Letters to the Editor

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Rett Syndrome in a Boy with a 47,XXY Karyotype

To the Editor:

Rett syndrome (RS [MIM 312750]) is a progressive encephalopathy characterized by severe mental retardation, autism, apraxia, seizures, stereotypical hand movements, and deceleration of head growth. Its prevalence is estimated at 1:10,000–15,000 female births (Hagberg 1995). The majority of cases are sporadic, but rare reports of familial recurrence have been made. In addition, all but 1 of the 10 MZ twins reported in the literature are concordant, whereas all 11 DZ twins reported are discordant for the disorder (Migeon et al. 1995). Laboratory investigations have not revealed any metabolic abnormalities in affected individuals.

Chromosomal abnormalities and/or association with another syndrome have already been reported in patients with RS: a translocation t(X;22)(p11.22;p11) by Journel et al. (1990), a translocation t(X;3)(p21.3;p25.2) by Zoghbi et al. (1990) and Ellison et al. (1993), a deletion del(3)(3p25.1-p25.2) by Wahlström et al. (1996), and a deletion del(13)(13q12.1-q21.2) by Herder et al. (1996). RS was described in association with fragile X by Alembick et al. (1995) and with Down syndrome by Easthaugh et al. (1996). No concordance for the chromosomal abnormalities has been found, however, since different chromosomes and/or breakpoints were involved in each case. Vorsanova et al. (1996) reported a boy with RS and karyotype 46,XY/47,XXY (the 47,XXY cell line was observed in 6%–12% of the studied lymphocytes).

Here, we describe a patient with RS and a 47,XXY karyotype. The propositus, a male patient born in January 1995, was referred for genetic studies at age 28 mo. His parents are healthy, were aged 30 years (father) and 29 years (mother) at the time of the birth, and are not consanguineous. The child was born at term, after an uneventful pregnancy. His birth weight was 3.330 g (25th–50th percentile), his Apgar indices were 6 (1st minute) and 7 (5th minute), and his birth occipitofrontal head circumference was 32 cm (2.5 percentile). The perinatal period was uneventful. The propositus is the fourth child, and his older sibs—two boys aged 16 and 9 years and one girl aged 13 years—are normal. There is no history of neuropsychiatric diseases in the families of the mother or the father. The propositus showed normal development until age 8 mo. At that time, he sat without support, played normally, and was able to grasp objects and to put food into his mouth. He had also started to say some words comprehensibly.

The family noticed that, at age 11 mo, he had lost purposeful hand movements and language skills. He also began to show regression in social contact. At age 1 year, he began to show stereotypical hand movements, bruxism, and constipation. At age 28 mo, he presented severe global retardation and slightly diffuse hypotonia. He was socially isolated and made few spontaneous movements (other than the stereotypical hand movements). He did not grasp or otherwise show interest in any object or toy. He could vocalize but did not form any words. He reacted to luminous and sonorous stimuli. When standing up with support, he presented axial ataxia. Bruxism and short episodes of apnea were observed during consultation. No focal neurological signs or alteration in cranial nerves were observed. His occipitofrontal head circumference was 45 cm (2.5 percentile), his weight was 12.220 g (35th percentile), and his height was 87 cm (25th percentile).

When the patient was last seen, at age 37 mo, the loss of purposeful hand movements, the manual apraxia, and the slight global hypotonia were persistent. The stereotypy of his hand movements was midline, was constant in vigil, and showed a slightly athetoid component. When walking with support, he presented ataxia/apraxia. He reacted to luminous and sonorous stimuli. The episodes of apnea were more frequent and more sustained. His occipitofrontal head circumference was 46 cm (2.5 percentile), his weight was 15.200 g (35th percentile), and his height was 94 cm (25th percentile). Results of electroretinogram, magnetic resonance imaging of the brain, and electroencephalogram were normal. The results for rubella, syphilis, HIV I and HIV II, cytomegalovirus, herpes, cerebrospinal fluid, and serum amino acid testing were all normal. Toxoplasmosis testing showed that the patient's IgG level was slightly increased. However, acquired neurological disorders resulting from congenital toxoplasmosis infection were

ruled out, since the boy was normal from birth until age ~8 mo.

Chromosomal analysis, including GTG banding, was performed on peripheral blood leukocytes as described by Seabright (1971). Karyotype analyses from all 300 banded metaphase preparations showed 47 chromosomes with an extra X chromosome (47,XXY).

To establish the origin of the nondisjunction, we analyzed DNA from the mother and the propositus with eight microsatellite markers from the dystrophin gene—5′DYSI; 5′DYSII; 3′DYSMS; STR 44; STR 45; STR 49; STR 50; and 3′-19n8. DNA from the father was not available. DNA analysis showed that the propositus had an allele that was not present in his mother, indicating, therefore, that the additional sex chromosome was paternal in origin—that is, it resulted from nondisjunction at the paternal first meiotic division.

For X-inactivation analyses, DNA was extracted from peripheral blood from the mother and the propositus, and 1 μg of digested (with *Alu*I and *Cfo*I) and nondigested DNA samples were used as templates for amplification of the androgen receptor (AR) highly polymorphic $(CAG)_n$ repeat, as reported (Allen et al. 1992; Edwards et al. 1992). All samples were run in duplicate in a 5% polyacrylamide gel (19:1 acrylamide:bis-acrylamide). A densitometer (Shimadzu CS-9000) was used to determine the ratio of X inactivation in each sample, and the mean of two readings was considered for each case. Since one allele may amplify more than the other, a correction factor was applied to compensate for unequal amplification of alleles. We did this for the mother and for the son, calculating, first, the ratio between the two alleles of the undigested DNA and correcting the final values for preferential PCR amplification (Pegoraro et al. 1994). We calculated the degree of X inactivation on the digested DNA by normalizing the sum of allele A plus allele B to 100%, as reported in Sumita et al. (1998). The analysis of the X-chromosome–inactivation pattern in blood DNA showed X-inactivation ratios of 73:27 in the mother and $41X^P$:$59X^M$ in the affected son.

To rule out a possible diagnosis of Angelman syndrome (AS), the methylation status of the locus *SNRPN* mapped within the PWS/AS region was assessed by Southern blotting. The probe used was a 0.6-kb *Eco*RI-NotI fragment that contains exon 1 of *SNRPN* (Glenn et al. 1996). Methylation assay for AS was analyzed at the *SNRPN* CpG island and a normal result was obtained, with the presence of the 0.9-kb band from the unmethylated paternal allele and a 4.2-kb band from the methylated maternal allele. This method confirms the diagnosis in ~80% of cases, since in the remaining 20% AS may be due to UBE3A mutations or other unknown mechanisms (Kishino et al. 1997; Matsuura et al. 1997)

The parental origin of additional sex chromosomes was studied by Lorda-Sanchez et al. (1992) in 47 patients with a 47,XXY chromosome constitution. In 23 (49%) cases, the error occurred during the first paternal meiotic division, as observed in the present case. No significant clinical differences were found among patients of distinct parental origin.

To date, RS has been convincingly described only in females. Some cases described as RS syndrome in males have been reported (Coleman 1990; Eeg-Olofsson et al. 1990; Philippart 1990; Topçu et al. 1991; Christen and Hanefeld 1995; Vorsanova et al. 1996). The clinical signs and symptoms, however, were but suggestive, atypical, and/or partial. In the present report, the clinical and laboratory findings do not overlap with any described for Klinefelter syndrome. AS was excluded with 80% certainty, and extensive testing did not disclose any other alternative etiology, such as infantile neuronal ceroid-lipofuscinosis. The clinical findings met the criteria of inclusion and exclusion for the diagnosis of RS (Trevathan and Naidu 1988).

Several authors (Zoghbi et al. 1990; Webb et al. 1993; Camus et al. 1996; Webb and Watkiss 1996; Krepischi et al. 1998) reported that, as a group, RS patients tended to present a higher frequency of moderate skewing (20%–35% or 65%–80%) of X inactivation in lymphocytes, when compared with their mothers and normal controls, and that this skewing, when present, favors, in most cases, preferential inactivation of the paternally inherited X chromosome. On the other hand, it has been suggested that extreme skewed X inactivation could prevent manifestation of the RS phenotype in mutant-gene female carriers, which would be consistent with RS being a male-lethal trait (Schanen and Franke 1998; Xiang et al. 1998). In the present report, analysis of X inactivation in the proband and his mother did not show extreme skewed X inactivation, suggesting that the proband might be the result of a new paternal or maternal germ line mutation event. However, as shown previously, it is not known whether the X-inactivation pattern found in DNA from blood is representative of other tissues and, furthermore, a skewed pattern of X-inactivation in blood is not rare in normal females (Naumova et al. 1996; Sumita et al. 1998). Therefore, although the occurrence of moderate skewing is more frequent in RS patients and extreme skewed X inactivation has been observed in obligate RS carriers (Sirianni et al. 1998), a correlation between X-inactivation skewing and the RS phenotype must be interpreted with caution.

An explanation for the exclusive occurrence of RS in females, without evidence of male lethality, was proposed by Thomas (1966) on the basis of the fact that de novo X-linked mutations occurring exclusively in male germ cells could only be passed on to, and result in, an affected daughter. Under such a hypothesis, the

absence of affected males is explained by the fact that sons do not inherit their X chromosomes from their fathers. Since our patient inherited one of his two X chromosomes from his father, his RS phenotype would be consistent with Thomas's hypothesis if the mutated gene was on the paternal X chromosome. On the other hand, RS-affected half sisters with the same mothers have been described (Archidiacono et al. 1991; Sirianni et al. 1998). However, under Thomas's hypothesis, it would be expected, in rare instances, to find families with half sisters with the same father, because of germinal mosaicism. This has already been demonstrated for other disorders such as achondroplasia (Philip et al. 1988) and Duchenne muscular dystrophy (Darras and Francke 1987) but apparently has not been reported for RS.

In a recent report, Sirianni et al. (1998) postulated that the relatively high frequency for RS would be explained by a high mutation rate in either male or female germ lines. In the present case, it was not possible to determine whether the mutation was inherited through paternal or maternal gametes.

With respect to the etiology of RS, several investigators have suggested the possibility of an alteration in the timing of replication of a gene (or genes) on the late X chromosome in RS patients (Riccardi 1986; Martinho et al. 1990; Kormann-Bortolotto 1992; Webb and Watkiss 1996). If this alteration represents the "misbehavior" of a gene (or genes) that should be inactive on the inactivated X chromosome but, when mutated, does not respond to XIST (the product of the X-inactivation–center gene), the consequence would be transient functional disomy at one or more loci. Partial functional disomy as a cause for RS (Webb et al. 1993) and other abnormal phenotypes, such as hypomelanosis of Ito or mental retardation, has already been suggested (Journel 1990; Schmidt and Du Sart 1992; Correa-Cerro et al. 1997; Wolff et al. 1998). If such a mechanism occurred in RS patients, this condition could be the result of functional disomy.

The present report, confirming an RS phenotype in a 47,XXY male, is consistent with the hypothesis that two X chromosomes are required for the manifestation of Rett syndrome.

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Electronic-Database Information

The URL for data in this article is as follows:

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

References

Alembick Y, Dott B, Stoll C (1995) Rett-like syndrome in fragile X syndrome. Genet Couns 6:207–210

Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW (1992) Methylation of *Hpa*II and *Hha*I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229–1239

Archidiacono N, Lerone M, Rocchi M, Anvret M, Ozcelik T, Francke U, Romeo G (1991) Rett syndrome: exclusion mapping following the hypothesis of germinal mosaicism for new X-linked mutations. Hum Genet 86:604–606

Camus P, Abbadi N, Perrier M-C, Chéry M, Gilgenkrantz S (1996) X chromosome inactivation in 30 girls with Rett syndrome: analysis using the probe. Hum Genet 97:247–250

Christen HJ, Hanefeld F (1995) Male Rett variant. Neuropediatrics 26:81–82

Coleman M (1990) Is classical Rett syndrome ever present in males? Brain Dev 12:31–32

Correa-Cerro LS, Rivera H, Vasquez AI (1997) Functional Xp disomy and de novo t(X;13)(q10;q10) in a girl with hypomelanosis of Ito. J Med Genet 34:161–163

Darras BT, Francke U (1987) A partial deletion of the muscular dystrophy gene transmitted twice by an unaffected male. Nature 329:556–558

Easthaugh P, Smith L, Leonard H (1996) Trisomy 21 associated with Rett syndrome phenotype. Paper presented at the World Congress on Rett Syndrome, Gothenburg, Sweden, 30 August–1 September

Edwards AL, Hammond HÁ, Jin L, Cakey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12:241–253

Eeg-Olofsson O, Al-Zuhair AGH, Teebi AS, Zaki M, Daoud AS (1990) A boy with Rett syndrome? Brain Dev 12:529–532

Ellison KA, Roth EJ, McCabe ERB, Chinault AC, Zoghbi HY (1993) Isolation of a yeast artificial chromosome contig spanning the chromosomal translocation breakpoint in a patient with Rett syndrome. Am J Med Genet 47:1124–1134

Glenn CC, Saitoh S, Jong MTC, Filbrandt MM, Surti U, Driscoll DJ, Nicholls RD (1996) Gene structure, DNA methylation, and imprinted expression of the human SNRPN gene. Am J Hum Genet 58:335–346

Hagberg B (1995) Rett syndrome: clinical peculiarities and biological mysteries. Acta Paediatr 84:971–976

Herder GA, Skjeldal O, Hagberg B, Tranebjærg L (1996) Congenital Rett syndrome phenotype–interstitial deletion chromosome 13 and retinoblastoma. Paper presented at the World Congress on Rett syndrome, Gothenburg, Sweden, 30 August–1 September

Journel H, Melki J, Turleau C, Munnich A, Grouchy J (1990) Rett phenotype with X/autosome translocation: possible mapping to the short arm of chromosome X. Am J Med Genet 35:142–147

Kishino T, Lalande M, Wagstaff J (1997): UBE3A/E6AP mutations cause Angelman syndrome. Nat Genet 15:70–73

Kormann-Bortolotto MH, Woods CG, Green SH, Webb T (1992) X-inactivation in girls with Rett syndrome. Clin Genet 42:296–301

Krepischi ACV, Kok F, Otto PG (1998) X chromosome inactivation patterns in patients with Rett syndrome. Hum Genet 102:319–321

Lorda-Sanchez I, Binkert F, Maechler M, Robinson WP, Schinzel A (1992) Reduced recombination and paternal age effect in Klinefelter syndrome. Hum Genet 89:524–530

Martinho PS, Otto PG, Kok F, Diament A, Marques-Dias MJ, Gonzalez CH (1990) In search of a genetic basis for the Rett syndrome. Hum Genet 86:131–134

Matsuura T, Sutcliffe JS, Fang P, Galjaard R-J, Jiang Y, Benton CS, Rommens JM, et al. (1997): De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nat Genet 15:74–73

Migeon BR, Dunn MA, Thomas G, Schmeckpeper BJ, Naidu S (1995) Studies of X inactivation and isodisomy in twins provide further evidence that the X chromosome is not involved in Rett syndrome. Am J Hum Genet 56:647–653

Naumova AK, Plenge RM, Bird LM, Leppert M, Morgan K, Willard HF, Sapienza C (1996) Heritability of X chromosome-inactivation phenotype in a large family. Am J Hum Genet 58:1111–1119

Pegoraro E, Schimke RN, Arahata K, Hayashi Y, Stern H, Marks H, Glasberg MR, et al (1994) Detection of new paternal dystrophin gene mutations in isolated cases of dystrophinopathy in females. Am J Hum Genet 54:989–1003

Philip N, Auger M, Mattei JF, Giraud F (1988) Achondroplasia in sibs of normal parents. J Med Genet 25:857–859

Philippart M (1990) The Rett syndrome in males. Brain Dev 12:33–36

Riccardi VM (1986) The Rett syndrome: genetics and the future. Am J Med Genet Suppl 24:389–402

Schanen C, Francke U (1998) A severely affected male born into a Rett syndrome kindred supports X-linked inheritance and allows extension of the exclusion map. Am J Hum Genet 63:267–269

Schmidt M, Du Sart D (1992) Functional disomies of the X chromosome influence the cell selection and hence the X inactivation pattern in females with balanced X-autosome translocations: a review of 122 cases. Am J Med Genet 42: 161–169

Seabright M (1971) A rapid banding technique for human chromosomes. Lancet 2:971–972

Sirianni N, Naidu S, Pereira JL, Pillotto RF, Hoffman EP (1998) Rett syndrome: confirmation of X-linked dominant inheritance, and localization of the gene to Xq28. Am J Hum Genet 63:1552–1558

Sumita DR, Vainzof M, Campiotto S, Cerqueira AM, Cánovas M, Otto PA, Passos-Bueno MR, et al (1998) Absence of correlation between skewed X inactivation in blood and serum creatine-kinase (CK) levels in Duchenne/Becker female carriers. Am J Med Genet 80:356–361

Thomas GH (1996) High male:female ratio of germ-line mutations: an alternative explanation for postulated gestational lethality in males in X-linked dominant disorders. Am J Hum Genet 58:1364–1368

Topçu M, Topaglu H, Renda Y, Berket M, Turani G (1991) The Rett syndrome in males. Brain Dev 13:62

Trevathan E, Naidu S (1988) The clinical recognition and differential diagnosis of Rett syndrome. J Child Neurol 3(Suppl):S6–16

Vorsanova SG, Demidova IA, Ulas V Yu, Soloviev IV, Kazantzeva LZ, Yurov Yu B (1996) Cytogenetic and molecular-cytogenetic investigation of Rett syndrome: analysis of 31 cases. Neuroreport 8:187–189

Wahlström J, Uller A, Tonnby B, Darnfors C, Martinsson T, Vujuic M (1996) Congenital Rett Syndrome phenotype-deletion short arm chromosome 3. Paper presented at the World Congress on Rett Syndrome, Gothenburg, Sweden, 30 August–1 September

Webb T, Watkiss E (1996) A comparative study of X inactivation in Rett syndrome probands and control subjects. Clin Genet 49:189–195

Webb T, Watkiss E, Woods CG (1993) Neither uniparental disomy nor skewed X-inactivation explains Rett syndrome. Clin Genet 44:236–240

Wolff DJ, Schwartz S, Montgomery T, Zackowski JL (1998) Random X inactivation in a girl with a balanced t(X;9) and an abnormal phenotype. Am J Med Genet 77:401–404

Xiang F, Zhang Z, Clarke A, Pereira JL, Naidu S, Budden S, Delozier-Blanchet CD, et al (1998) Chromosome mapping of Rett syndrome: a likely candidate region on the telomere of Xq. J Med Genet 35:297–300

Zoghbi HY, Percy AK, Schultz RJ, Fill C (1990) Patterns of X chromosome inactivation in Rett syndrome. Brain Dev 12:131–135

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