Letters to the Editor

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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

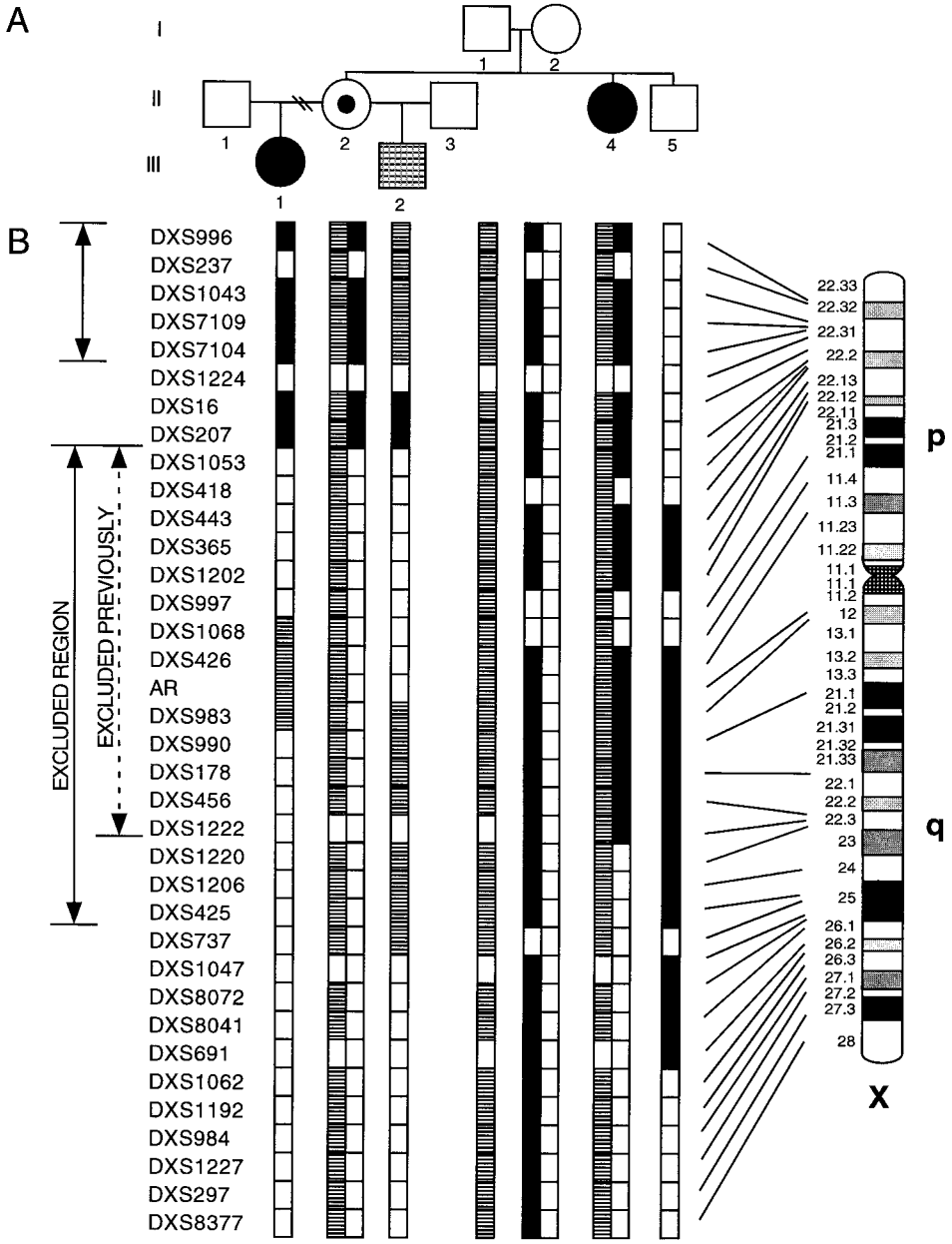

Figure 1 Exclusion mapping of X-chromosome markers. *A,* Pedigree for the RTT kindred. Probands with classic RTT are denoted by blackened symbols; and the severely affected male is designated by a cross-hatched symbol. *B,* Genotyping results for 36 microsatellite markers. The alleles of I-1 are indicated 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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