

Acknowledgments

A.B.K. is funded by a National Kidney Research Fund Twis-
tington Higgins Fellowship. We would like to thank the renal
units throughout the United Kingdom, which kindly provided
patients and clinical information for this study.

A. B. KOZIELL,¹ R. GRUNDY,³ T. M. BARRATT,^{2,*}
AND P. SCAMBLER¹

¹Molecular Medicine, ²Nephrourology, ³Haematology
and Oncology Units, Institute of Child Health,
London

Electronic-Database Information

Online Mendelian Inheritance in Man (OMIM): <http://www.ncbi.nlm.nih.gov/Omim> (for FS [MIM 136680], DDS [MIM 194080], DMS [MIM 256370], and FSGS [MIM 603278])

References

- Baird PN, Groves N, Haber DA, Housman DE, Cowell JK (1992a) Identification of mutations in the WT1 gene in tumours from patients with the WAGR syndrome. *Oncogene* 7: 2141–2149
- Baird PN, Santos A, Groves N, Jadresic L, Cowell JK (1992b) Constitutional mutations in the WT1 gene in patients with Denys-Drash syndrome. *Hum Mol Genet* 1:301–305
- Barboux S, Niaudet P, Gubler MC, Grunfeld JP, Jaubert F, Kuttann F, Fekete CN, et al (1997) Donor splice site mutations are responsible for Frasier syndrome. *Nat Genet* 17: 467–470
- Bruening W, Pelletier J (1996) A non-AUG translational initiation event generates novel WT1 isoforms. *J Biol Chem* 271:8646–8654
- Denys P, Malvaux P, van den Berghe H, Tanghe W, Proemans W (1967) Association d'un syndrome anatomo-pathologique de pseudo-hermaphrodisme masculin, d'un tumeur de Wilms' d'un nephropathie parenchymateuse et d'un mosaïcisme XX/XY. *Arch Pediatr* 24:729–739
- Drash A, Sherman F, Harmann W, Blizzard R (1970) A syndrome of pseudohermaphroditism, Wilm's tumour, hypertension and degenerative renal disease. *J Pediatr* 76:585–593
- Frasier SD, Bashore RA, Mosier HD (1964) Gonadoblastoma associated with pure gonadal dysgenesis in monozygotic twins. *J Pediatr* 64:740–745
- Fuchshuber A, Jean G, Gribouval O, Gubler MC, Broyer M, Beckmann JS, Niaudet P, et al (1995) Mapping a gene (SRN1) to chromosome 1q25-q31 in idiopathic nephrotic syndrome confirms a distinct entity of autosomal recessive nephrosis. *Hum Mol Genet* 4:2155–2158
- Groves N, Baird PN, Hogg A, Cowell JK (1992) A single base pair polymorphism in the WT1 gene detected by single-stranded conformational polymorphism analysis. *Hum Genet* 90:440–442
- Jeanpierre C, Beroud C, Niaudet P, Junien C (1998a) Software and database for the analysis of mutations in the human WT1 gene. *Nucleic Acids Res* 26:271–274
- Jeanpierre C, Denamur E, Henry I, Cabanis M-O, Luce S, Cécille A, Elion J, et al (1998b) Identification of constitu-
- tional WT1 mutations, in patients with isolated diffuse mesangial sclerosis, and analysis of genotype/phenotype correlations by use of a computerized mutation database. *Am J Hum Genet* 62:824–833
- Jinno Y, Yun K, Nishiwaki K, Kubota T, Ogawa O, Reeve AE, Niikawa N (1994) Mosaic and polymorphic imprinting of the WT1 gene in humans. *Nat Genet* 6:305–309
- Kikuchi H, Takata A, Akasaka Y, Fukuzawa R, Yoneyama H, Kurosawa Y, Honda M, et al (1998) Do intronic mutations affecting splicing of WT1 exon 9 cause Frasier syndrome? *J Med Genet* 35:45–48
- Klamt B, Koziell AB, Poulat F, Wieacker P, Scambler P, Berta P, Gessler M (1998) Frasier syndrome is caused by defective alternative splicing leading to an altered ratio of WT1 +/- KTS splice isoforms. *Hum Mol Genet* 7:709–714
- Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Houseman DE, Jaenisch R (1993) WT1 is required for early kidney development. *Cell* 74:679–691
- Little MH, Williamson KA, Mannens M, Kelsey A, Gosden C, Hastie ND, van Heyningen V (1993) Evidence that WT1 mutations in Denys-Drash syndrome patients may act in a dominant-negative fashion. *Hum Mol Genet* 2:259–264
- Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA (1998) Wilms' tumor 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. *Cell* 93:445–454
- Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, Bard J, et al (1990) The candidate Wilms' tumour gene is involved in genitourinary development. *Nature* 346:194–197
- Schumacher V, Scharer K, Wuhl E, Altrogge H, Bonzel KE, Guschmann M, Neuhaus TJ, et al (1998) Spectrum of early onset nephrotic syndrome associated with WT1 missense mutations. *Kidney Int* 53:1594–1600

Address for correspondence and reprints: Dr. Ania Koziell, Molecular Medicine Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH. E-mail: A.Koziell@ich.ucl.ac.uk

*Present affiliation: Department of Oncology, The Birmingham Children's Hospital NHS Trust, Birmingham, England.

© 1999 by The American Society of Human Genetics. All rights reserved. 0002-9297/99/6406-0032\$02.00

Am. J. Hum. Genet. 64:1781–1785, 1999

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 Journal 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 Eas-though 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 μ g of digested (with *AluI* and *CfoI*) 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 *EcoRI*-*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 (Journal

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.

Acknowledgments

The collaboration of Drs. Mariz Vainzof, Maria Rita Passos-Bueno, and Lygia V. Pereira and of Constanca Urbani is gratefully acknowledged. This research was supported with grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo, Programa de Apoio à Núcleos de Excelência, and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

JOSÉ SALOMÃO SCHWARTZMAN,¹ MAYANA ZATZ,²
LUCIANA DOS REIS VASQUEZ,²

RAQUEL RIBEIRO GOMES,² CÉLIA P. KOIFFMANN,²
CINTIA FRIDMAN,² AND PRISCILLA GUIMARÃES OTTO²
¹Universidade Mackenzie, and ²Departamento de
Biologia, Instituto de Biociências,
Universidade de São Paulo, São Paulo

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 *HpaII* and *HhaI* 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, Abbadí 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, Lalonde 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, Diamant 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 pa-

ternal 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

Address for correspondence and reprints: Dr. Mayana Zatz, Centro de Estudos do Genoma Humano, Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, CEP:05508-900, São Paulo, SP, Brazil. E-mail: mayazatz@usp.br

© 1999 by The American Society of Human Genetics. All rights reserved. 0002-9297/99/6406-0033\$02.00

Am. J. Hum. Genet. 64:1785–1786, 1999

Combining the Sibling Disequilibrium Test and Transmission/Disequilibrium Test for Multiallelic Markers

To the Editor:

Horvath and Laird (1998) describe the SDT (sibling disequilibrium test) which, like the sibling-association test (Curtis 1997), is a test for association in addition to linkage even when applied to sibships larger than sib pairs. These tests thus differ from the sibling transmission disequilibrium test (S-TDT [Spielman and Ewens 1998]), which is a test for linkage but not for association (unless attention is restricted to sib pairs). The possible advantage that the SDT has over Curtis's test is that it uses all affected sibs in the sibship, although it does not allow for special provision to be made to detect a recessive effect by testing whether there is an excess of affected sibs homozygous for one particular allele. Horvath and Laird demonstrate how the SDT can be applied to a multiallelic marker and how, in the case of a biallelic marker, the SDT and TDT can be combined, but they do not show how the tests can be combined for a multiallelic marker. Curtis described by using logistic regression how his test could be combined with multiallelic TDT data as implemented in the extended TDT (ETDT [Sham and Curtis 1995]), and here we show, using their multivariate sign test, that it is straightforward to apply Horvath and Laird's own approach to combine the multiallelic SDT with multiallelic TDT data.

Horvath and Laird use the "component" sign test (Bickel 1965; Randles 1989) as follows. For N sibships and a marker with m alleles, let s_i^j be 1, 0, or -1, according to whether, in the i th sibship, the frequency of allele j in affected sibs is higher than, equal to, or lower than that in unaffected sibs. Then define $\mathbf{S} = (S^1, S^2, \dots, S^{m-1})$ where $S^j = \sum_{i=1}^N s_i^j$ and a matrix \mathbf{W} having elements $W_{jk} = \sum_{i=1}^N s_i^j s_i^k$. The multiallelic SDT statistic is then $T = \mathbf{S} \mathbf{W}^{-1} \mathbf{S}$, which is asymptotically χ_{m-1}^2 under the null hypothesis of no association or no linkage. In order to extend this approach to include TDT data, we note that we can apply exactly the same formula to a sample of