Letters to the Editor

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Rett Syndrome: Confirmation of X-Linked Dominant Inheritance, and Localization of the Gene to Xq28

To the Editor:

Rett syndrome (MIM 312750) is a neurodevelopmental disorder of unknown cause that primarily affects girls (Naidu 1997). The clinical picture is enigmatic for the normal perinatal period, followed by rapid deceleration of head growth during early childhood, with loss of purposeful hand movements and apraxia. Approximately 99.5% of cases are isolated, with no other affected relative. The mode of inheritance has been hotly debated, with models of both X-linked and sex-influenced autosomal inheritance advanced to explain the preponderance of isolated female cases. We describe a family with the largest number of female siblings affected with Rett syndrome identified to date, and we have used data from this family, as well as from families previously described (Ellison et al. 1992; Schanen et al. 1997; Xiang et al. 1998), to demonstrate X-linked dominant inheritance and to localize the responsible locus to Xq28.

A Brazilian family presented with three daughters showing clinical features characteristic of Rett syndrome. All three affected children showed rapid deceleration of head growth, with subsequent progressive mental deterioration. Two of them (individuals II-6 and II-7; fig. 1*A*) were examined at the Kennedy Krieger Institute. The two living affected daughters (II-6 and II-7), who were examined at 9 and 5½ years of age, showed no purposeful hand movements, with persistent hand stereotypes and rubbing of the torso. They showed spontaneous episodes of hyperventilation while awake. They had a severe attention deficit and no language development. They had significant muscle wasting and an inability to walk. Both had intellectual and adaptive behavior at the 1–6-mo level. Although the younger daughter (II-7) still was able to reach for food, she was without other purposeful hand use. She also had marked air swallowing, with abdominal bloating.

DNA was collected for genetic analyses of these two affected girls, their parents, an additional affected sister (II-5), who subsequently died at 12 years of age, and two normal female siblings. To determine whether this family was consistent with X-linked dominant maternal inheritance, we genotyped the five sisters and their parents for 47 polymorphic markers distributed throughout the X chromosome (fig. 1). Markers were selected from the Genome Database so as to obtain informative markers spaced apart at a maximum distance of 10 cM. Phase was established unambiguously in the mother, and each maternal meiotic breakpoint was mapped for each daughter (fig. 1*A*). Concordance analysis showed that only Xq28 was shared among the three affected girls. This same region was not shared with the unaffected sisters.

We then used the genotype data to conduct a multipoint linkage analysis of the Brazilian family. The relative order of microsatellite markers along the X chromosome and their genetic distances (in centimorgans) were derived from published maps (Fain et al. 1995), by use of a model of X-linked dominant inheritance with the mother assigned the status "nonpenetrant carrier" (fig. 2*A*). The GENEHUNTER package (Kruglyak et al. 1996) was used for multipoint linkage analyses across the entire X chromosome. Only the Xq28 region of the X chromosome showed a positive LOD score ($Z = 1.2$; fig. 2*A*). The threshold for statistically significant evidence of linkage of X-linked traits is $Z > 2.0$. Although the Xq28 region did not reach this threshold, the majority of the remainder of the X chromosome showed $Z < -1.0$. Thus, the statistical *difference* (Kobayashi et al. 1995) between Xq28 and the majority of the remainder of the X chromosome was >2.0, lending some statistical support to the results of the concordance analysis of this single family.

Since 99.5% of Rett cases are isolated female patients, the determination of whether new maternal or paternal mutations are responsible for the disease or of whether the mother is a carrier is, in general, impossible. In the Brazilian family, the presence of three affected daughters suggests that the mother is a carrier, rather than that either parent is a gonadal mosaic. There are four other families that show X-linked inheritance, with nonpenetrant mothers, for which genotype data for the X chromosome have been published previously (fig. 1*B;* Ellison et al. 1992; Schanen et al. 1997; Xiang et al. 1998). We used this published genotype data, together with the data

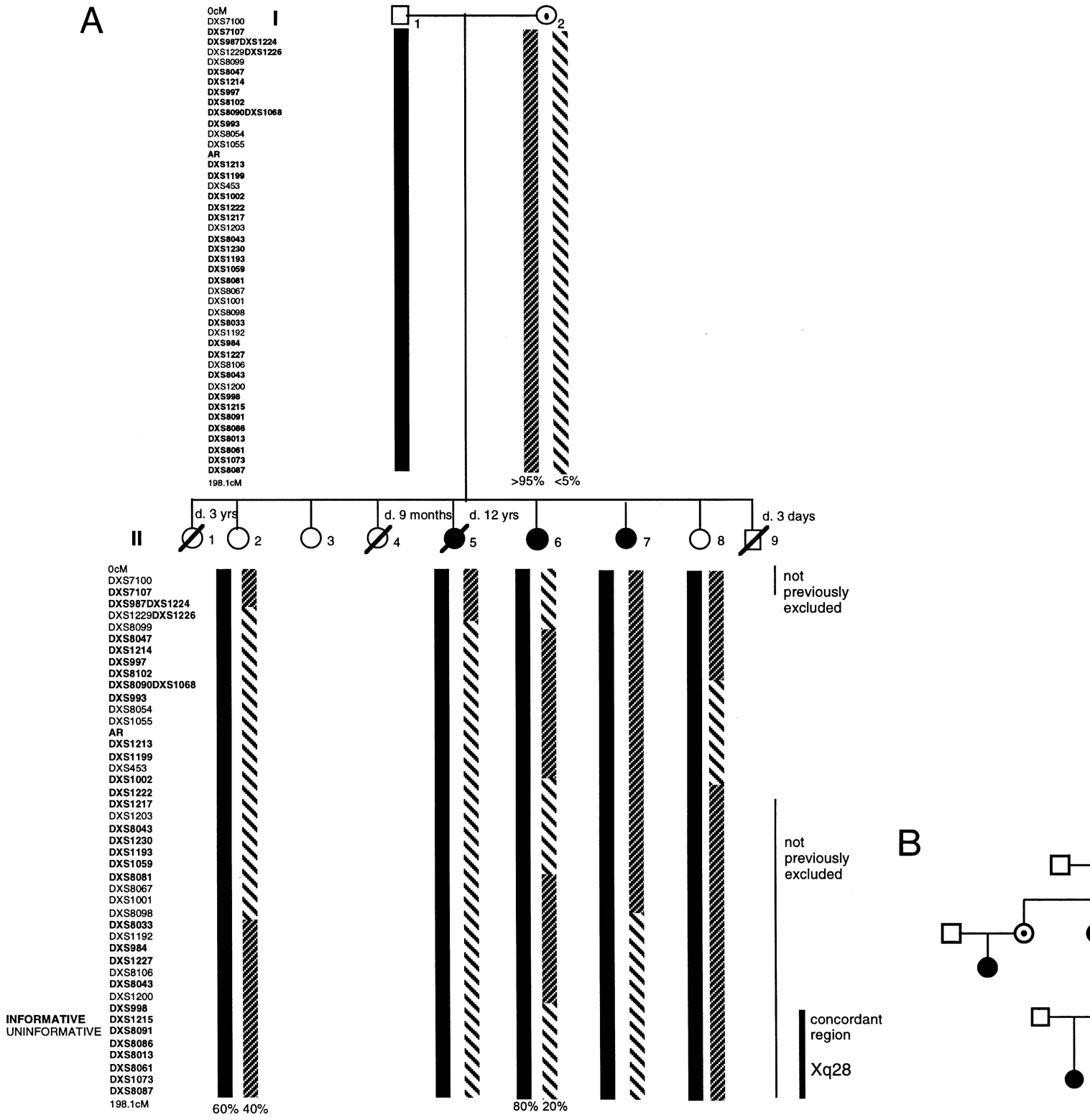

Figure 1 Pedigrees of X-linked families segregating Rett syndrome. *A*, Brazilian pedigree used in this study. The polymorphic markers tested are given, and schematic diagrams of the extended haplotype for each X chromosome are shown. Informative markers are shown in boldface type. The regions of the X chromosome not excluded in previous studies (Xpter–Xp22.2, Xq22.3–Xqter) are indicated ("not previously excluded"; Schanen et al. 1997). The only region of the X chromosome concordant for the Rett syndrome trait is indicated (Xq28). Also shown are the results of quantitative X-inactivation studies, with the percentage of peripheral blood cells with each X active shown below the haplotype schematic (individuals I-2, II-2, and II-6). *B*, X-linked Rett syndrome pedigrees (two sets of affected half sisters with different fathers [Ellison et al. 1992] and two families with an affected aunt/niece pair [Schanen et al. 1997; Xiang et al. 1998]), for which clinical descriptions and genotype data have been reported previously. Genotype data from these pedigrees were used for generation of LOD scores for Xq28 (fig. 2*B*).

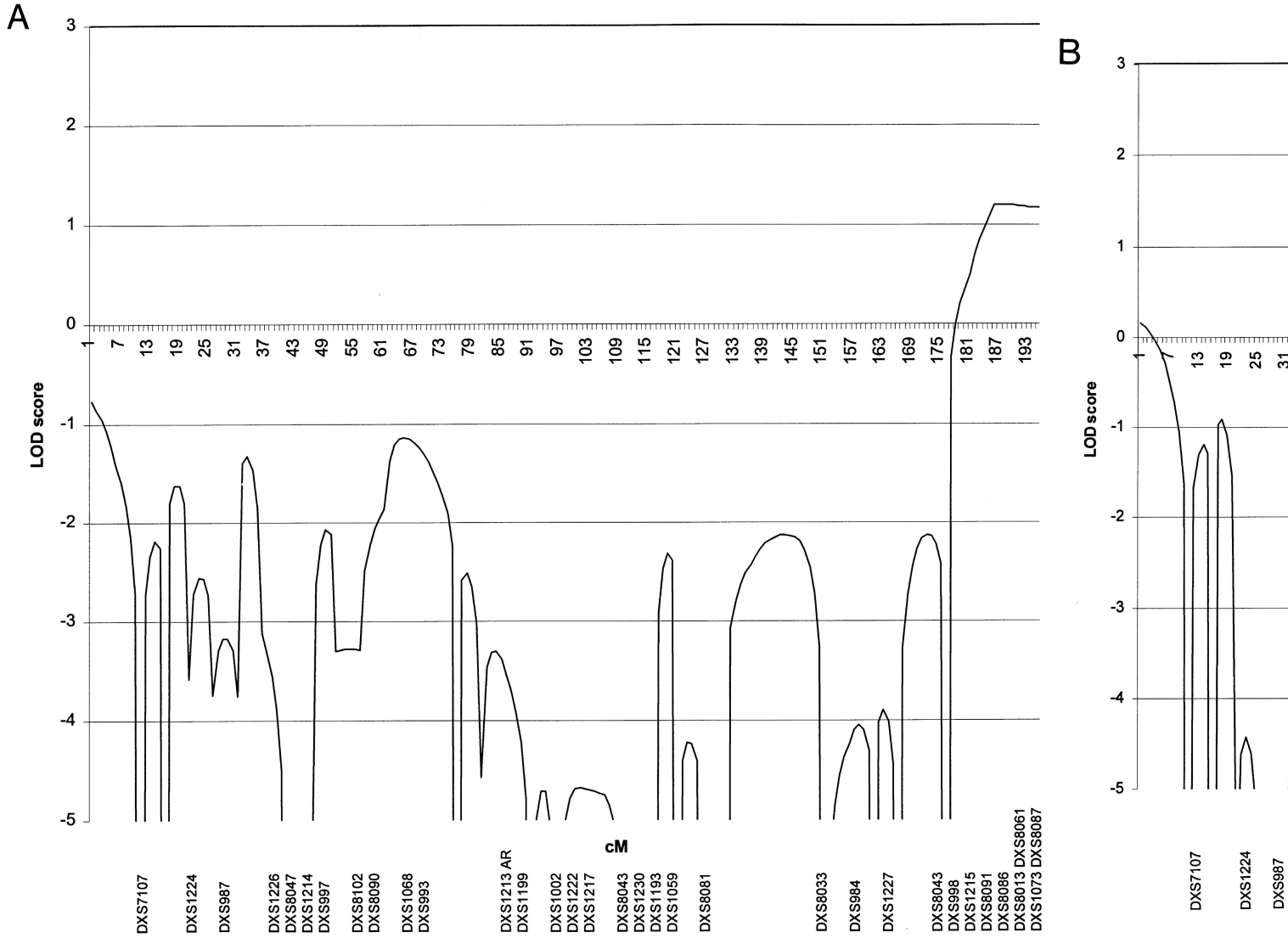

Figure 2 Multipoint linkage analysis across the X chromosome. *A,* Multipoint linkage analysis for the Brazilian family. Xq28 is the only region showing a positive LOD score; all other regions show a negative LOD score. *B,* Combined multipoint linkage analysis for five families, the Brazilian family and four families described elsewhere (see fig. 1*B*). For the pedigree with the aunt/niece pair, the aunt's mother (i.e., the niece's grandmother) showed random X inactivation and was hypothesized to be a gonadal mosaic (Schanen et al. 1997). This woman had an unaffected son, who we excluded from the combined linkage analysis because we were uncertain of his affection status. The combined linkage analysis shows statistically significant ($Z = 2.9$ at recombination fraction 0) support for linkage of the Rett syndrome gene to Xq28.

reported here, to generate a cumulative multipoint score for Xq28 (fig. 2*B*). This analysis showed that all families were consistent with localization of the Rett syndrome gene to Xq28 (DXS998-ter), with a cumulative LOD score of $Z = 2.9$ (fig. 2*B*). These data complement the exclusion-mapping data described by Xiang et al. (1998) and strongly suggest that Rett syndrome is a genetically homogenous disorder and that the gene responsible maps to Xq28.

For the Brazilian family described here, the pedigree is consistent with the mother being a nonpenetrant carrier of Rett syndrome. If the mother is a nonpenetrant carrier of Rett syndrome, then skewed X inactivation toward the normal X chromosome should be found, as has been seen in other obligate carriers (see fig. 1*B;* Zoghbi et al. 1990; Schanen et al. 1997). To test this, we performed quantitative X-inactivation assays, using a fluorescent androgen-receptor assay (Pegoraro et al. 1994). With this assay, the methylation status of the androgen-receptor promoter adjacent to a highly polymorphic CAG repeat in the 5′ end of the coding region of the androgen-receptor gene was determined by use of methylation-sensitive restriction enzymes *Hpa*II and *Cfo*I. PCR products were electrophoresed, both before and after digestion, on an ABI 373A automated sequencer, and peak heights were analyzed by use of

GeneScan software (Applied Biosystems). Corrections for preferential amplification of specific alleles and quantitation of X inactivation were completed as described elsewhere (Pegoraro et al. 1994). We found the mother to have highly skewed X inactivation (>95%:<5%; figs. 1 and 3). We had studied previously the X-inactivation patterns in 65 normal female volunteers, using this same assay, and had shown that none of these 65 individuals showed skewing at levels of 95%:5% or greater (Pegoraro et al. 1997). Furthermore, we set the phase of the androgen-receptor markers with the Xq28 region and showed that the mother had the *unaffected* X active in 95% of cells (fig. 1). Thus, our finding of highly skewed X inactivation in the mother, with preferential use of the unaffected X chromosome, strongly suggests that she is a nonpenetrant carrier of Rett syndrome. X-inactivation analyses also were performed for one unaffected daughter and an additional affected daughter: neither showed highly skewed X inactivation (figs. 1 and 3).

Many models have been used to explain the enigmatic incidence of Rett syndrome in isolated female patients. Most prominent has been that of an X-linked dominant trait that is lethal to males and that results in Rett syndrome in carrier females. Since affected females are considered to lack reproductive opportunities, a high new-mutation rate, in either male or female germ lines, would be needed in order to maintain a relatively high disease frequency. This could explain the preponderance of isolated cases and the failure to observe a high recurrent spontaneous-abortion rate (affected males), since relatively few mothers of children with Rett syndrome are carriers of the disease. We used data from the Rett syndrome pedigrees showing X-linked inheritance (fig. 1 *A* and *B*). Our data are consistent with previously published data suggesting that nonpenetrant obligate car-

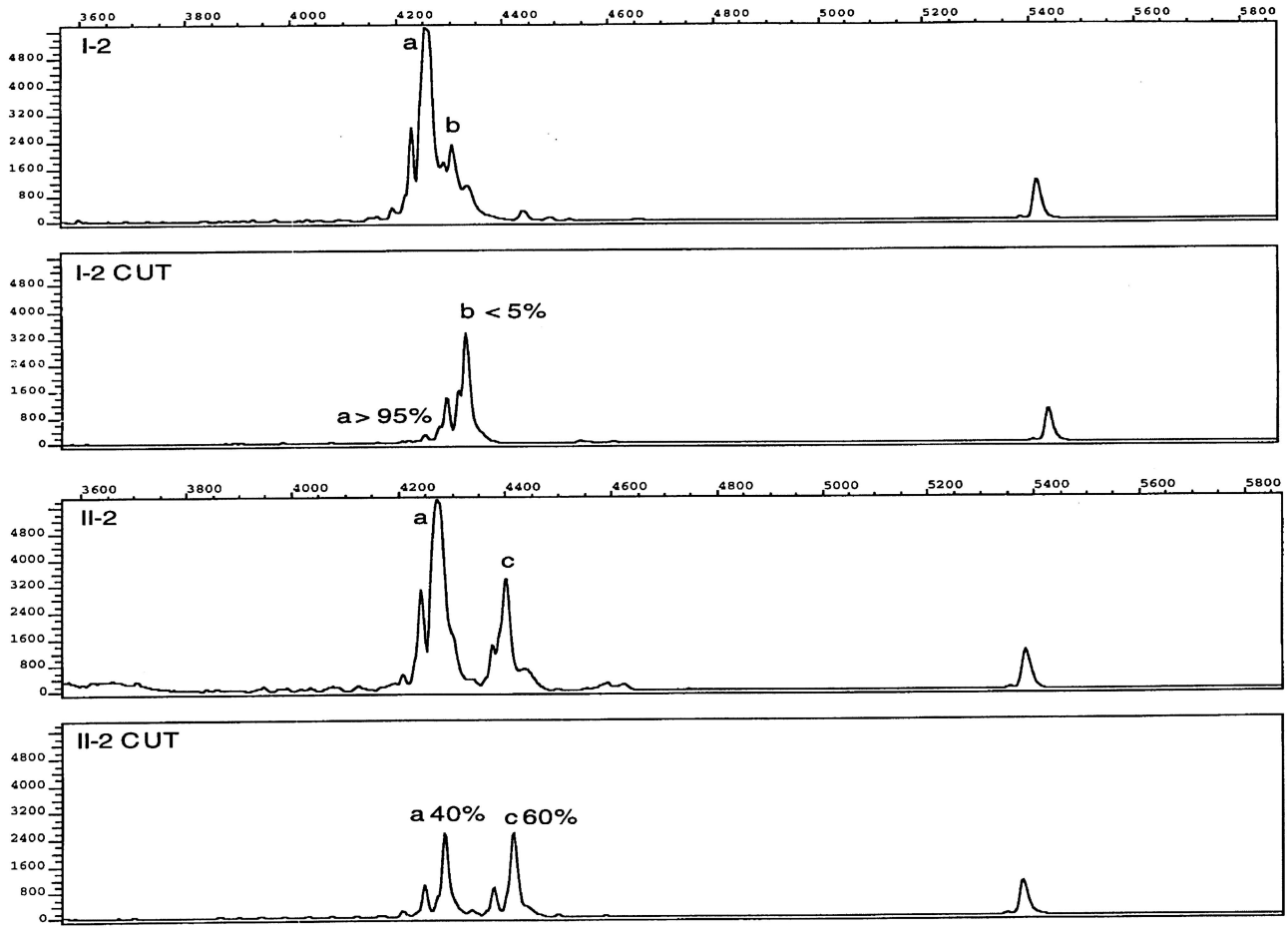

Figure 3 X-inactivation assay for two members of the Brazilian pedigree. Quantitation of X-inactivation patterns in individuals I-2 (unaffected mother) and II-2 (unaffected daughter) is shown. The top panel of each pair shows the results for alleles of the androgen receptor, and the lower panel of each pair shows the results for digestion with methylation-sensitive restriction enzymes prior to amplification. Preferential amplification of one allele is common when this assay is used (see allele a in top panel for individual I-2); thus, X-inactivation patterns must be normalized. The mother (I-2) of the children with Rett syndrome shows highly skewed X inactivation (>95%:5%) with preferential use of the normal X chromosome. The unaffected daughter (II-2) shows random X inactivation.

riers in these pedigrees show skewed X inactivation, whereas Rett syndrome patients show random (equal) X-inactivation patterns. Our data extend previous data by showing that the extreme X inactivation is toward the *normal* X chromosome in the obligate-carrier mother (I-2) in our Brazilian pedigree (fig. 1*A*). The preponderance of female children in this pedigree also is consistent with Rett syndrome being a male-lethal trait. Finally, our finding of a significant LOD score ($Z = 2.9$ at recombination fraction 0) for linkage of Rett syndrome to Xq28 (DXS998-ter) provides convincing statistical support for an X-linked dominant model and suggests that the disease likely is genetically homogeneous.

Typically, females affected with Rett syndrome show random X-inactivation patterns, strongly suggesting that there is no selective *disadvantage* for cells carrying the Rett trait on their *active* X chromosome (Zoghbi et al. 1990). On the other hand, obligate asymptomatic carriers show skewed X inactivation, and data from our family show that this skewing is toward the *unaffected* X chromosome. Since affected girls show no selective advantage for the normal X chromosome, speculation of why *asymptomatic* carriers show an apparent selective advantage for the *normal* X chromosome is important. Traits causing highly skewed X inactivation in females have been documented recently, but no overt phenotype has been found to be inherited in a Mendelian fashion (Pegoraro et al. 1997; Plenge et al. 1997). Indeed, the chance co-occurrence of a skewed X-inactivation trait and an X-linked recessive disease in carrier females may explain why many carriers manifest an X-linked disease (Hoffman et al. 1996). We hypothesize that the rare incidence of familial cases of Rett syndrome is due to the necessity for two concomitant traits to be present in an asymptomatic carrier; that is, the presence of one trait for Rett syndrome and one trait for skewed X inactivation (which may or may not map to the X chromosome) forces preferential activation of the unaffected X and thereby permits obligate carriers to reproduce. We had shown previously that such skewed X-inactivation traits may lead to fetal loss of males; the mechanisms of the association of recurrent pregnancy loss and skewed X-inactivation traits are still being investigated (Pegoraro et al. 1997).

Our evidence supporting the presence of a gene on Xq28 should set the stage for the identification of the responsible gene. Candidate genes for Rett syndrome likely will be involved in postnatal CNS development, since the major abnormalities appear to be confined to the developing brain (Naidu 1997). It is pertinent to note that Xq28 is a very gene- and disease-rich region. Further genetic mapping designed to narrow the disease-gene region within Xq28 may prove difficult because of the paucity of families. However, the candidate-gene approach, using either genes characterized for CNS involvement or anonymous expressed sequence tags mapped to Xq28, ultimately should prove successful.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, http://gdbwww.gdb.org (for the polymorphic markers used in genotyping)

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

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