horwitz et al. 1996; Plon 1997). On the basis of 21 parent-child pairs with acute myelogenous leukemia and 9 parent-child pairs with chronic lymphocytic leukemia identified from the literature, Horwitz et al. rejected the hypothesis that there was no age-at-onset difference between the two generations, in either data set. Several published data sets were pooled to test whether there is a difference in parent-child pairs affected with Hodgkin lymphoma (HL). Because the occurrence of HL parent-child pairs is a rare event, several published data sets were pooled to test whether there is a difference, in cancer age at onset, between parents and children who are affected with HL. Thirty parent-child pairs with confirmed di-

Table 1

Pooled Parent-Child Pairs with Hodgkin Lymphoma

Reference	Parent (Age at Diagnosis [years])	Child (Age at Diagnosis [years])
Devore and Doan (1957)	Father (33)	Son (27)
	Mother (59)	Son (28)
	Father (60)	Daughter (29)
	Father (50)	Daughter (23)
Razis et al. (1959)	Mother (38)	Son (20)
	Mother (52)	Son (14)
	Father (40)	Daughter (38)
	Mother (52)	Son (46)
	Mother (40)	Son (13)
	Mother (47)	Daughter (19)
	Father (53)	Daughter (16)
Vianna et al. (1974)	Father (65)	Son (43)
	Mother (40)	Son (21)
	Father (43)	Son (23)
	Father (45)	Daughter (18)
	Father (50)	Son (18)
	Mother (41)	Son (28)
	Mother (41)	Daughter (16)
Hors et al. (1980)	Mother (47)	Daughter (15)
	Father (50)	Son (18)
	Father (44)	Son (21)
	Mother (46)	Daughter (24)
Haim et al. (1982)	Mother (26)	Daughter (28)
	Father (44)	Daughter (19)
Hors and Dausset (1983)	Father (39)	Son (18)
		Son (12)
Cimino et al. (1988)	Father (67)	Daughter (30)
	Father (41)	Daughter (9)
	Father (34)	Daughter (9)
	Father (41)	Son (9)

agnosis and well-documented age at diagnosis were included in this study. Age at onset and data sources are listed in table 1. In all pairs except one, HL children reveal a younger age at onset. The mean age at onset is 46 years in parents and 22 years in children. This significant difference between the age at diagnosis of parents and that of children was detected by use of the Mann-Whitney test ($N = 30$, $U = 40.5$, $P < .0001$). One may argue that the smaller number of parents of relatively young age among the pairs reported in the 50s may be due to reduced fitness, as a consequence of poorer treatment. To address this issue, the analysis was repeated after removal of these pairs. The age-at-onset difference between the two generations remained significant (Mann-Whitney; $N = 12$, $U = 2.0$, $P < .0001$), and the mean age at onset was 43.2 years in parents and 17.7 years in children. Therefore, the results presented in this letter support the hypothesis of anticipation in familial HL. Nevertheless, as pointed out by Penrose (1948), false claims of genetic anticipation may be the result of various selection biases. A more optimal study design should be based on prospectively selected cases, as discussed by Horwitz et al. (1996). In addition, infectious agents such as the Epstein-Barr virus (EBV) have been implicated in the etiology of familial HL. The observed anticipation may also be related to simultaneous parent-child exposure to viral infection. To unfold this intriguing relation, further study should focus on cases who test negative for EBV.

Literature search has its place in terms of retrieval of data for a metanalysis. However, in 36 publications, only 30 parent-child pairs were eligible for inclusion in this study, because pairs selected on the basis of certain age criteria were not suitable for the testing of anticipation. Another drawback to the use of published data is that the age at diagnosis of relatives is sometimes not reported, which results in a loss of information. Given the rarity and complexity of the disease, a large international collaboration is required, to fully demonstrate the anticipation effect as well as to elucidate the role of genetic factors in the etiology of familial HL.

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The 8765delAG Mutation in BRCA2 Is Common among Jews of Yemenite Extraction

To the Editor:

The proportion of high-risk families with BRCA2 mutations varies widely among populations. In Iceland, 8% of unselected breast cancer (BC) patients and 64% of patients with a definite family history of BC carry a founder mutation in BRCA2—995del5 (Thorlacius et al. 1997). In the Ashkenazi Jews, the 6174delT mutation is found in 24% of high-risk families and in 6% of unselected BC patients (Abeliovich et al. 1997; Levy-Lahad et al. 1997). Other ancient BRCA2 mutations have been summarized by Szabo and King (1997). Whereas some of the BRCA2 mutations were found in BC-only families, including the majority of families with male and female BC (Ford et al. 1998), other BRCA2 mutations, such as 6174delT, were found in BC/OC patients (i.e., those with BC and/or ovarian cancer [OC]).

In this letter, we describe the 8765delAG mutation in BRCA2, a founder mutation in Jews of Yemenite origin.

During the screening of BC/OC patients for mutations in the BRCA2 gene, PCR products of two patients (III-9 in family BC10 and III-6 in family BC149) of Yemenite extraction had mobility shifts, as determined by single-strand conformation polymorphism (SSCA) (fig. 1*a*). Sequencing of these fragments revealed a deletion of 2 bp (AG), one of three AGs starting at position 8761 (fig. 1*b*). The mutation was analyzed in genomic DNA of the patients and of their family members, by a *Bsm*AI restriction assay using a primer into which a mismatch was introduced (fig. 1*c*). Patient II-4 in family BC703 and patient III-2 in family BC703, who were referred to us because of their ethnic affiliation and positive family history, were analyzed directly for the mutation. The pedigrees of the three families are presented in figure 2. We could not find any relationship among the three families. In families BC10 and BC149, only BC was reported. In family BC703, one of the sisters had BC and

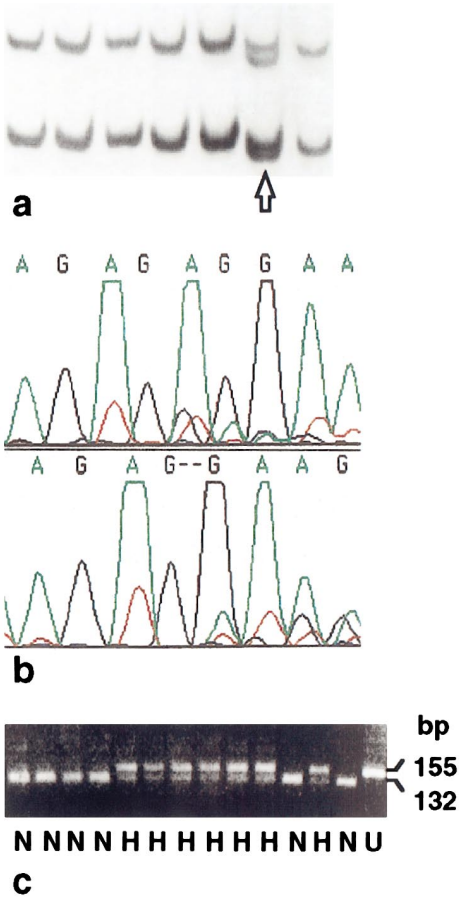

Figure 1 *a,* SSCA. The arrow indicates the fragment with mobility shift in patient III-1 of family BC10; and the other lanes contain DNA samples of unrelated BC/OC patients. PCR primers for amplification of exon 20 were retrieved from the Breast Cancer Information Core (1997); they are 20F, 5′-cactgtgcctggcctgatac-3′; and 20R, 5′-atgttaaattcaaagtctcta-3′. Amplification conditions were 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 30 s; the size of the PCR product was 296 bp. SSCA was performed as described elsewhere (Zlotogora et al. 1995). *b,* Sequence of the 8765delAG mutation in exon 20 of BRCA2 The PCR fragments with mobility shift in SSCA were separated on 8% polyacrylamide gel, were excised from the gel, and were run on 1% low-melt-temperature agarose in tris-acetate/EDTA buffer. The DNA was cleaned with β-Agarase (NEB) and was precipitated with isopropanol. The purified PCR fragments were sequenced by the dideoxy terminator cycle–sequencing method with AmpliTaq DNA polymerase, FS (ABI Prism Ready Reaction Kit), and then were analyzed by use of an automatic DNA sequencer (ABI PRISM 310). The primers for sequencing were the same as those for SSCA. *c,* Restriction analysis (with *Bsm*AI) of the 8765delAG mutation in family members of the identified carriers. U = uncut; N = normal; and H = heterozygote. A mismatch was introduced into one of the primers, and, as a result, the normal allele acquired a *Bsm*AI restriction site. The PCR primers were 20F and misR (5′-gctgcttccttttcttcg*t-3′), and the size of the PCR product was 155 bp for the normal allele and 153 bp for the mutant allele. The PCR products were cut by *Bsm*AI (NEB) and were separated on NuSieve:agarose 3:1, were stained by ethidium bromide, and were visualized under a UV lamp. In the heterozygote, two bands—153 bp and 132 bp—were seen.

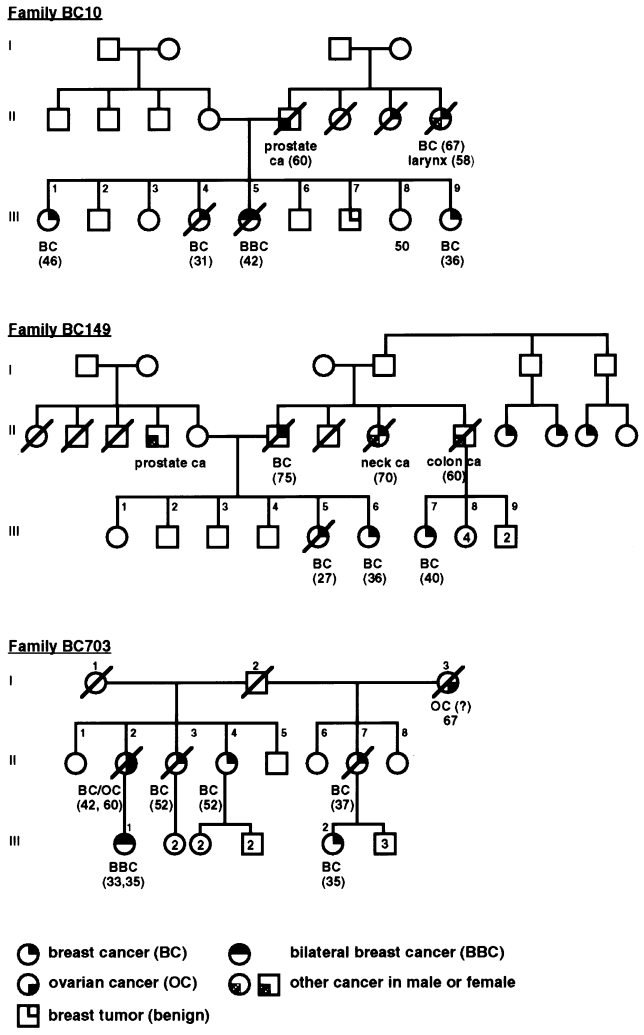

Figure 2 Pedigrees of three families with the 8765delAG mutation. Numbers in parentheses are the ages (in years) at diagnosis.

OC. In the three sibships there were 27 sisters (including the index cases); 13 of them had BC, 2 had bilateral BC, and 1 had BC and OC. The ages at diagnosis were 27–52 years, with a mean of 38.4 years. In all three families, the fathers were apparent carriers. In family BC10, the father had prostate cancer at the age of 60 years; in family BC149, the father had BC at the age of 75 years. The father (I-2) in family BC703 died at the age of 80 years of a cerebrovascular accident (stroke). Other cancers in the families were colon, neck, and laryngeal cancer.

Nine BC patients of Yemenite origin (two of whom were of mixed origin) and without family history of BC/OC were analyzed for the 8765delAG mutation, and none was found to be a carrier (table 1). In a sample of 140 healthy individuals of Yemenite origin, 1 carrier was identified. The control DNA samples were collected from unrelated and unselected individuals and were identified interms of a code number. The frequency (0.7%) of the 8675delAG mutation that was observed in this sample should be validated in a larger sample.

In addition, we tested the 8765delAG mutation in 41 Jewish BC patients—28 Ashkenazi Jews and 13 Sephardic and Oriental Jews (table 1)—who did not carry any of the Ashkenazi founder mutations (185delAG and 5382insC, in BRCA1 ; and 6174delT, in BRCA2). This group of patients met some of the criteria of hereditary BC, such as positive family history of BC and/or OC in three first-degree relatives, bilateral BC, both BC and OC, BC and other primary cancer, or early age at diagnosis (<30 years); some of these patients have been described elsewhere (Abeliovich et al. 1997). None of these patients was a carrier of the 8765delAG mutation, in support of the conclusion that the 8765delAG mutation is confined to the Yemenite Jews.

The haplotypes (D13S171 and D13S260) of the chromosomes bearing the 8765delAG mutation were analyzed in the three families (Lerer et al. 1994). The families all share the same haplotype: allele 7 with $(CA)_{n=5}$, of D13S171, and allele 7, with $(CA)_{n=21}$, of D13S260. In the anonymous carrier in the control group, we could not determine the haplotype, but, in both loci, one of the alleles was the same as that of the mutation's haplotype in the carrier patients. It thus has been concluded that this is a founder mutation in the Yemenite Jews. Among the Jewish people, the Yemenite Jews are a relatively small group that, until their immigration to Israel (during the last century), lived for many years in isolation. The same mutation previously has been described in two French Canadian patients (Phelan et al. 1996). Family members of the two French Canadian patients included 22 females with BC only, with mean age at diagnosis 49.2 years. It thus seems that the risk that the 8765delAG mutation confers on carriers is mainly (but not exclusively) with regard to BC. On the basis of the limited number of patients studied, it seems that the penetrance of the 8765delAG mutation is relatively high, since the carriers had a strong family history of BC; 13 of 27 first-degree relatives had BC, and the age at diagnosis was very early.

It would be of interest to compare the haplotype of the 8765delAG mutation in the Yemenite Jews with that in the French Canadians, although it is highly unlikely that the two groups share a mutation of common ancestral origin. Since the mutation is a deletion of AG in a stretch of AGAGAG, the chance of recurrent mutation resulting in AG deletion in this site might be higher than that in a site having a single AG.

Nine BC patients of Yemenite origin—eight of whom were diagnosed at age <50 years, including one patient with bilateral BC and one patient with two other primary tumors—were not carriers of this mutation, which

Table 1

Jewish BC Patients Who Were Analyzed for the 8765delAG Mutation, According to Clinical Diagnosis and Ethnic Affiliation

	No. (Age [Years] at Diagnosis)				
	Ashkenazim	Sephardim	Orientals	Yemenites	Total
Positive family history	16 (40–64)	4 (40–64)	2 (40–64)		22
BC:					
Unilateral	2 (25–29)	2[a] (25–29)	3 (25–29)	7 (20, 32, 35, 44, 46, 49, 55)[b]	14
Bilateral	9[c]	1	1 (29–65 [BC 40–43, OC 50–58])[d]	1 (BC 34, 38; OC 38)	11
BC and OC	1[e]				2
BC and other primary tumors				1[f]	1
Total	28	7	6	9	50

[a] One patient had a positive family history (i.e., at least three first-degree relatives with BC and/or OC).
[b] Two patients were of mixed origin (i.e., Yemenite/Ashkenazi and Yemenite/non-Ashkenazi).
[c] Four patients had a positive family history (i.e., at least three first-degree relatives with BC and/or OC).
[d] One patient had a positive family history (i.e., at least three first-degree relatives with BC and/or OC).
[e] One patient had a positive family history (i.e., at least three first-degree relatives with BC and/or OC).
[f] The other primary tumors were colon cancer and leukemia.

might indicate that the 8675delAG mutation is not the only BRCA mutation in the Yemenite Jews. Indeed, one BC patient of Yemenite origin (who was not included in this study) was identified as a carrier of the 5382insC mutation in BRCA1.

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Electronic-Database Information

Breast Cancer Information Core, http://www.nhgri.nih.gov/intramural_ research/lab_transfer/Bic

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Localization of a Gene (*CORD7*) for a Dominant Cone-Rod Dystrophy to Chromosome 6q

To the Editor:

The cone-rod dystrophies are a heterogeneous group of retinal disorders, often leading to registrable blindness, that are characterized by an initial loss of cone photo-

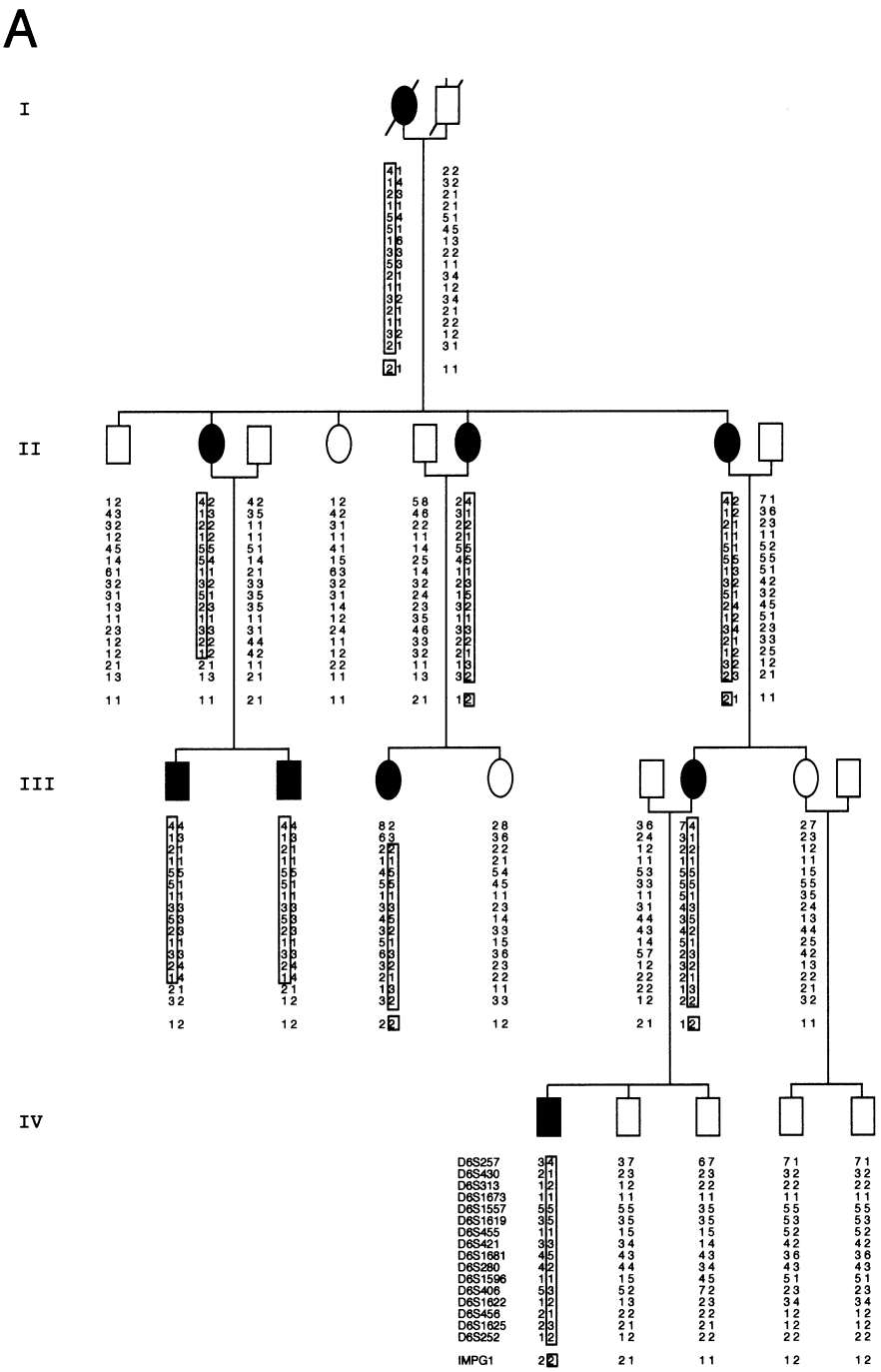
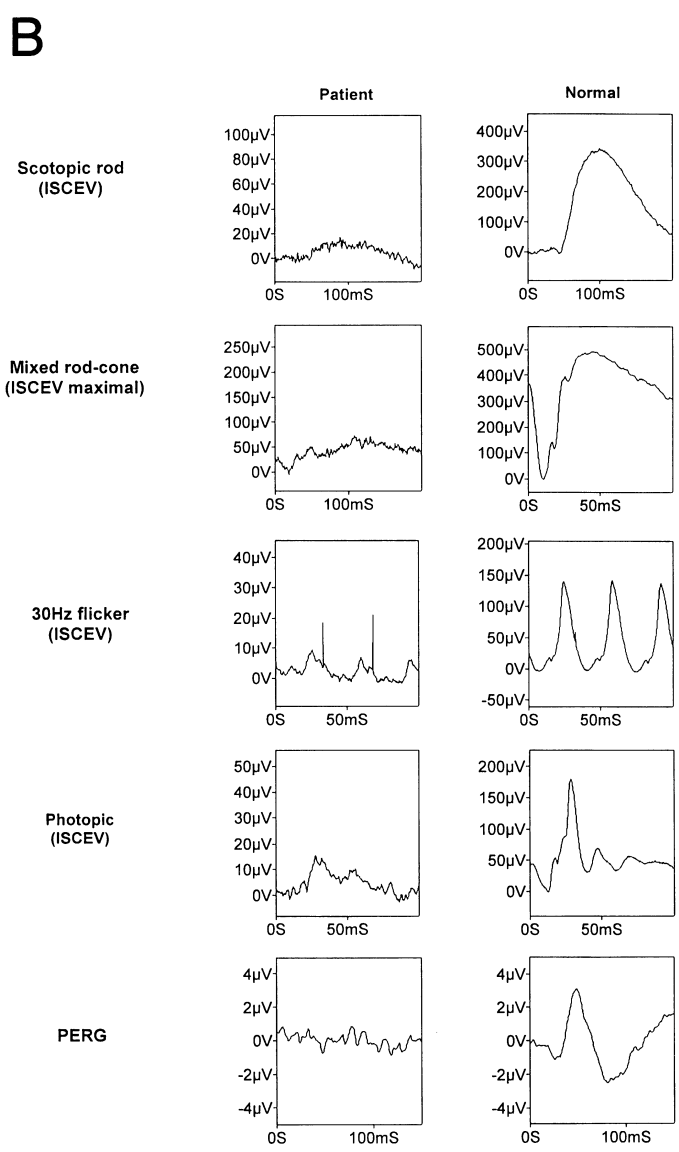


Figure 1 *A, CORD7* pedigree and haplotype results for 16 microsatellite-marker loci situated on chromosome 6q. The identities of microsatellite markers are shown to the left of individual IV-1. The haplotype that appears to be segregating with the disease in this family is boxed. The bottom entry in the list represents the C and G alleles of the *IMPG1* gene. The brackets indicate inferred haplotypes, for individuals I-1 and I-2. *B,* ERG traces from patient II-6 and from a normal control. Except for the pattern ERG ("PERG"), note that different axis scales are used for patient and control traces.

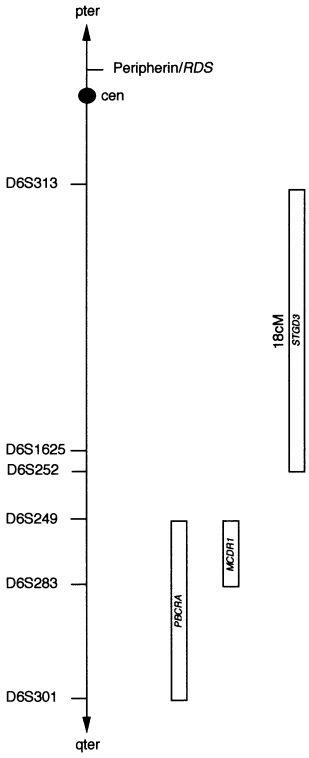
receptors, followed by the degeneration of rod photoreceptors. Recent genetic studies have mapped the disorder to a number of different chromosomal locations (Evans et al. 1994; Kelsell et al. 1997), although, to date, mutations have been identified in only three genes, *peripherin/RDS* (Nakazawa et al. 1994, 1996*a,* 1996*b;* Kohl et al. 1997), *CRX* (Freund et al. 1997), and retinal guanylate cyclase (Kelsell et al., in press). In the present



study, a new chromosomal localization for an autosomal dominant cone-rod dystrophy is reported. In accordance with the guidelines of the Nomenclature Review Committee, "*CORD7*" has been assigned as the gene designation for this disorder.

A four-generation British family was recruited for the study. Affected members of the family first became aware of reduced color vision and visual acuity between the ages of 20 and 40 years. As the disorder progressed, they reported difficulty seeing in bright light, and one individual (IV-1; fig. 1*A*) reported visual problems in dim light. At the onset of symptoms, retinal pigmentary changes were already present around the fovea, simulating a bull's eye dystrophy, which developed to macular atrophy. Electrophysiological tests in advanced disease showed that scotopic rod responses were barely detectable and that all cone responses were severely attenuated (fig. 1*B*) but with no change in implicit time. Pattern electroretinography (ERG) was extinguished, in keeping with the severe macular dysfunction. No significant intraocular asymmetry was present.

EDTA blood samples were obtained from eight affected family members, eight unaffected family members, and four spouses, for linkage analysis (fig. 1). DNA was extracted from these samples with a Nucleon II extraction kit (Scotlab Bioscience). Genotyping was per-



	Recombination fraction							
Marker	0.00	0.05	0.10	0.20	0.30	0.40	Zmax	θ max
D6S257	-∞	2.02	2.02	1.65	1.09	0.44	2.05	0.07
D6S430	-∞	2.02	2.02	1.65	1.09	0.44	2.05	0.07
D6S313	1.51	1.35	1.18	0.83	0.45	0.12	1.51	0.00
D6S1673	0.90	0.81	0.72	0.51	0.29	0.09	0.90	0.00
D6S1557	1.51	1.35	1.18	0.83	0.45	0.12	1.51	0.00
D6S1619	2.11	1.91	1.70	1.24	0.72	0.22	2.11	0.00
D6S455	2.41	2.19	1.95	1.44	0.89	0.35	2.41	0.00
D6S421	2.07	1.89	1.70	1.30	0.85	0.38	2.07	0.00
D6S1681	3.61	3.30	2.97	2.26	1.45	0.59	3.61	0.00
D6S280	3.31	3.02	2.72	2.05	1.31	0.52	3.31	0.00
D6S1596	1.13	1.04	0.93	0.71	0.47	0.24	1.13	0.00
D6S406	2.41	2.19	1.95	1.44	0.89	0.35	2.41	0.00
D6S1622	2.11	1.91	1.70	1.24	0.76	0.32	2.11	0.00
D6S456	2.22	2.03	1.84	1.43	0.96	0.43	2.22	0.00
D6S1625	-∞	2.02	2.02	1.66	1.11	0.47	2.05	0.07
D6S252	-∞	-0.09	0.30	0.45	0.32	0.11	0.45	0.20

Figure 2 Macular dystrophies mapping to human chromosome 6. The table to the right shows the two-point LOD scores for linkage between *CORD7* and marker loci situated on chromosome 6q. The 7-cM region to which *CORD7* maps and the previous 18-cM localization for *STDG3* are depicted schematically to the left of the table. The approximate positions of the other macular dystrophy genes mapping to chromosome 6, with their flanking marker loci, also are shown (not to scale). The peripherin/*RDS* gene maps to the short arm of chromosome 6, and the regions for *MCDR1* and *PBCRA* map below the *STDG3* locus.

formed with microsatellite-marker loci, as described elsewhere (Kelsell et al. 1995). In brief, 100 ng DNA samples were PCR amplified and labeled by $\alpha[^{32}P]$-dCTP incorporation. These amplified products then were separated by denaturing PAGE and were visualized by autoradiography.

Data were collected with LINKSYS 3.1 (Attwood and Bryant 1988), and two-point linkage analysis was performed with the MLINK subprogram of LINKAGE package 5.10 (Lathrop et al. 1984). Allele frequencies were calculated from the four spouses in this family as well as from an additional 10 normal individuals taken from four other British families. The cone-rod dystrophy phenotype in this family was analyzed as an autosomal dominant trait with complete penetrance and a frequency of .001 for the affected allele.

Since a variety of retinal degenerations have been mapped to chromosome 6 (Nichols et al. 1993; Small et al. 1993; Weleber et al. 1993; Wells et al. 1993; Nakazawa et al. 1994; Stone et al. 1994; Kelsell et al. 1995; Reig et al. 1995; Kohl et al. 1997), we chose this region of the genome as the first candidate area for linkage analysis. Two-point linkage data for the family studied (data not shown) excluded the 6p12 region occupied by *peripherin/RDS* and the 6q14-q16.2 region occupied by the genes for North Carolina macular dystrophy (*MCDR1;* Small et al. 1993) and progressive bifocal chorioretinal atrophy (*PBCRA;* Kelsell et al. 1995). Significant linkage was obtained at the Stargardt-like dominant progressive macular dystrophy region on chromosome 6q13-q15 (Stone et al. 1994). Two-point LOD scores obtained after genotyping 16 microsatellite-marker loci are shown in figure 2. Significant linkage was obtained at two of these marker loci, with a maximum LOD score of 3.61 (recombination fraction of 0.00) at *D6S1681*. The haplotypes that define the most likely chromosomal interval for the disease-causing gene are indicated in figure 2. Affected individual III-3 is recombinant at *D6S430* (as well as at the more centromeric marker locus *D6S257*), placing the disease gene telomeric to *D6S430*. Affected individual II-2 is recombinant for *D6S1625* (as well as the more telomeric marker locus *D6S252*), placing the disease gene centromeric to *D6S1625*. Three of the marker loci

(*D6S1619, D6S1681,* and *D6S456*) that give maximum LOD scores at zero recombination were completely informative in these recombinant individuals. Therefore, the disease-causing gene in this family has been localized between *D6S430* and *D6S1625*, a region estimated to be 7 cM in size (Dib et al. 1996).

IMPG1 is an interphotoreceptor matrix proteoglycan gene that has been localized to chromosome 16q14.2-q15 (Felbor et al., in press). It therefore is a good functional candidate for retinal dystrophies mapping to this region of the genome (see Gehrig et al., in press). Exon 13 contains a frequent C/G polymorphism (Gehrig et al., in press) that enabled us to follow the segregation of the *IMPG1* gene in our CORD7 family. The exon was amplified by use of primers and reaction conditions, as described elsewhere (Gehrig et al., in press); sequencing was performed with the PCR-amplification primers by use of AmpliTaq FS polymerase cycle sequencing with dye-labeled dideoxyterminators, and the products were visualized on an Applied Biosystems model 373 sequencer. In figure 1, the C and G alleles are indicated as 1 and 2, respectively. The presence of a crossover in affected individual II-2 places the *IMPG1* gene telomeric to *CORD7* and excludes it as the disease gene.

An autosomal dominant Stargardt-like disease (STGD3) (Stone et al. 1994) also maps to this region of chromosome 6. Clinically, this disorder is quite distinct from CORD7; it is described as a childhood-onset maculopathy with white/yellow flecks in the midperipheral retina. In contrast, the CORD7 disease is of middle-age onset, and no flecks are present in the macula or peripheral retina of affected individuals. However, the possibility that different mutations in the same gene are responsible for STGD3 and CORD7 cannot be ruled out, since clinical heterogeneity is not an infrequent finding. For example, different mutations in the *peripherin/RDS* gene result in retinitis pigmentosa, macular dystrophy, cone-rod dystrophy, pattern dystrophy, or central areolar choroidal dystrophy (Nichols et al. 1993; Weleber et al. 1993; Wells et al. 1993; Nakazawa et al. 1994; Reig et al. 1995; Kohl et al. 1997), and a similar situation is seen for the *ABCR* gene, in which different mutations cause either recessive Stargardt macular degeneration (Allikmets et al. 1997) or recessive retinitis pigmentosa (Martinez-Mir et al. 1998).

Two other retinal dystrophies principally affecting the posterior pole of the eye have been mapped just telomeric to *STGD3* (Small et al. 1993; Kelsell et al. 1995; also see fig. 2), and cytogenetic alterations affecting the chromosome 6q region have been associated with a variety of other retinal diseases (Milosevic and Kalicanin 1975; Hagemeijer et al. 1977; Pierpont et al. 1986; Tranebjaerg et al. 1986). This has led to the suggestion that there may be a family of retinal genes located on chromosome 6q (Small et al. 1992; Kelsell et al. 1995).

The mapping of *CORD7* in this study adds to the expanding list of localizations for cone-rod dystrophies. Clearly, more family studies are required to determine the quantitative importance of each locus. The cloning of the disease genes should aid in our understanding of the etiology of this diverse set of degenerative disorders.

Acknowledgments

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Evidence for Linkage of Spelling Disability to Chromosome 15

To the Editor:

Dyslexia (reading and spelling disability) is one of the most frequently diagnosed disorders in childhood. It is generally agreed that dyslexia has a substantial genetic contribution, although the exact mode of inheritance remains obscure. The phenotype of dyslexia is complex, and different phenotype dimensions can be distinguished. In the *Journal,* Grigorenko et al. (1997) recently reported linkage for distinct components of dyslexia to chromosomes 6 and 15: the phonological-awareness phenotype was mapped to chromosome 6p21-p22, and the single word–reading phenotype was assigned to chromosome 15q21. The chromosome 6 linkage of phonological awareness was supported by multipoint affected-pedigree-member analysis using markers D6S109, D6S461, D6S299, D6S464, and D6S306. With chromosome 15 markers and the single word–reading phenotype, a LOD score of 3.15 was obtained for marker D15S143 at a recombination fraction (θ) of 0, under an autosomal dominant–inheritance model.

We conducted a linkage study for another component of dyslexia—namely, spelling disability—in seven multiplex families from Germany. Twin studies of dyslexia

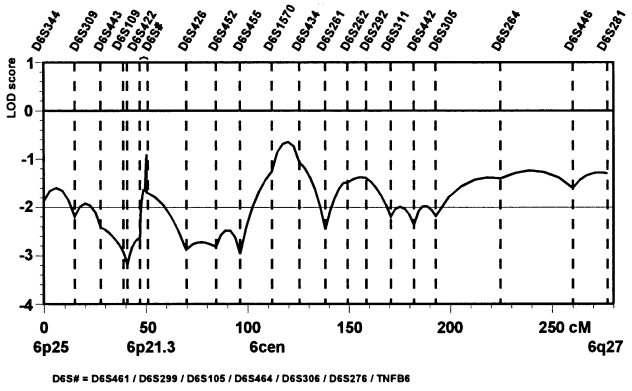

Figure 1 Graphic representation of parametric multipoint linkage analysis of chromosome 6 markers and spelling disability.

have indicated that deficits in spelling are substantially heritable and that the heritability of spelling deficits is higher than the heritability of reading deficits (Stevenson et al. 1987; DeFries et al. 1991). In the families in our study, we genotyped 26 microsatellite markers covering the entirety of chromosome 6 and 13 microsatellite markers covering the entirety of chromosome 15. The highest density of markers was in the regions where Grigorenko et al. (1997) had obtained positive results.

Seven families were chosen from our study sample (Schulte-Körne et al. 1996). Selection criteria were an extended family history of spelling disability and a pedigree suggestive of autosomal dominant transmission (e.g., a three-generational history of familial spelling problems). Diagnosis was based on psychometric tests (IQ test and spelling test) and on a questionnaire (Schulte-Körne et al. 1997). For children to grade 6, the spelling test required the spelling of 30–40 words with specific difficulties with regard to German spelling rules and the German language. For children beyond grade 6 and for adults, a standardized German word–recognition test (Jäger and Jundt 1981) was administered. The nonverbal Culture Fair Intelligence Test (CFT) (Weiß and Osterland 1977; Weiß 1987) was chosen as intelligence test, in order to reduce the influence of verbal abilities and cultural and educational influences on IQ-test performance. Individuals were classified as affected either if their actual spelling achievement (percentile rank as measured by the spelling test) was $\geqslant 1$ SD below the expected spelling achievement based on IQ or if, on the basis of the questionnaire data (adults only; $n = 9$), they had a history of spelling disorder. This definition includes compensated adults (those with a history of spelling disorder but with a discrepancy <1 SD).

Expected spelling achievement was computed by use of a regression model (spelling on IQ) with an assumed .42 correlation between the two measures (Glogauer 1977). The regression equation was derived from a large normative German sample that was independent from our sample. The underlying regression equation is as follows: spelling (T-norm) .42 × (SD IQ/SD spelling) × (IQ − 100) + residual (Schulte-Körne et al. 1996).

After informed consent had been obtained, EDTA blood samples were collected from 67 family members. Of these, 51 were classified as affected. Lymphocyte DNA was extracted by standard methods. Microsatellite markers were typed by use of a model 377 Applied Biosystems automatic sequencer.

Parametric two-point linkage analysis was performed with the LINKAGE package (Lathrop et al. 1984). Parametric and nonparametric multipoint linkage analyses were performed with the GENEHUNTER program (Kruglyak et al. 1996). The *P* values were based on an exact test as described by Kruglyak et al. (1996). For the parametric analyses, the following assumptions were made: autosomal dominant inheritance, disease penetrance .91, phenocopy rate .11, and disease-allele frequency .0298.

On chromosome 6, no significant evidence for linkage was obtained. None of the two-point LOD scores was >0.24 (results not shown). The parametric multipoint analysis showed negative results over the entirety of chromosome 6 (fig. 1). A maximum multipoint LOD score of −0.64 was observed between D6S1570 and D6S434 on the long arm of chromosome 6. A second relative peak, of −0.95, was observed between D6S105 and D6S464 at 6p22-p21. Nonparametric analysis also failed to reveal significant evidence for linkage. The maximum multipoint LOD score peaked at 0.39 ($P = .30$) between D6S1570 and D6S434 and at 0.70 ($P = .21$) between D6S105 and D6S464. Although the data for chromosome 6 were negative, results for chromosome 15 markers supported a locus on 15q21. The two-point LOD scores for spelling disability and markers on chromosome 15q are shown in table 1. The highest two-point LOD score was 1.26 with marker D15S143, at $\theta = 0$. A multipoint LOD score of 1.78 ($P = .0042$) was

Table 1

Results of Two-Point Linkage for Chromosome 15 Markers

	LOD Score at θ =			
Marker	0	.01	.05	.1
D15S214	−.43	−.36	−.15	.01
D15S132	.44	.46	.49	.46
D15S143	1.26	1.23	1.09	.88
D15S126	−.03	.03	.17	.24
D15S117	-1.13	−.97	−.51	-0.17

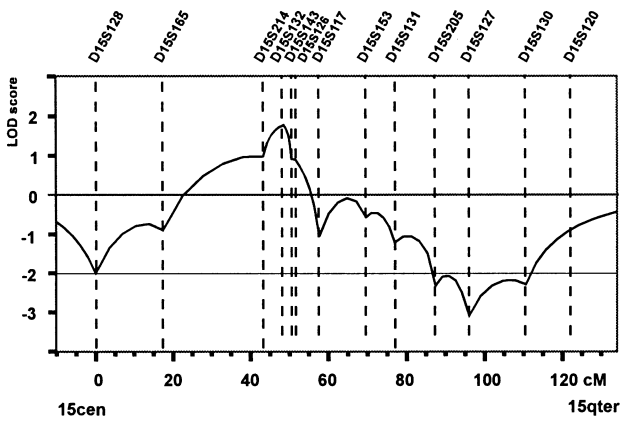

Figure 2 Graphic representation of parametric multipoint linkage analysis of chromosome 15 markers and spelling disability.

achieved with a maximum multipoint LOD score at D15S132 (fig. 2). Linkage to chromosome 15 was also supported by nonparametric analysis. The multipoint maximum LOD score peaked at 2.19 ($P = .03$) at marker D15143.

Our results confirm those of Grigorenko et al. (1997), supporting linkage between chromosome 15q21 markers and dyslexia. The P value of .0042, equivalent to the LOD score of 1.78, obtained in the multipoint LOD-score analysis meets the criteria for confirmation of linkage (Lander and Kruglyak 1995). Given that a third independent study (Smith et al. 1991) had shown linkage of dyslexia to the same chromosomal region, this locus might be considered an established locus for the disorder. The convergence of our results and the findings by Smith et al. (1991) and Grigorenko et al. (1997) is especially interesting, considering that different phenotype definitions were applied. However, spelling and reading disability are strongly correlated ($r = .50 - .80$) (Malmquist 1958), and the results suggest that at least some of the shared variance is responsible for linkage of both phenotypes to chromosome 15. In our study, we found no convincing evidence for linkage of spelling disability to markers on chromosome 6. Although phonological awareness and spelling disability are also moderately correlated ($r = .55$; authors' unpublished data), our results are at least suggestive of the possibility that the shared variance probably is not responsible for the linkage to chromosome 6. However, if the gene residing on chromosome 6 has only a minor effect on spelling disability, then our sample size might have been too small for detection of such an effect. The latter explanation might be supported by a previous study of reading disability, in which evidence for quantitative-trait loci on chromosome 6p21.3 was revealed in a large sample of sib pairs (Cardon et al. 1994). Interestingly, our own results show a relative peak in the same region of chromosome 6.

In conclusion, our results do not support a strong effect by a putative chromosome 6 dyslexia gene on the phenotype of spelling disability. However, we present independent evidence in support of a dyslexia gene on chromosome 15q21. This gene seems to be relevant for spelling (our results) as well as for word reading (Smith et al. 1991; Grigorenko et al. 1997).

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