Letters to the Editor

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A Family with Isolated Hyperparathyroidism Segregating a Missense *MEN1* Mutation and Showing Loss of the Wild-Type Alleles in the Parathyroid Tumors

To the Editor:

Familial isolated primary hyperparathyroidism (FIHP, or HRPT1; MIM 145000) is characterized by hypercalcemia, elevated parathyroid hormone (PTH) levels, and uniglandular or multiglandular parathyroid tumors. The diagnosis involves the exclusion of other familial disorders characterized by primary hyperparathyroidism, mainly multiple endocrine neoplasia type 1 (MEN1) and hyperparathyroidism–jaw tumor syndrome (HPT-JT, or HRPT2). To date, >70 FIHP families have been reported (Huang et al. 1997), and FIHP has been proposed to be either a distinct genetic entity or a variant of MEN1 or of HPT-JT. MEN1 is characterized by tumors of the parathyroids, the endocrine pancreas and duodenum, and the anterior pituitary. Other associated features include adrenocortical tumors, lipomas, and carcinoids. The *MEN1* gene has been mapped to 11q13 (Larsson et al. 1988) and was recently cloned (Chandrasekharappa et al. 1997; The European Consortium on MEN1 1997). Frequent loss of heterozygosity (LOH) in MEN1-related tumors (Friedman et al. 1994) and the inactivating mutations found in patients and tumors (Agarwal et al. 1997; Heppner et al. 1997) suggest that *MEN1* is a tumor-suppressor gene. HPT-JT is characterized by solitary parathyroid adenomas/carcinomas and fibro-osseous jaw tumors and occasionally by renal lesions, namely, Wilm tumors, polycystic kidney disease, and renal hamartomas (Szabo et al. 1995; Teh et al. 1996*a*). The *HRPT2* gene, which has been mapped to 1q21-q32 but which has not yet been cloned, is also considered to be a tumor-suppressor gene (Teh et al. 1996*a*).

We recently have found that, in two FIHP families characterized by solitary adenomas, the disease was linked to 1q21-q32, suggesting that a subset of FIHP forms a variant of HPT-JT (Teh et al. 1998*b*). On the other hand, linkage to *MEN1* has also been implicated in one FIHP family, but without conclusive evidence (Kassem et al. 1994). The recent cloning of the *MEN1* gene has allowed mutation analysis of FIHP kindreds, but, to date, no *MEN1* mutation has been found in the nine small families analyzed (Agarwal et al. 1997; Teh et al. 1998*a*). We report a large family in which seven members are affected with primary hyperparathyroidism without association of other tumors, and we present genetic data to demonstrate that this is a MEN1 variant.

The family is of Caucasian origin and resides in England. Seven family members from two generations were found to have primary hyperparathyroidism (fig. 1 and table 1). The present age and age at diagnosis of parathyroid disease is detailed for each family member in table 1. Five family members have had parathyroid glands surgically removed, whereas two declined surgery. The index case (II-3) had three enlarged parathyroid glands removed at the first operation, and, subsequently, a mediastinal parathyroid tumor was removed, because of persistent disease. Subject II-2 had four enlarged glands removed, and subjects III-4, III-5, and III-8 each had three or three and a half enlarged glands removed. Histopathologically, the parathyroid glands were classified as hyperplastic and did not demonstrate any evidence of cysts or malignancy. Patients II-4 and III-9 declined surgery and were diagnosed as affected, on the basis of borderline hypercalcemia (2.6 mmol/liter each; reference range 2.20–2.60 mmol/liter) in combination with repeated increased PTH levels (113 pg/ml and 99 pg/ml, respectively; normal range 10–50 pg/ml). Subject I-1, who died from myocardial infarction at the age of 77 years, was known to have renal calculi, suggesting that he was also affected. None of the patients have clinical or biochemical evidence of MEN1 or MEN2. The family has been followed, at the Department of Medicine at King's College, with annual hormonal profiles, determined since 1994. Fasting serum gut-hormone profiles (insulin, pancreatic polypeptide, vasoactive intestinal polypeptide, gastrin, glucagon, somatostatin, and neurotensin) are all within normal ranges. Pentagastrin-stimulated serum calcitonin levels are all undetectable. Twenty-four–hour urinary catecholamine metabolites are within the normal ranges. Computed tomography and magnetic-resonance imaging of the abdomen did not detect any tumor of the pancreas and adrenal glands. No patients have evidence of a pituitary

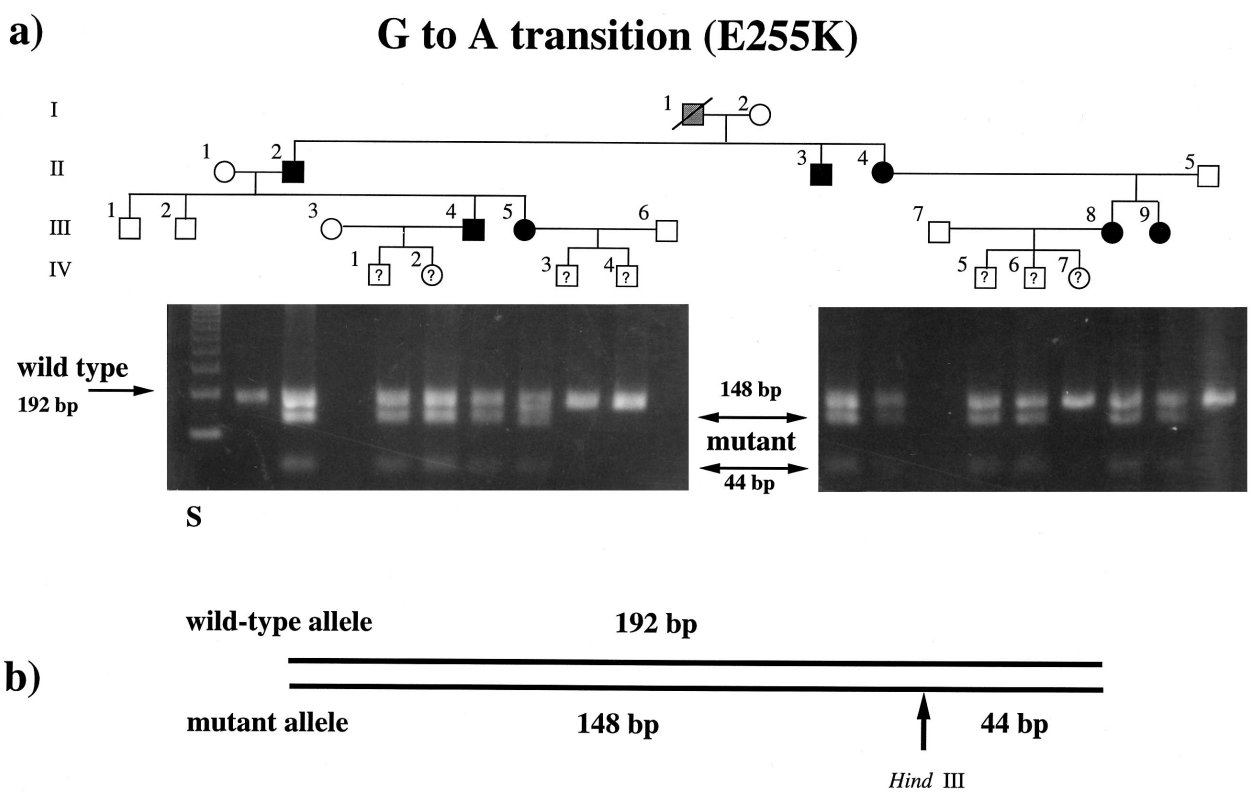

Figure 1 *a,* Pedigree showing the family with autosomal dominantly inherited isolated hyperparathyroidism. Blackened symbols indicate affected family members, and unblackened symbols indicate unaffected family members. The gray-shaded symbol indicates that the individual (I-1) probably is affected, and the symbols with a question mark (?), in generation IV, indicate that the individuals are still at risk. The results from mutation analysis using restriction cleavage are shown below the pedigree. *b,* Illustration showing the *Hin*dIII site created by the E255K mutation.

tumor, either on skull radiography or on magnetic-resonance imaging, and their pituitary hormonal profiles, including prolactin, growth hormone, and adrenocorticotropic hormone, are within the normal range. In addition, no family member has any clinical evidence of HPT-JT. Orthopentography of the jaw was carried out on all affected family members, but no case of jaw tumor was found.

Consent was obtained from the participating family members, and the study was approved by the local ethics committee. Genomic DNA was extracted from peripheral leukocytes of 17 individuals and from fresh frozen tumor tissues as well as tissue blocks from the parathyroid operations of four affected individuals (table 2). Constitutional DNA was genotyped for the polymorphic microsatellite markers D11S956, *PYGM,* D11S787, and *INT2,* within the *MEN1* region at 11q13 (The European Consortium on MEN1 1996), and D1S218, D1S222, D1S428, D1S412, D1S413, and D1S510, from the *HRPT2* region in 1q21-q32 (Teh et al. 1996*a*). Paired constitutional and tumor DNA samples were analyzed for LOH by use of D11S956, *PYGM, INT2,* D11S787, D11S419, *HBB,* D11S1378, and *TYR.*

Linkage to the *HRPT2* locus in 1q21-q32 was excluded by significantly negative LOD scores (<-2) and by haplotyping (data not shown). We thus focused on the other candidate region, that is, the *MEN1* locus in chromosome 11q13. The seven affected members all shared the disease-associated haplotype constructed for four markers flanking the *MEN1* gene (not shown). LOH was identified with the same markers, in 5 of 11 tumors analyzed (table 2 and fig. 2). For all informative cases, these 5 tumors also showed LOH for the six additional chromosome 11 loci tested, suggesting loss of one entire chromosome 11 homologue (fig. 2). Combined analyses of the constitutional and tumor genotypes revealed that the losses invariably involved the wild-type alleles derived from the unaffected parent. Two-point linkage calculations were then performed by incorporation of the results from constitutional genotyping and LOH analysis, by use of a modification of the LINKAGE program (Cottingham et al. 1993; Rohde et al. 1995).

Table 1

Clinical and Genetic Details of the Family Members in This Study

Patient	Sex	Present Age (years)	Age at Diagnosis (years)	Serum Calcium (mmol/liter)[a]	*MEN1* Mutation	Affected Haplotype	No. of Glands Removed	Complications
I-1	Male	Deceased	NA	NA	NA	NA	NA	Renal calculi
II-2	Male	70	66	2.86	Yes	Yes	4	Renal calculi
II-3	Male	52	46	3.20	Yes	Yes	4	Renal calculi and hypertension
II-4	Female	71	69	2.60[b]	Yes	Yes	Declined surgery	…
III-4	Male	40	38	2.84	Yes	Yes	$3\frac{1}{2}$	…
III-5	Female	39	38	3.00	Yes	Yes	$3\frac{1}{2}$	…
III-8	Female	51	37	NA	Yes	Yes	3	Renal calculi and hypertension
III-9	Female	47	45	2.60[b]	Yes	Yes	Declined surgery	…
IV-1	Male	16	…	2.39	Yes	Yes	…	…
IV-2	Female	14	…	2.39	Yes	Yes	…	…
IV-3	Male	12	…	2.45	No	No	…	…
IV-4	Male	10	…	2.45	No	No	…	…
IV-5	Male	28	…	2.39	Yes	Yes	…	…
IV-6	Male	15	…	NA	Yes	Yes	…	…
IV-7	Female	19	…	2.39	No	No	…	…

NOTE.—NA = not available or not applicable.

[a] Corrected to serum albumin of 40 g/liter (normal range 2.20–2.60 mmol/liter). PTH levels are not given, since these were measured by use of different assays at different centers.

[b] Patient had borderline hypercalcemia and increased PTH levels (113 pg/ml in II-4 and 99 pg/ml in III-9; normal range 10–50 pg/ml).

For D11S956 and *PYGM,* the maximum LOD scores of 2.48 and 1.99, respectively, were obtained at a recombination fraction of .00. Taken together, the results indicate the involvement of a tumor-suppressor gene in 11q13, presumably the *MEN1* gene, in this family.

The *MEN1* gene was screened for mutations by use of single-strand conformation analysis (SSCA) and sequencing, as described elsewhere (The European Consortium on MEN1 1997). By SSCA an aberrant shift was detected in the exon 4 fragment (192 bp). This shift was present in all seven affected cases and in four at-risk individuals in generation IV (aged 14, 15, 16, and 28 years; table 1). However, the shift was not detected in unrelated spouses in the family or in 150 unrelated individuals. Direct sequencing revealed a missense mutation in codon 255 (GAG→AAG) of exon 4, causing an amino acid change from glutamic acid to lysine (E255K or c.763G→A). This G→A transition also gave rise to a *Hin*dIII restriction-cleavage site (AAGCTT) for the mutant allele. As the result of the enzyme cleavage, two bands of 144 bp and 44 bp were obtained that were consistent with the SSCA results (fig. 1). The results from the mutation analysis were completely in agreement with those obtained by haplotyping of the 11q13 markers (table 1).

It is now established that mutations in some familial cancer genes can give rise to similar but distinct clinical variants. For example, specific mutations of the *RET* proto-oncogene are associated with each of the three variants of multiple endocrine neoplasia type 2 (MEN2), that is, MEN2A, MEN2B, and familial medullary carcinoma of the thyroid (Eng 1996). For MEN1, many researchers have tried to determine clinically distinct variants. Reports of FIHP and familial pituitary tumors, for example, are abundant, but, to date, there is no conclu-

Table 2

Results from LOH Studies of the Parathyroid Tumors, Using Microsatellite Markers within the *MEN1* Region at 11q13

Patient and Gland Number	D11S956	*PYGM*	D11S787	*INT2*	Allele Lost
II-2:					
1	+	+	+	+	
II-3:					
1	LOH	LOH	LOH	LOH	Wild-type
2	LOH	−	LOH	−	Wild-type
3	+	−	+	−	
4	+	−	LOH	−	Wild-type
III-4:					
1	LOH	LOH	−	LOH	Wild-type
2	+	+	+	−	
3	LOH	LOH	−	LOH	Wild-type
III-5:					
1	+	+	+	−	
2	+	+	+	−	
3	+	+	+	−	

NOTE.—A plus sign (+) indicates retained heterozygosity, and a minus sign (−) indicates not informative or not done.

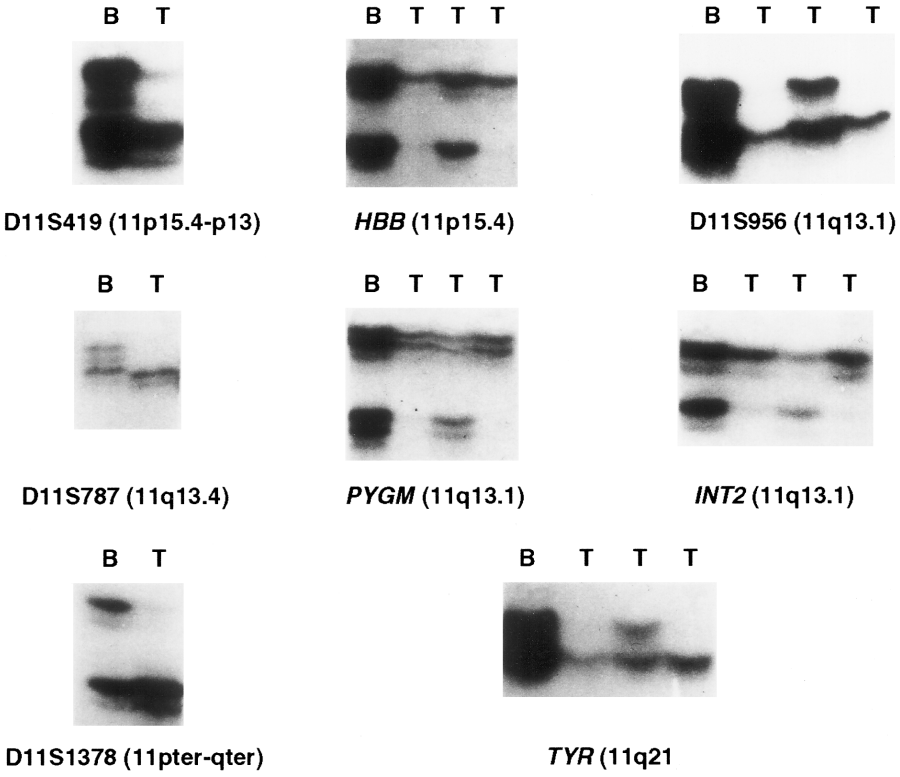

Figure 2 Autoradiograms showing LOH in the whole of chromosome 11 in parathyroid tumor 1 from patient II-3 (D11S419, D11S787, and D11S1378) and in two of the three tumors from patient III-4 (*HBB,* D11S956, *PYGM, INT2,* and *TYR*). Lane B, Leukocyte DNA. Lane T, Tumor DNA.

sive genetic evidence to confirm that they are a variant of MEN1 (Teh et al. 1998*c*).

To our knowledge, this is the first study to demonstrate that FIHP can occur as a variant of MEN1, and in this family FIHP is associated with a *MEN1* missense mutation. The disease transmission follows an autosomal dominant pattern with high penetrance, as in MEN1. Clinically, the hyperparathyroidism runs a rather mild course, as evidenced by two affected subjects who declined surgery and yet developed no obvious complications. Pathologically, the multiglandular parathyroid disease found is also consistent with that of MEN1 (Teh et al. 1996*b*). Furthermore, LOH results of the parathyroid tumors indicated the involvement of the *MEN1* gene, which has been considered to be a tumor-suppressor gene. The loss of the wild-type alleles in the parathyroid tumors from two individuals is consistent with Knudson's two-hit mutation theory. Thus, in these tumors, one copy of the *MEN1* gene is mutated with E255K, whereas the other copy is lost. We thus propose that FIHP could be divided into at least two forms, on the basis of histopathological and genetic findings. The MEN1 variant is characterized by multiglandular hyperplastic disease resulting from a *MEN1* mutation and, clinically, by a milder course of hyperparathyroidism. The HPT-JT variant characterized by solitary adenomas is linked to the *HRPT2* locus in 1q21-q32 and more frequently presents with profound hypercalcemia or hypercalcemic crisis.

To date, the function of the *MEN1* gene remains unknown. A wide range of *MEN1* mutations, spreading across all nine coding exons, have been reported, although a large proportion of them are frameshift or nonsense, indicating that they are inactivating mutations. The missense mutation found in this family (E255K) has never been reported either in MEN1 families (Basset et al. 1998; Teh et al. 1998*c*; Genome Database) or in sporadic counterparts of MEN1-related tumors, including parathyroid tumors (Heppner et al. 1997; Farnebo et al. 1998). By comparison with the murine *Men1* sequence, this mutation was shown to affect a conserved amino acid (C.L., unpublished data). Although the significance of this mutation, which alters glutamic acid to lysine in codon 255, is not known, our findings suggest that it contributes relatively mildly to parathyroid hyperplasia and not to other MEN1-related

neoplasias. However, the family members carrying the mutation should be considered as potential MEN1 patients and should have close long-term follow-up. Future functional studies of the mutation in the family reported here, compared with others, will provide information relevant to elucidating the biological roles of the *MEN1* gene, in various endocrine tissues.

Acknowledgments

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BIN T. TEH,[1,*] CHRISTOPHER T. ESAPA,[3,*] RICHARD HOULSTON,[4] ULLA GRANDELL,[1] FILIP FARNEBO,[1,2] MAGNUS NORDENSKJÖLD,[1] CHRISTOPHER J. PEARCE,[5] DAVID CARMICHAEL,[6] CATHARINA LARSSON,[1] AND PHILIP E. HARRIS[3]

Departments of [1]Molecular Medicine and [2]Surgery, Karolinska Hospital, Stockholm; [3]Department of Medicine, King's College School of Medicine and Dentistry, London; [4]Institute of Cancer Research, Sutton, United Kingdom; [5]Ipswich Hospital, Ipswich, United Kingdom; [6]Southend Hospital, Prittlewell Chase, Westcliffe-on-sea, United Kingdom

Electronic-Database Information

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

Genome Database, http://www.gdb.org/ (for *MEN1* mutations [120173])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FIHP [MIM 145000])

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Address for correspondence and reprints: Dr. Catharina Larsson, Endocrine Tumor Unit, Department of Molecular Medicine, Karolinska Hospital, CMM L8:01, S-171 76 Stockholm, Sweden. E-mail: Catharina.Larsson@cmm.ki.se
* These two authors contributed equally to this work.

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Prevalence of Mutations in TIGR/Myocilin in Patients with Adult and Juvenile Primary Open-Angle Glaucoma

To the Editor:

Primary open-angle glaucoma (POAG) is an important cause of irreversible blindness worldwide (Quigley 1996). The disease results in a characteristic degeneration of the optic nerve that is usually associated with an elevation of intraocular pressure. Pressure within the eye is dependent on the rate of production of a fluid (aqueous humor) by the ciliary body and on the rate of removal of the fluid by the trabecular meshwork.

Relatives of POAG patients have an increased risk of developing glaucoma, which suggests that genetic factors are an important component of POAG susceptibility (Leske 1983). Adult-onset POAG is inherited as a non-Mendelian trait, whereas forms of juvenile-onset POAG exhibit autosomal-dominant inheritance (Wiggs et al. 1995). One locus for juvenile glaucoma was initially mapped to 1q23 (Sheffield et al. 1993; Richards et al. 1994; Wiggs et al. 1994) and was subsequently refined to a 3-cM interval (Belmouden et al. 1997). In recent studies, evaluation of candidate genes mapped to this region has led to the identification of mutations in the TIGR/Myocilin gene (Stone et al. 1997). This gene was originally cloned, from cultured trabecular meshwork cells, as a steroid-response protein, named "trabecular meshwork-induced glucocorticoid response protein" (TIGR; Nguyen et al. 1993). The gene was isolated subsequently from a retinal cDNA library and was shown to be localized to the cilium connecting the inner and outer segments of photoreceptor cells (named "myocilin"; Kubota et al. 1997). Mutations have been detected in the TIGR/myocilin gene in juvenile- and adult-onset glaucoma pedigrees, and in populations of sporadic adult- and juvenile-onset patients (Adam et al. 1997; Stone et al. 1997; Suzuki et al. 1997; Alward et al. 1998; Morissette et al. 1998). Because the prevalence of mutations in TIGR/myocilin has not yet been investigated in a large number of pedigrees affected by juvenile- and adult-onset glaucoma, we have performed SSCP and sequence analysis in 152 affected families and in 104 individuals with macular degeneration but with normal intraocular pressures and optic nerves.

The pedigrees used for this study are all of North American origin and were ascertained and sampled at the New England Medical Center and the Duke University Medical Center. The diagnostic criteria for POAG included intraocular pressure >22 mm/Hg and glaucomatous optic-nerve damage with consistent visual-field loss. Gonioscopic evaluation showed open angles (at least grade III) without any associated abnormalities. Individuals were identified as affected by adult-onset POAG if onset of the disease occurred after age 35 years, and as affected by juvenile-onset POAG if onset occurred before age 35 years. All pedigrees included in this study had at least two affected individuals. The control population underwent a complete ocular examination and did not show evidence of elevation of intraocular pressure or of optic-nerve disease.

For mutation detection, a BAC clone (244L10) containing the TIGR/myocilin gene was identified by the

Table 1

Mutations Identified in Juvenile- and Adult-Onset POAG

POAG Type and Pedigree	Mutation	Proband Age at Diagnosis (years)	Proband Intraocular Pressure at Diagnosis (mmHg)
Juvenile-Onset POAG:			
4	Pro370Leu (1109 C/T)	6	38
18	Tyr437His (1309 T/C)	16	35
Adult-Onset POAG:			
27	Thr377Met (1131 C/T)	42	24
125	Gln368STOP (1102 C/T)	49	24
5052	Gln368STOP (1102 C/T)	78	28
5055	Gln368STOP (1102 C/T)	53	31

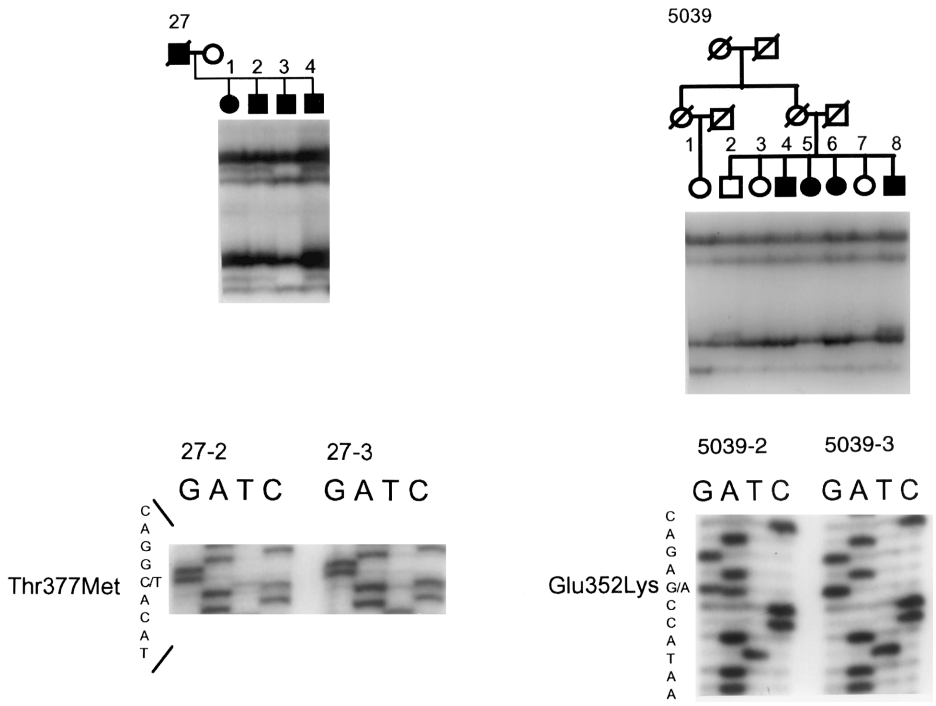

Figure 1 SSCP and DNA sequence analysis of POAG pedigrees 27 and 5039. Affected individuals are shown as blackened circles or squares. SSCP variants are seen in 27-1, 27-2, 27-4, 5039-2, and 5039-8. The DNA sequence of the sense strand shows aT→C transition in 27-2 that results in a change in amino acid 377 (threonine→methionine). The same sequence change is found in 27-1 and 27-4 (data not shown). This sequence change is not found in 27-3. The DNA sequence of the sense strand shows a G→A transition in 5039-2 that is not found in 5039-3, which results in a change in amino acid 352 (glutamate→lysine). This sequence change is also found in 5039-8 but is not found in 5039-1, 5039-4, 5039-5, 5039-6, or 5039-7 (data not shown). All DNA sequence changes were confirmed by sequencing the antisense strand.

screen of an arrayed BAC library (Research Genetics, Inc.). The BAC DNA was used as a source for the genomic sequence, and three exons separated by two introns were identified. Oligonucleotide primers (sequences available on request from Janey L. Wiggs), developed from the intron sequences flanking the intron/exon boundaries and from the cDNA sequence, were used to selectively amplify overlapping fragments of the coding sequence and the exon/intron splice sites. Sixty-eight families (25 juvenile-onset and 43 adult-onset) were screened for mutations in the entire coding sequence of the gene, and an additional 84 adult-onset families were screened for mutations in the third exon. Abnormal SSCP patterns were observed in 17 of the 152 families screened. To identify sequence variants, direct DNA sequencing was performed bidirectionally, by use of the Amersham Life Science dideoxy sequencing kit. All DNA sequence alterations that resulted in a change of amino acid were found in the third exon of the gene. Deletions, insertions, or splice-site mutations were not found. In the population of individuals without glaucoma, nine individuals had a T→C transition at position 1041, which resulted in a wobble mutation (TYR347TYR). No other base-pair changes were found in the control population.

Two different missense mutations were found in 2 of the 25 pedigrees affected by juvenile-onset POAG (table 1). One of these (TYR437HIS) has previously been identified in two pedigrees from North America (Stone et al. 1997; Alward et al. 1998). A second mutation (PRO370LEU) was identified in a three-generation POAG pedigree of English ancestry that settled in Maine >100 years ago. This mutation has also been identified in one Japanese family (Suzuki et al. 1997) and in two unrelated French families (Adam et al. 1997). The recurrence of this mutation in pedigrees of varied ethnicity suggests that the loss of the proline at this position may severely affect the function of the protein. One juvenile pedigree had a C→T transition at position 366, which resulted in a wobble mutation (122GLY122).

In our analysis, only 8% of juvenile-onset pedigrees had identifiable mutations in the TIGR/myocilin gene. This prevalence is lower than that reported in a study by Adam et al. (1997) that showed mutations in five of eight pedigrees, demonstrating linkage to the GLC1A locus. In our study, the majority of pedigrees are too

small for accurate detection of linkage to any particular locus. The small number of pedigrees, in our study, with mutations in TIGR/myocilin suggests that additional genes are likely to be responsible for this disease. Genetic heterogeneity of juvenile-onset POAG has previously been suggested (Graff et al. 1995; Wiggs et al. 1995; Avramopoulous et al. 1996; Richards et al. 1996; WuDunn et al. 1996).

Sequence alterations that resulted in a change of amino acid were identified in 5 of 127 families affected by adult-onset POAG. Three of these families had the GLN368STOP mutation, previously reported in pedigrees of North American origin (Stone et al. 1997; Alward et al. 1998). The three pedigrees we studied do not have common ancestry. We did not detect the GLN368STOP mutation in any individuals affected by juvenile-onset glaucoma.

Two adult-onset pedigrees have sequence alterations that do not segregate with the phenotype. Neither of these sequence changes were seen in 104 control patients. Three members of pedigree 27 had a C→T transition, which resulted in a change of the threonine at amino acid 377 to a methionine. Of the four affected individuals in this family, only three were found to have this alteration. No abnormalities in this gene were found in the remaining affected individual (fig. 1), and it remains a possibility that individual 27-3 is a phenocopy. Pedigree 5039 was found to have a DNA sequence-pair change, which caused the glutamate at position 352 to be replaced by a lysine. Of the four affected individuals in this family, one has the mutation, whereas three do not (fig. 1). Of the four unaffected individuals, one has the mutation but, at age 48 years, does not have any evidence of the disease. This sequence change could represent an extremely rare polymorphism, not present in our study or control populations. In support of this hypothesis, this family is of African American origin, whereas 90% of the study and control populations are Caucasian. The TYR437TYR wobble variant was found in eight adult-onset pedigrees.

Previous reports have suggested that 3%–5% of patients with adult-onset glaucoma have mutations in the TIGR/myocilin gene (Stone et al. 1997; Suzuki et al. 1997; Alward et al. 1998). Our results confirm that mutations in the TIGR/myocilin gene are an uncommon cause of adult-onset POAG. These results are consistent with the current heterogeneity of adult-onset POAG. In recent studies, five loci for adult-onset POAG have been discovered: GLC1B (Stoilova et al. 1996), GLC1C (Wirtz et al. 1997), GLC1D (Trifan et al. 1998), GLC1E (Sarfarazi et al. 1998), and GLC1F (Wirtz et al. 1998). The identification of these and other genes responsible for various forms of glaucoma may lead to valuable insights into the pathophysiology of these important blinding disorders.

Acknowledgments

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Janey L. Wiggs,[1,2] R. Rand Allingham,[3] Douglas Vollrath,[5] Katherine H. Jones,[3] Monica De La Paz,[3] Jeremy Kern,[2] Kara Patterson,[2] Virna L. Babb,[5] Elizabeth A. Del Bono,[2] Bob W. Broomer,[3] Margaret A. Pericak-Vance,[4] and Jonathan L. Haines[6]

[1]*School of Medicine and Sackler School of Graduate Biomedical Sciences, Tufts University, and* [2]*Department of Ophthalmology, New England Medical Center, Boston;* [3]*Duke University Eye Center and* [4]*Center for Human Genetics, Duke University, Durham;* [5]*Department of Genetics, Stanford University School of Medicine, Stanford; and* [6]*Vanderbilt University, Nashville*

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Address for correspondence and reprints: Dr. Janey L. Wiggs, New England Medical Center, 750 Washington Street, Box 450, Boston MA 02111. E-mail: jwiggs_gla@opal.tufts.edu

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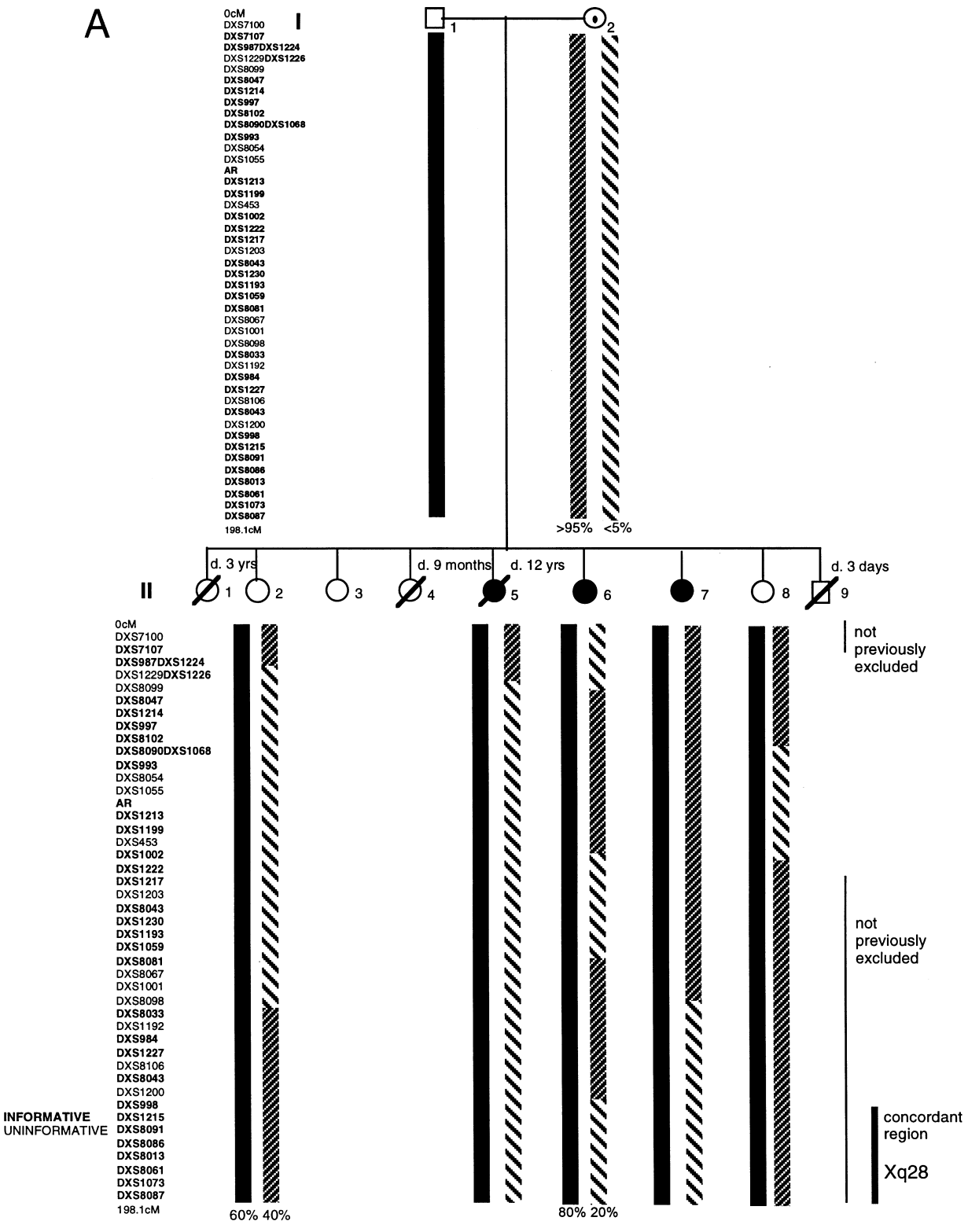
Rett Syndrome: Confirmation of X-Linked Dominant Inheritance, and Localization of the Gene to Xq28

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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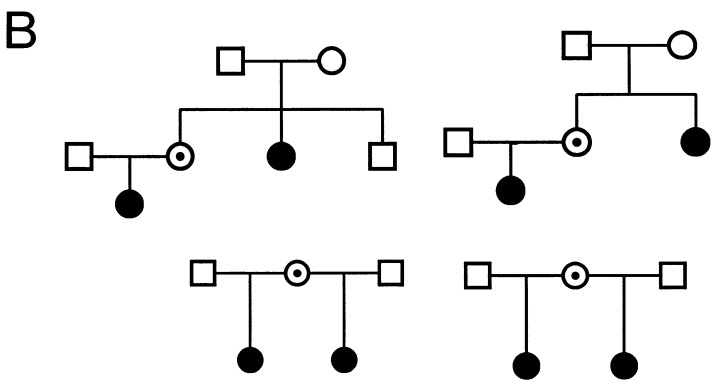


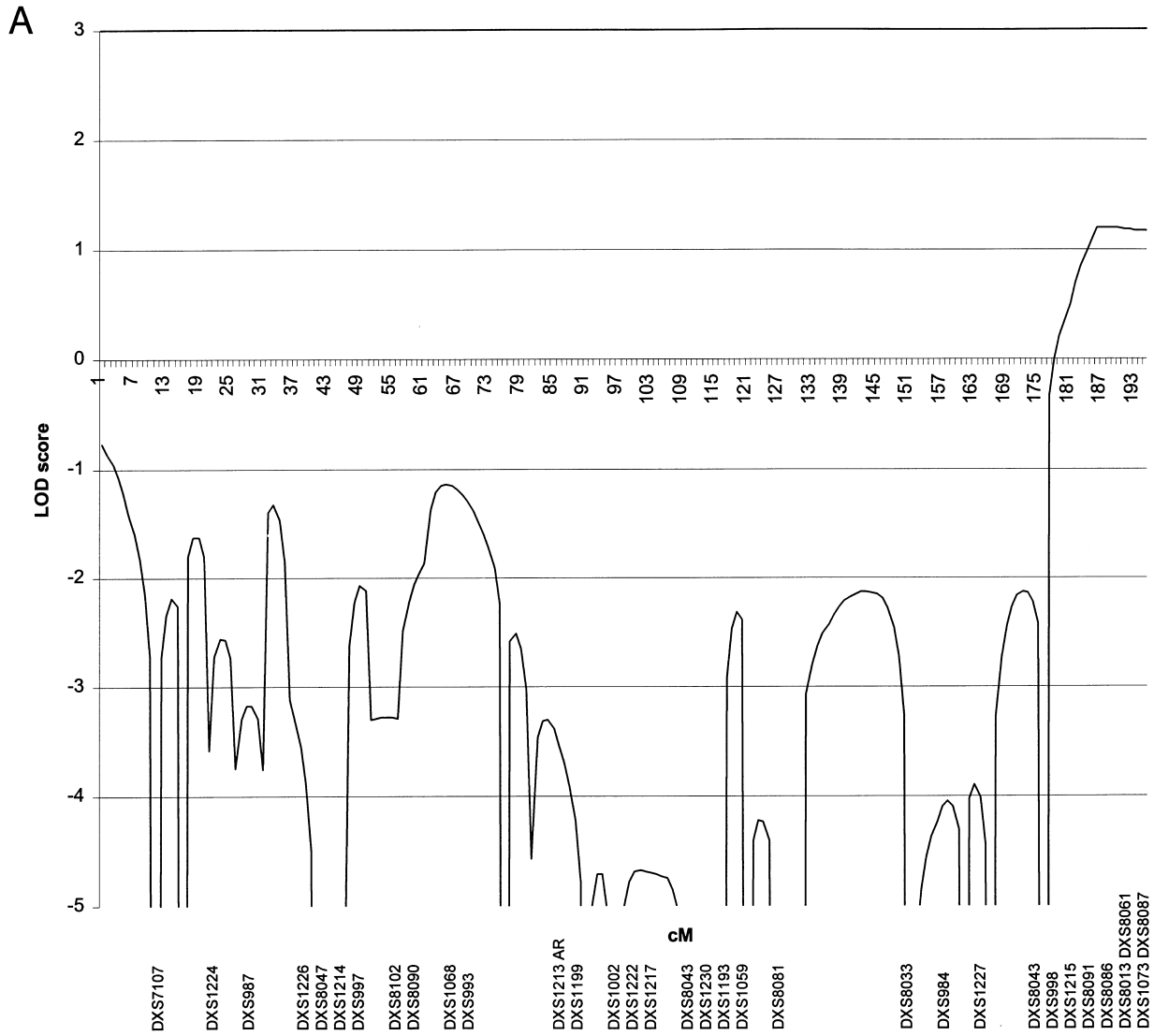
Figure 1 Pedigrees of X-linked families segregating Rett syndrome. *A,* Brazilian pedigree used in this study. The polymorphic markers tested are given, and schematic diagrams of the extended haplotype for each X chromosome are shown. Informative markers are shown in boldface type. The regions of the 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*).

(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 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.

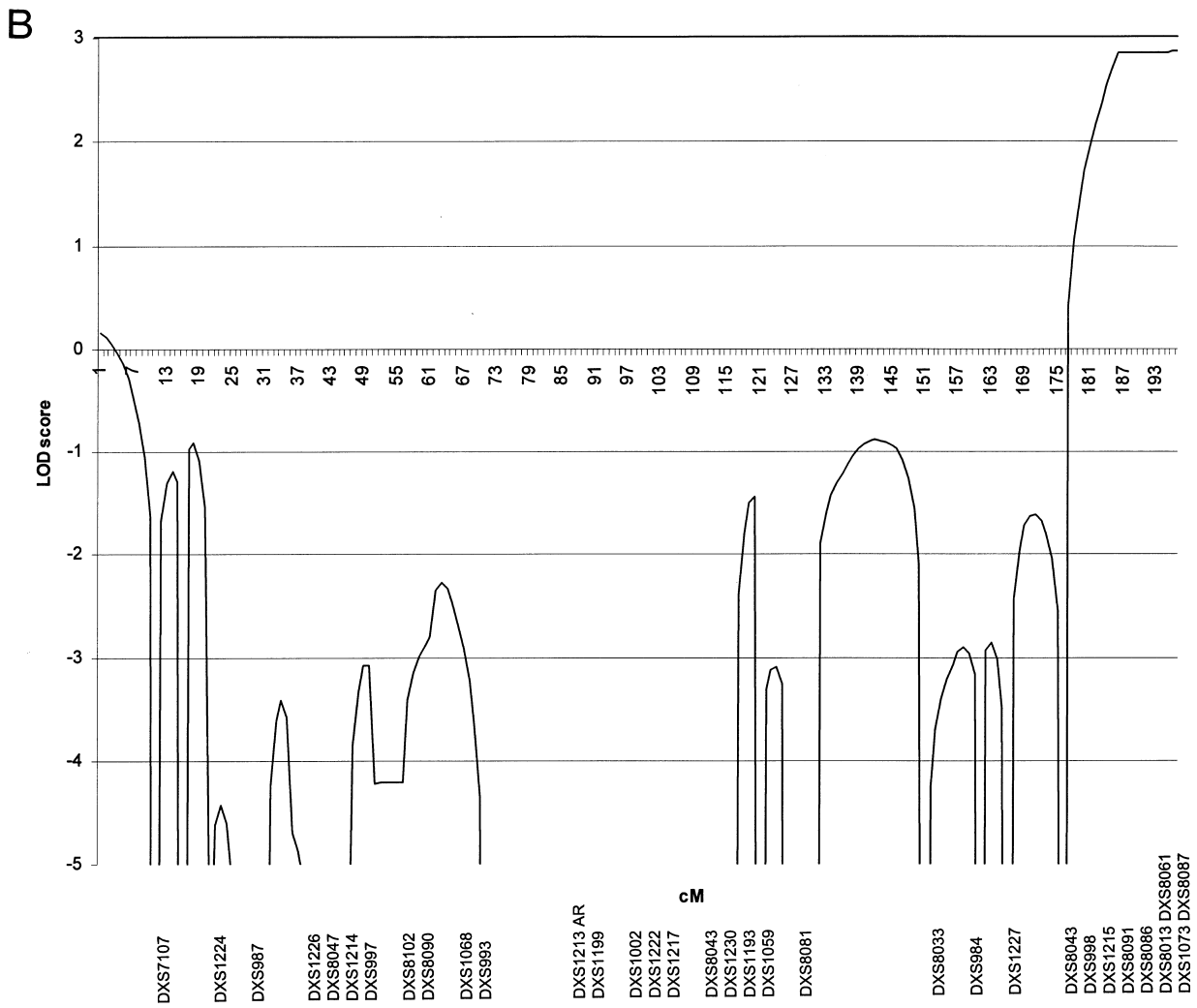


Figure 2 Multipoint linkage analysis across the X chromosome. *A,* Multipoint linkage analysis for the Brazilian family. Xq28 is the only region showing a positive LOD score; all other regions show a negative LOD score. *B,* Combined multipoint linkage analysis for five families, the Brazilian family 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.

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 carriers 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

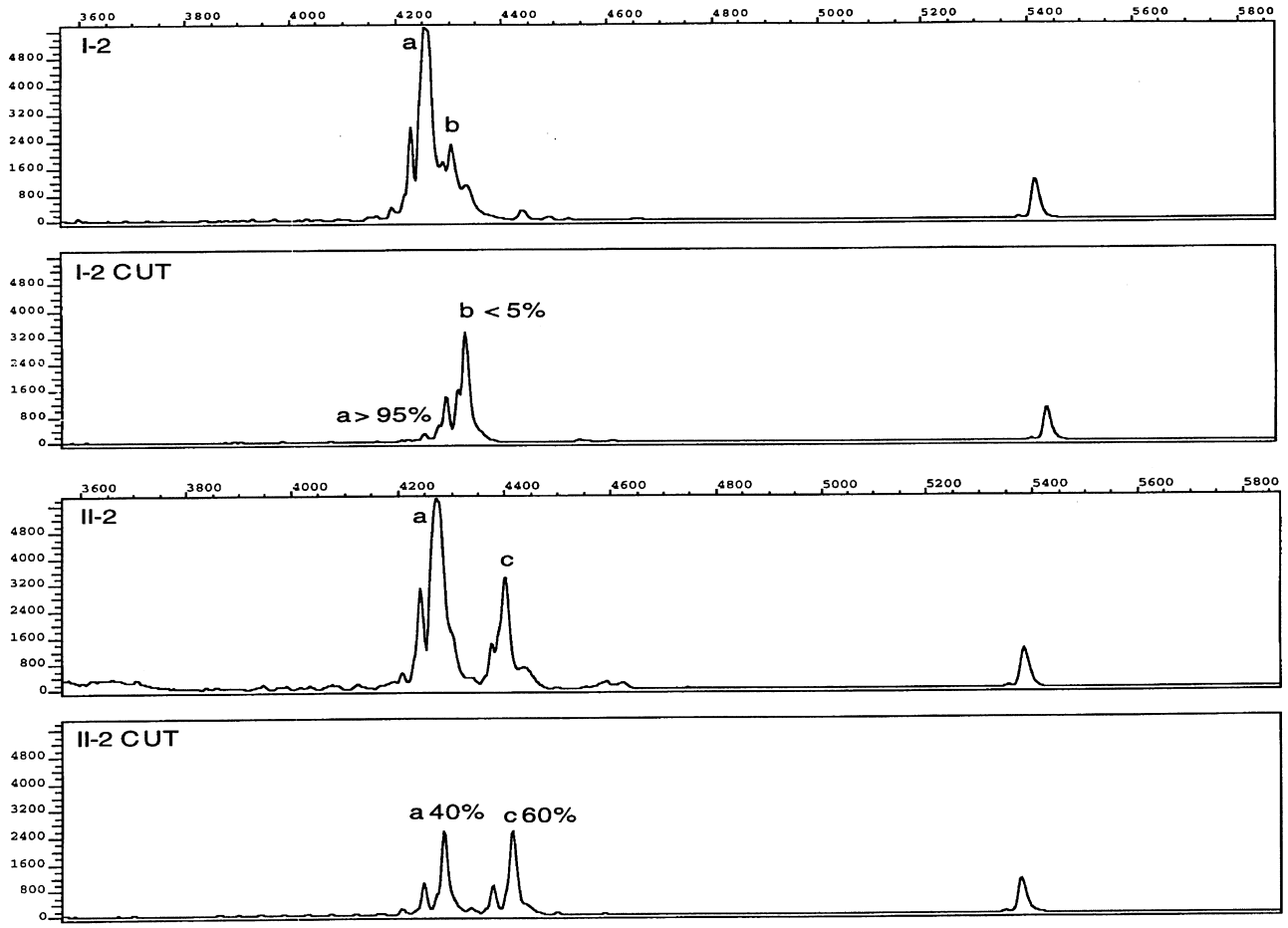


Figure 3 X-inactivation assay for two members of the Brazilian pedigree. Quantitation of X-inactivation patterns in individuals I-2 (unaffected mother) and II-2 (unaffected daughter) is shown. The top panel of each pair shows the results for alleles of the androgen receptor, and the lower panel of each 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.

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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NICKY SIRIANNI,[1] SAKKUBAI NAIDU,[2] JOSELUIZ PEREIRA,[3] RUI FERNANDO PILLOTTO,[4] AND ERIC P. HOFFMAN[1]

[1]*Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh;* [2]*Kennedy Krieger Institute, Johns Hopkins Medical Institutions, Baltimore; and* [3]*Hospital General do Portao, Curitiba, and* [4]*Medical Genetics Unit, Clinical Hospital, The Federal University of Parana, Parana, Brazil*

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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Address for correspondence and reprints: Dr. SakkuBai Naidu, Kennedy Krieger Institute/Neurology, 707 North Broadway, 5th Floor Tower, Baltimore, MD 21205. E-mail: naidu@kennedykrieger.org

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Marshall Syndrome and a Defect at the COL11A1 Locus

To the Editor:

Accurate syndromic diagnosis is important in the provision of appropriate genetic counseling but is essential when the molecular basis of a syndrome is being evaluated. With this in mind, we read with interest Griffith et al.'s (1998) article on Marshall syndrome, which dem-

onstrates a splice-donor site mutation, in the COL11A1 gene, that cosegregates with the abnormal phenotype in nine individuals in three generations with "characteristic features." Marshall syndrome overlaps with at least three other disorders of craniofacial development, including Stickler syndrome, which itself demonstrates phenotypic and genetic heterogeneity. Mutations in the genes encoding COL11A1, COL11A2, and COL2A1 have been reported (Spranger et al. 1994; Vikkula et al. 1995; Richards et al. 1996). In the family that they studied, Griffith et al. (1998) established linkage to the COL11A1 locus and concluded that their results demonstrate allelism of Marshall syndrome with the subset of Stickler syndrome families with COL11A1 mutations. We propose a different interpretation of their data.

We have reported a family in which six members in four generations are affected with Marshall syndrome (Shanske 1997). We also have reviewed the literature, in an attempt to clarify the debate about the existence of Marshall syndrome, as well as its overlap with three similar disorders—Stickler, Weissenbacher-Zweymuller, and Wagner syndromes. For example, ophthalmological abnormalities including high myopia, as well as midfacial hypoplasia, micrognathia with or without palatal clefting, and nonspecific skeletal abnormalities have been reported in both Marshall and Stickler syndromes. In spite of these overlaps, each of these disorders has distinctive features. Striking ocular hypertelorism and abnormalities of ectodermal derivatives have been reported only in Marshall syndrome. The distinctiveness of Marshall and Stickler syndromes is strongly supported by the work of Ayme and Preus (1984). It is significant that Marshall's (1958) article emphasized the finding of ectodermal dysplasia in seven members in three generations of a single family and that, thus far, the family that we studied represents the only other reported insatnce of ectodermal abnormalities. The phenotype described by Griffith includes only "mild" orbital hypertelorism and no evidence of ectodermal derivative abnormalities.

We suggest, therefore, that the family reported by Griffith et al. most likely does not have Marshall syndrome. Rather, the linkage with the COL11A1 locus and the demonstrated defect in the α1(XI) collagen polypeptide suggest that the mutation in this family may be allelic with the subset of Stickler syndrome families associated with COL11A1 mutations.

Alan Shanske, Anna Bogdanow, Robert J. Shprintzen, and Robert W. Marion
Center for Congenital Disorders
Montefiore Medical Center
Albert Einstein College of Medicine
Bronx, NY

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Address for correspondence and reprints: Dr. Alan Shanske, Center for Congenital Disorders, Montefiore Medical Center, Albert Einstein College of Medicine, 111 East 210th Street, Bronx, NY 10467. E-mail: AShanske@aol.com

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Reply to Shanske et al.

To the Editor:

Clinical overlap often confounds accurate diagnosis. For a number of pleiotropic genetic syndromes, sophisticated biochemical, molecular, and cytogenetic tests have mitigated this problem. However, for a laboratory result to be of value to the practitioner, there must be no doubt about the accuracy of the diagnosis for those patients on which the laboratory test was validated. In their foregoing letter to the Editor, Shanske et al. express concern about our clinical diagnosis of Marshall syndrome in a family in which we found a mutation in a gene encoding a subunit of type XI collagen. To address their concern, we shall review the clinical findings in the family described by Marshall (1958), for whom the syndrome is named, and compare these findings to those reported for our patients (Griffith et al. 1998) and for the patients described by Shanske et al. (1997), who also were thought to have Marshall syndrome.

Marshall (1958) reported "a kindred of seven individuals in three generations, who showed the following ocular abnormalities: congenital and juvenile cataracts,…basic myopia; fluid vitreous; and one instance of retinal detachment…; these patients also had defective hearing, a congenital defect of the nose and the associated facies, and other evidence suggestive that they may represent incomplete examples of hereditary anhidrotic ectodermal dysplasia" (p. 143). In addition to anhidrotic ectodermal dysplasia, Marshall included in his differential diagnosis "congenital syphilis, gargoylism, achondroplasia, and (distinct facies) even alone in an otherwise normal body" (pp. 144–145). Not included in his differential diagnosis were Stickler syndrome (Stickler et al. 1965) and Weissenbacher-Zweymüller syndrome (Weissenbacher and Zweymüller 1964), which had not yet been described. After careful clinical assessment and examination of the dermal histology of two family members, Marshall stated, "This kindred lacks the triad found basically in the major, anhidrotic type of ectodermal dysplasia: hypotrichosis, hypodontia, and hypohidrosis. There is some evidence of the presence of the latter two conditions, but this is not strongly convincing" (p. 155). Did these patients really have a form of ectodermal dysplasia, or did Marshall expand the differential diagnosis of congenital nasal defect by describing a new nonectodermal dysplasia syndrome that now bears his name? We and others (Cohen et al. 1974; Zellweger et al. 1974; Lyons-Jones 1997, pp. 252–253) think that the latter conclusion is more likely. Shanske et al. (1997) disagree and refer to a four-generation kindred with ectodermal abnormalities and hypertelorism, which they feel is consistent with Marshall syndrome.

As detailed below, a comparison of the principal findings reported by Marshall (1958) with the findings reported for our patients (Griffith et al. 1998) reveals high concordance, whereas comparison with the patients reported by Shanske et al. (1997) shows low concordance. Marshall's patients and our patients all had congenital or juvenile cataracts and fluid vitreous; none of the patients described by Shanske et al. had these conditions. Marshall's patients and our patients all had significant hearing loss; none of the patients described by Shanske et al. had hearing loss. Marshall's patients had "ample and normal hair," as did our patients; the patients described by Shanske et al. all had "sparse" hair or a "paucity of hair." Two of Marshall's patients were studied radiographically; each had nasal bones that were "small, short, and far back of their normal position." These patients also had "prominence of the frontal bossae," which served to "accentuate the flatness or depression of the bridge of the nose," and "thickening of the outer table of the skull and absent frontal sinuses." In our report (Griffith et al. 1998), we included a patient photograph and cranial CT scan that showed nearly identical features. In contrast, the patients described by Shanske et al. had "significant frontal recession" and normal skeletal surveys.

Why did Shanske et al. conclude that their patients had Marshall syndrome? One reason seems to be the presence of ectodermal abnormalities, including sparse hair, eyebrows, and eyelashes, in their patients. However, Marshall's patients did not have these ectodermal abnormalities. Instead, Marshall thought that his patients had an altered ability to sweat. When comparing his patients with a 32-year-old female control, Marshall observed that sweat production was "diminished, perhaps 25 percent below normal" (p. 148). However, the patients described by Shanske et al. did not have problems with sweating. A second reason that Shanske et al. favored a diagnosis of Marshall syndrome was the presence of myopia in their patients; however, in addition to basic myopia, Marshall's patients had cataracts and fluid vitreous, which the patients described by Shanske et al. lacked. A third reason that Shanske et al. considered Marshall syndrome was the presence of ocular hypertelorism in all of their patients. After inspecting the published facial photographs of six of Marshall's patients, Shanske et al. noted that one of the six (patient 6) had "striking ocular hypertelorism." We tend to agree with this assessment and suspect that Marshall's patients 2, 4, and 7 also might have had mild ocular hypertelorism. However, "mild orbital hypertelorism" was included in the figure legend accompanying the published photograph of one of our patients (Griffith et al. 1998, p. 819).

We feel strongly that this detailed comparison supports the clinical diagnosis of Marshall syndrome in our kindred. A separate issue is whether the clinically defined Marshall, Stickler, Weissenbacher-Zweymüller, and Wagner syndromes represent locus heterogeneity, allelic heterogeneity, or variable expression of the same mutation. In our report (Griffith et al. 1998), we discussed the similarity and possible identity of Marshall syndrome and Stickler syndrome. Yet, there are precedents for consideration of the possibility that clinically distinct phenotypes also can result from allelic heterogeneity; for example, FGFR3 mutations cause both achondroplasia and thanatophoric dysplasia. On the basis of published studies, we suggest the following conclusions: First, Stickler syndrome exhibits locus heterogeneity with mutations identified and shown to cosegregate in at least three different genes, COL2A1, COL11A1, and COL11A2; thus far, COL11A2 mutations have been identified only in families lacking eye involvement but otherwise having other component features of Stickler syndrome. Second, the autosomal recessive disorder oto-spondylo-megaepiphyseal dysplasia (Giedion et al. [1982] concluded that the patient described by Weissenbacher and Zweymüller [1964] had this disorder) also

appears to be due to mutation within the COL11A2 gene. Third, Wagner syndrome is genetically distinct from both Stickler and Marshall syndromes, having been mapped by linkage analysis to a novel locus on human chromosome 5, by use of DNA from the kindred originally reported by Wagner (Brown et al. 1995). This kindred had ocular involvement without the systemic findings reported to occur with Marshall and Stickler syndromes (Graemiger et al. 1995). Last, in at least one kindred, Marshall syndrome is caused by a mutation within COL11A1 (Griffith et al. 1998).

We fully agree with Shanske et al. (1997) that accurate syndrome diagnosis is necessary in order to draw meaningful conclusions about the molecular pathogenesis of a disease phenotype. Results from one family are not sufficient to determine whether COL11A1 will be the sole Marshall syndrome locus or whether identical mutations in COL11A1 (or in other loci) can cause Marshall syndrome in one instance and Stickler syndrome in another. If the unexpected, yet exciting, findings from studies of other collagens are any indication (e.g., O'Reilly et al. 1997), studies of collagen XI will continue to yield intriguing results. For now, we speculate that mutations in COL11A1 cause Marshall syndrome, and we invite our clinical and scientific colleagues to assist us in testing this hypothesis.

M. L. Warman,[1] G. E. Tiller,[2] and A. J. Griffith[3]

[1]*Case Western Reserve University, Cleveland;* [2]*Vanderbilt University School of Medicine, Nashville; and* [3]*National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda*

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Address for correspondence and reprints: Dr. M. L. Warman, Department of Genetics, Case Western Reserve University School of Medicine, Room BRB-719, 2109 Adelbert Road, Cleveland, OH 44106. E-mail: mlw14@po.cwru.edu

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Prenatal Diagnosis and Carrier Detection for a Point Mutation in *UBE3A* Causing Angelman Syndrome

To the Editor:

Angelman syndrome (AS; MIM 105830) is a neurobehavioral disorder, characterized by severe mental retardation, absence of speech, seizures, and gait ataxia (Williams et al. 1995). AS is associated with maternal deficiency of the E6-AP ubiquitin-protein ligase (*UBE3A* gene; MIM 601623; Kishino et al. 1997; Matsuura et al. 1997). Molecular analysis can distinguish five classes of patients: those having a large deletion of a common interval of ~4 Mb on maternal chromosome 15q11-q13 (~70% of cases), those having paternal uniparental disomy (UPD) (3%–5%), those having imprinting mutations (7%–9%), those having intragenic mutations in *UBE3A* (2%–4%), and those having a clinical diagnosis of AS but with none of the above molecular abnormalities (10%–20%). The first three classes of AS patients demonstrate abnormal methylation patterns, by Southern blot analysis (Sutcliffe et al. 1994; Beuten et al. 1996) or by PCR amplification of bisulphite-treated DNA (Kubota et al. 1997), and are easily distinguished from the AS patients who are candidates to have intragenic mutations in *UBE3A*. Point mutations in *UBE3A* may be de novo or may be present on the paternal chromosome of mothers of affected children, which leads to a 50% recurrence risk in subsequent pregnancies, in the latter circumstance.

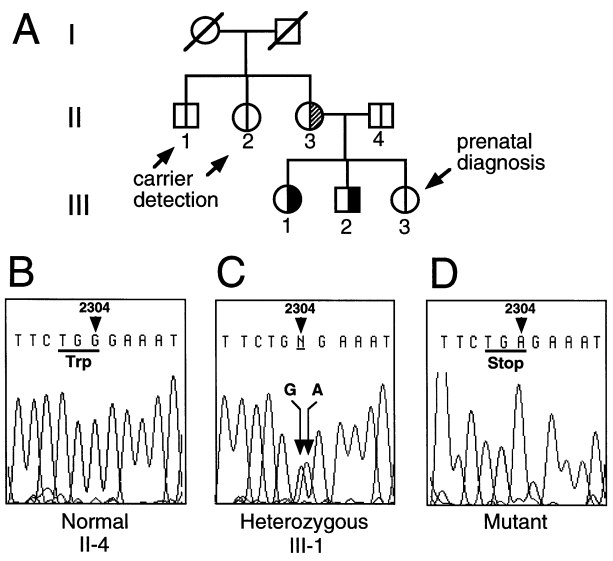

Figure 1 Prenatal diagnosis and carrier detection. *A,* Pedigree: half-blackened symbols (III-1 and III-2) indicate affected with AS and heterozygous for the W768X mutation, half-hatched symbol (II-3) indicates normal phenotype and heterozygous for the W768X mutation, and divided nonblackened symbols (II-1, II-2, II-4, and III-3) indicate normal phenotype and absence of the W768X mutation. *B* and *C,* Direct sequencing of genomic DNA for the normal father and for one of the affected children, respectively. *D,* DNA sequence for the cloned mutation.

The *UBE3A* gene includes 10 exons that encode the major open reading frame and additional upstream exons that are primarily noncoding but may contribute to alternative isoforms (Yamamoto et al. 1997). Recent delineation of the exon/intron organization (Yamamoto et al. 1997) and the flanking intronic sequence of the major coding exons of the *UBE3A* gene (Matsuura et al. 1997), provide information for the design of PCR primers to amplify the coding exons and splice boundaries (see GenBank AF016703 to AF016708). We have used intronic PCR primers (available from the authors upon request; to be published elsewhere) to amplify and directly sequence genomic DNA, as described previously (Matsuura et al. 1997).

A family of mixed Ashkenazi and Iraqi Jewish descent with two children affected with AS was referred for genetic counseling. The mother was pregnant and requested prenatal diagnosis. Molecular analysis was performed by use of DNA isolated from the two affected children and their mother. Methylation analysis of the family by use of Southern blot hybridization was normal, and ruled out common deletion, UPD, and imprinting mutation as molecular abnormalities in this family (data not shown). Sequence analysis for the 10 major coding exons of *UBE3A* identified a nonsense mutation in exon 15. The mutation was a G→A substitution at nucleotide 2304 (numbering based on GenBank X98032), which caused a nonsense mutation at tryptophan codon 768 (W768X) at the protein level. Figure 1 illustrates the normal sequence from the father and the heterozygous mutation in the patient, with direct sequencing of amplified genomic DNA and the sequence for the cloned mutation. The W768X mutation was present in the mother of the affected children but was not present in her two siblings (II-1 and II-2 in fig. 1).

Cytogenetic analysis of cultured aminocytes revealed a normal female karyotype. Mutation analysis on DNA from cultured aminocytes indicated that the fetus did not inherit the W768X mutation from the mother. On the basis of this information, the mother continued the pregnancy and gave birth to a healthy female infant. The absence of the W768X mutation was confirmed by testing peripheral blood DNA from the infant, after birth.

In 70%–80% of cases of AS, the presence of the common ~4-Mb deletion, paternal UPD, or imprinting mutation, can be identified through the use of methylation analysis, FISH, and DNA marker studies (American Society of Human Genetics/American College of Medical Genetics Test and Technology Transfer Committee 1996). The recurrence risk is low for the common interstitial deletion and for UPD but can be extremely high in the case of chromosomal translocation or in some imprinting mutations (Buiting et al. 1998). The remaining 20%–30% of AS cases represent a difficult diagnostic problem, because mutation analysis for *UBE3A* is only available on a limited basis, and because the sequencing of all of the coding exons identifies a disease-causing mutation in only ~30% of the methylation-negative cases, in our experience. It is unknown whether the remaining AS patients have other molecular abnormalities or represent potential misdiagnoses. When disease-causing mutations are identified, as in the family reported here, the information is extremely valuable for genetic counseling and prenatal diagnosis.

In patients with typical AS clinical findings and normal methylation studies, the family history should be thoroughly investigated for the possibility of AS in distant maternal relatives, because the imprinted pattern of inheritance may result in the occurrence of multiple affected individuals, who are quite distantly related (Meijers-Heijboer et al. 1992). Although mutation studies for *UBE3A* would be valuable in all such families, they are particularly indicated in families with more than one individual affected with AS. In one report, mutations were found in 80% of multiplex families and in 14% of sporadic cases (Malzac et al. 1998), and we have found mutations in 75% and 23% for these two groups, respectively (P. Fang et al., unpublished data). In families where we have found disease-causing mutations, the index case has represented a de novo mutation in about half of the families, with the mother carrying the mu-

tation in the remaining group (P. Fang et al., unpublished data). Thus, the risk of recurrence in families may be as high as 50% or may be relatively low, and the two circumstances are easily distinguished if a mutation is identified. In instances where no mutation is identified but where the clinical findings are typical of AS, considerable uncertainty prevails. Recurrence of AS is uncommon in this group but does occur.

To conclude, mutation analysis of *UBE3A* can be extremely informative for establishing a diagnosis of AS and for genetic counseling. If a disease-causing mutation is identified and is present in the mother, prenatal diagnosis is readily accomplished.

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TING-FEN TSAI,[1] ANNICK RAAS-ROTHSCHILD,[2] ZIVA BEN-NERIAH,[2] AND ARTHUR L. BEAUDET[1]
[1]*Department of Molecular and Human Genetics, Baylor College of Medicine and Howard Hughes Medical Institute, Houston; and* [2]*Department of Human Genetics, Hadassah University Hospital, Jerusalem*

Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for human genomic *UBE3A* sequences [AF016703–AF016708 and X98032])
Online Mendelian Inheritance in Man http://www.ncbi.nlm.nih.gov/Omim (for AS [MIM 105830] and for *UBE3A* [601623])

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Address for correspondence and reprints: Dr. Arthur L. Beaudet, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Room T619, Houston, TX 77030. E-mail: abeaudet@bcm.tmc.edu

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Estimation of Pairwise Relationships in the Presence of Genotyping Errors

To the Editor:

Boehnke and Cox (1997) described a likelihood method for the inference of pairwise relationships from data for a genome scan. This tool is useful for verification of the relationships in a linkage study, to identify pedigree errors, which, if undetected, could reduce the power to detect genes. While they showed that the method is robust for genotyping errors when the relationships full sibs, half sibs, and unrelated are inferred, a single genotyping error will lead to zero likelihood for the relationships MZ twins and parent/offspring. A simple modification of the method eliminates this problem and thus allows the accurate inference of the relationships MZ twins and parent/offspring, even in the presence of genotyping errors.

The crucial calculation in the method of Boehnke and

Cox (1997), with regard to genotyping errors, is $P(X_k | I_k = i)$, the probability that two individuals have genotypes $X_k = (X_{k1}, X_{k2})$ at locus k, given that they share i alleles identical by descent at the locus. To simplify the notation, let $p_i(x) = P(X_k = x | I_k = i)$. When two individuals share no alleles identical by state at a locus, $p_1(x) = p_2(x) = 0$, which results in a zero likelihood for relationships such as MZ twins and parent/offspring. If we allow $p_i(x) > 0$ for all possible x, this problem is eliminated.

In the calculation of the likelihood for a putative relationship, we propose to replace the values for $p_i(x)$ used by Boehnke and Cox (1997) with the following:

$$p_0^*(x) = p_0(x) ,$$

$$p_1^*(x) = (1 - \epsilon)p_1(x) + \epsilon p_0(x) ,$$

$$p_2^*(x) = (1 - \epsilon)p_2(x) + \epsilon p_0(x) ,$$

where ϵ denotes twice the approximate genotyping error rate.

To test this idea, we performed a computer simulation. By use of the 366 autosomal markers in Weber screening set version 9 (Yuan et al. 1997), 10,000 relative pairs were simulated for each of five relationships: MZ twins, parent/offspring, full sibs, half sibs, and unrelated. The sex-averaged map locations were taken from the study by Broman et al. (1998); allele frequencies were estimated by use of eight of the CEPH families. The intermarker spacings were 9.4 ± 3.6 cM; the marker heterozygosities were .78 ± .06. Genotypes were simulated by use of an error rate of 2%. For each relative pair, the likelihood for the five relationships were calculated by use of ϵ = .02, .04, and .08.

For the values of ϵ considered, all five relationships were classified correctly in all 10,000 replicates. Thus, our proposal is successful in extending Boehnke and Cox's (1997) method to the inference of MZ twins and parent/offspring pairs, in the presence of genotyping errors.

KARL W. BROMAN AND JAMES L. WEBER
Marshfield Medical Research Foundation
Marshfield, WI

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Address for correspondence and reprints: Karl W. Broman, Center for Medical Genetics, Marshfield Medical Research Foundation, 1000 North Oak Avenue, Marshfield, WI 54449. E-mail: BromanK@cmg.mfldclin.edu

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