Am. J. Hum. Genet. 58:998–1007, 1996

Molecular Analyses of 17p11.2 Deletions in 62 Smith-Magenis Syndrome Patients

Ramesh C. Juyal,[1,*] Luis E. Figuera,[1] Xueya Hauge,[1] Sarah H. Elsea,[1] James R. Lupski,[2,3,5] Frank Greenberg,[2,3] Antonio Baldini,[2,5] and Pragna I. Patel[1,2,4,5]

Departments of [1]Neurology, [2]Molecular and Human Genetics, and [3]Pediatrics and [4]Division of Neuroscience and [5]Human Genome Center, Baylor College of Medicine, Houston

Summary

Smith-Magenis syndrome (SMS) is a clinically recognizable, multiple congenital anomalies/mental retardation syndrome caused by an interstitial deletion involving band p11.2 of chromosome 17. Toward the molecular definition of the interval defining this microdeletion syndrome, 62 unrelated SMS patients in conjunction with 70 available unaffected parents were molecularly analyzed with respect to the presence or absence of 14 loci in the proximal region of the short arm of chromosome 17. A multifaceted approach was used to determine deletion status at the various loci that combined (i) FISH analysis, (ii) PCR and Southern analysis of somatic cell hybrids retaining the deleted chromosome 17 from selected patients, and (iii) genotype determination of patients for whom a parent(s) was available at four microsatellite marker loci and at four loci with associated RFLPs. The relative order of two novel anonymous markers and a new microsatellite marker was determined in 17p11.2. The results confirmed that the proximal deletion breakpoint in the majority of SMS patients is located between markers D17S58 (EW301) and D17S446 (FG1) within the 17p11.1-17p11.2 region. The common distal breakpoint was mapped between markers cCI17-638, which lies distal to D17S71, and cCI17-498, which lies proximal to the Charcot Marie-Tooth disease type 1A locus. The locus D17S258 was found to be deleted in all 62 patients, and probes from this region can be used for diagnosis of the SMS deletion by FISH. Ten patients demonstrated molecularly distinct deletions; of these, two patients had smaller deletions and will enable the definition of the critical interval for SMS.

Introduction

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation syndrome characterized by a deletion on the short arm of chromosome 17 involving band p11.2 (Smith et al. 1982, 1986; Patil and Bartley 1984; Stratton et al. 1986). To date, ~100 patients have been reported (Patil and Bartley 1984; Smith et al. 1986; Stratton et al. 1986; Hamill et al. 1988; Lockwood et al. 1988; Colley et al. 1990; Allen et al. 1991; de Rijk-van Andel et al. 1991; Greenberg et al. 1991, 1993; Kondo et al. 1991; Moncla et al. 1991, 1993; Finucane et al. 1993*a*, 1994; Fan and Farrell 1994; Juyal et al. 1995*a*, 1995*b*). This disorder has a characteristic behavioral and physical phenotype that includes moderate to severe mental retardation, self-injurious behavior (such as hand biting, head banging, onychotillomania, and polyembolokoilamania), sleep disturbances, delayed speech and motor development, ear malformations, brachycephaly, and brachydactyly. Ocular pathologies of myopia and retinal detachments (Finucane et al. 1993*a*) and a characteristic spasmodic upper body squeeze, or "self-hugging behavior" (Finucane et al. 1994), have also been reported in SMS patients. We have previously suggested that SMS is likely a contiguous gene syndrome, although evidence for Mendelian inheritance of one or more individual phenotypic features is not available (Greenberg et al. 1991). The estimated frequency of SMS is 1/25,000, but it is quite likely that the syndrome is underdiagnosed because of its relatively recent description and requirement for high-resolution cytogenetics and/or FISH to detect the deletion.

To date, four expressed genes have been mapped within the common SMS deletion region: the small nuclear RNA U3 gene (*snU3*) (Chevillard et al. 1993), the human homologue of the *Drosophila melanogaster flightless-I* gene (*FLI*) (Chen et al. 1995), the gene encoding a human microfibril–associated protein, h*MFAP4* (Zhao et al. 1995), and most recently the gene encoding cytosolic serine hydroxymethyltransferase (*cSHMT*) (Elsea et al. 1995). The phenotypic implications for hemizygosity at these gene loci in SMS patients are as yet unknown.

Flow cytometric evaluation of selected SMS patients has determined that the deletions can span a broad range from <1.5 Mb (undetectable by flow cytometry) up to

Received November 3, 1995; accepted for publication February 9, 1996.

Address for correspondence and reprints: Dr. Pragna I. Patel, Department of Neurology, Baylor College of Medicine, Room NB431A, One Baylor Plaza, Houston, TX 77030. E-mail: pragna@bcm.tmc.edu

*Present address: National Institute of Immunology, New Delhi

0002-9297/96/5805-0012$02.00

9 Mb (B. Trask and P. I. Patel, unpublished data). We have previously molecularly evaluated 31 unrelated SMS patients and determined that most patients were deleted for five markers in 17p11.2: FG1 (D17S446), 1516 (D17S258), pYNM67-R5 (D17S29), pA10-41 (D17S71), and pS6.1-HB2 (D17S445) (Greenberg et al. 1991; Guzzetta et al. 1992). Parental origin of deletion was determined in 15 patients. The deletion was found to be of paternal origin in nine patients and of maternal origin in six patients (Greenberg et al. 1991), with no apparent phenotypic differences dependent on parent of origin for the deletion, which suggests that imprinting does not play a role in the major clinical manifestations of SMS.

In order to determine which genes are critical in causing the phenotype in a contiguous gene syndrome such as SMS, it is crucial to identify patients with small deletions who would enable narrowing the interval responsible for the major phenotypic features. Toward this goal, we studied a total of 62 SMS patients, which is the largest patient cohort analyzed to date. Thirty-four of these 62 patients were largely uncharacterized at the molecular level. In order to assess the deletion status and its parental origin in SMS patients, a multifaceted approach was undertaken, which combined the use of somatic cell hybrid analysis, genotyping of microsatellite markers, RFLP analysis, and FISH. The analysis included 14 markers in proximal 17p. We also determined the parental origin of deletions, to further investigate whether imprinting plays a role in this genetic disorder.

Our studies defined a common deletion region between markers EW301 (D17S58) and an anonymous marker cCI17-498 in the majority of SMS patients. We also identified 10 patients with molecularly distinct deletions, 2 of whom have apparently much smaller deletions. Both of the latter patients were initially diagnosed as mosaic for the 17p11.2 deletion by G-banding analysis but were subsequently shown by FISH to be deleted in all cells examined (Finucane et al. 1993*b*; Juyal et al. 1995*a*, 1995*b*).

Subjects, Material, and Methods

Subjects

A total of 62 SMS patients, 42 females and 20 males, were subjected to molecular evaluation of the chromosome 17p11.2 deletion. This included 28 patients who had been characterized previously at the molecular level with available 17p markers (Greenberg et al. 1991; Zori et al. 1993; Juyal et al. 1995*a*, 1995*b*). All patients were evaluated by at least one expert clinician, and the diagnosis was supported by demographic, anthropometric, morphological, developmental, behavioral, and neurological findings. Peripheral blood samples were obtained from the SMS patients and available parent(s) after obtaining informed consent. The blood was used to establish Epstein-Barr virus–transformed lymphoblastoid cell lines by standard methods (Anderson and Gusella 1984). Genomic DNA was isolated either directly from the blood sample obtained or from the established cell line by use of a standard phenol-chloroform extraction method (Sambrook et al. 1989).

Somatic Cell Hybrids

The hybrids used in this study, 88H5, Hy357-2D, and MH22-6, and their rodent parents, Cl-1D (mouse) and a23 (hamster), have been described elsewhere (Patel et al. 1992). Hy117-4D is derived from a patient with hereditary neuropathy with liability to pressure palsies and has a deletion of 1.5 Mb in 17p12 (Roa et al. 1993). Somatic cell hybrids containing the deleted chromosome 17 of SMS patients have been described elsewhere (Guzzetta et al. 1992; Zori et al. 1993).

DNA Probes

Five polymorphic DNA markers, pEW301 (Fain et al. 1987), pYNM67-R5 (Ray et al. 1990), c1516 and p1516-R4 (Franco et al. 1990; Patel et al. 1990*b*), and pA10-41 (Barker et al. 1987), corresponding to loci D17S58, D17S29, D17S258, and D17S71, respectively, were used in this study to determine the deletion status and the parental origin of the deletion in SMS patients. A 2.8-kb *Eco*RI fragment from pEW301, a 4-kb *Eco*RI insert from pYNM67-R5, the entire cosmid c1516, a 4.9-kb *Eco*RI fragment from p1516-R4, and a 345-bp *Eco*RI insert from pUC10-41 were each used in Southern analysis to detect the polymorphisms described in table 1. The 4.5-kb *Eco*RI/*Hin*dIII fragment insert from the plasmid HU3-3H was used as a probe for the *snU3* RNA locus (Yuan and Reddy 1989). Probes were prepared by PCR amplification of the inserts from pEW301, pYNM67-R5, and pUC10-41 by use of the universal and reverse sequencing primers. The entire cosmids c1516 and cCI17-638 were used as probes. A 7.5-kb *Eco*RI fragment from cCI17-498 was used as a probe.

Southern Analysis

Southern blotting and hybridization were carried out essentially as described by Patel et al. (1990*a*). Probes were labeled using either Quick Prime (Pharmacia) or Rediprime (Amersham). The probes were preassociated with 0.25 mg/ml human placental DNA for 1 h at 65°C to quench repeat sequences. Blots were prehybridized in hybridization solution containing 1 M NaCl, 1% SDS, 10% dextran sulphate, and 0.1 mg herring sperm DNA/ml at 65°C. Hybridization was carried out for 16–18 h at 65°C. Blots were washed for 20 min in 2 × SSC, 0.1% SDS at 65°C, followed by a stringent wash in 0.1 × SSC, 0.1% SDS, for 10–15 min at 65°C. Finally, the blots were exposed to Kodak XAR5 film or Hyperfilm

Table 1

Markers Used for Deletion Analysis of SMS Patients

Locus and Marker	Method	Enzyme	Allele Size (kb)	Reference
PMP22:				
c49E4	FISH	...	...	Suter et al. (1994)
cC117-498	FISH	...	...	Inazawa et al. (1993)
cC117-638	FISH	...	...	Inazawa et al. (1993)
D17S71:				
OS2-GA3	PCR	...	...	Guzzetta et al. (1992)
A10-41	RFLP	*Msp*I	2.4/1.9	Barker et al. (1987)
c97D11	FISH	...	...	Present study
D17S620:				
UT159	PCR	...	...	Gerken et al. (1995)
D17S258:				
c1516	RFLP	*Hin*dIII	22.0/12+10	Patel et al. (1990)
1516-R4	RFLP	*Msp*I	3.3/2.4	Franco et al. (1990)
c142F7	FISH	...	...	Present study
cSHMT:				
c108D4, c155D9	FISH	...	...	Elsea et al. (1995)
FLI:				
c62F2	FISH	...	...	Chen et al. (1995)
D17S447:				
cH1	FISH	...	...	Guzzetta et al. (1991)
D17S29:				
pYNM67-R5	RFLP	*Taq*I	3.4/2.0+1.3	Ray et al. (1990)
c116B9	FISH	...	...	Present study
snU3:				
HU3-3H	Southern	...	...	Yuan et al. (1989)
c94A12	FISH	...	...	Present study
MFAP4:				
c28A5	FISH	...	...	Zhao et al. (1995)
D17S446:				
FG1	PCR	...	...	Guzzetta et al. (1991)
c105G12	FISH	...	...	Present study
D17S805:				
AFM23tal	PCR	...	...	Gyapay et al. (1994)
D17S58:				
c113B12, c132F2	FISH	...	...	Present study
pEW301	RFLP	*Taq*I	4.5/3.1	Fain et al. (1987)

(Amersham) with one or two intensifying screens (DuPont) at −70°C.

Microsatellite Genotyping

For genotyping at microsatellite marker loci, genomic DNA (100 ng) was amplified using 1 U *Taq* polymerase in a cocktail containing 0.05 mM of the forward primer, 0.1 mM of the reverse primer, and 0.2 mM of ^{32}P-labeled forward primer, 0.25 mM of each deoxynucleotide triphosphate, and 10 × PCR-Buffer (Perkin-Elmer) in a final volume of 20 μl. PCR amplification of genomic DNA samples was performed in a 96-well format in a Perkin-Elmer 9600 thermal cycler, using the following conditions: a first step of denaturation (94°C, 5 min), followed by 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min), and elongation (72°C, 1 min) followed by a final elongation step (72°C, 7 min). After PCR, 7 μl of loading buffer (formamide/bromophenol blue/xylene cyanol) was added to 3 μl of the reaction mixture, and 5 μl of the sample was denatured for 5 min at 95°C, cooled rapidly on ice, and electrophoresed in a 5% polyacrylamide sequencing gel.

FISH Analysis

The chromosome preparations were made from transformed lymphoblastoid cell lines or lymphocytes of SMS patients by use of standard cytogenetic techniques. Cosmids corresponding to each of the loci D17S58, D17S446, *MFAP4*, D17S29, D17S447, *FLI*, *cSHMT*, D17S71, OS2-GA3, cCI17-638, and cCI17-498 indicated in table 1 were used as test probes for FISH analysis. For unambiguous identification of chromosome 17,

a control probe c123F8 (locus TK) mapping to the distal end of 17q was included in each hybridization. DNA (500 ng) from each cosmid probe was nick-translated with biotin dATP for 2 h at 16°C. Of this, 100 ng of labeled DNA (for each slide) was ethanol precipitated at −70°C for 15 min, using 2 μg of human Cot-1 DNA and 3 μg herring sperm DNA and resuspended in 10 μl of hybridization solution containing 50% formamide, 2 × SSC, and 10% dextran sulphate. The probe was denatured at 80°C for 5 min and preassociated at 37°C for 15–30 min prior to hybridization. For cosmid c113B12 representing locus D17S58, instead of human Cot-1 DNA, 4.5 μg of human placental DNA was used, and the probe was added for hybridization without preassociation. The chromosomal DNA was denatured in 70% formamide/2 × SSC at 80°C for 2 min, and the slides were dehydrated for 5 min each in 70%, 90%, and 95% ethanol at −20°C prior to hybridization. The hybridization was carried out for 16–24 h at 37°C in a moist chamber. Posthybridization washes included one in 50% formamide/2 × SSC for 15 min at 42°C, followed by a stringency wash in 1 × SSC for 15 min at 60°C. In order to detect the signals, the chromosomes were treated with alternate layers of blocking agent and fluoroscein isothiocyanate (FITC)–Avidin for 30 min, each at 37°C in a moist chamber and washed in 4 × SSC, 0.1% Tween-20, for 15 min at 42°C. Finally, the chromosomes were counterstained with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) and viewed under a Zeiss fluorescence microscope equipped with a digital imaging system. The images were obtained as described by IJdo et al. (1992). Essentially, the grey-scale digital images were obtained using a cooled charge-coupled-device camera (Photometrics, chip Kodak KAF1400) and commercial (Invision) and in-house-developed software running on a Sun workstation. DAPI and FITC were used to obtain color-merged images. The use of a triple bandpass/dichronic filter set with separated individual exciters (Chroma Technology), for the two-color fluorochromes mounted on a filterwheel allows the acquisition of images in perfect registration. At least 20 metaphase plates were scored for each hybridization.

Results

Deletion Analysis of SMS Patients

Thirty-four of the 62 patients in the study were largely uncharacterized at the molecular level. They were analyzed for all markers indicated in figure 1. The remaining 28 patients were analyzed only at the *MFAP4* gene locus, at the D17S805, OS2-GA3 (D17S71), and UT159 (D17S620) microsatellite marker loci and with the anonymous markers cCI17-638 and cCI17-498 (Inazawa et al. 1993). For 21 of these 28 patients, somatic cell hybrids retaining the deleted chromosome 17 were available and were used to determine the deletion status of the marker by PCR or Southern analysis as described elsewhere (Guzzetta et al. 1992; Patel et al. 1992; Zori et al. 1993).

RFLP Analysis to Determine Deletion Status

Sixteen of the 34 new patients for whom parental DNA was available were subjected to RFLP analysis to determine the deletion status at four loci: EW301 (D17S58), YNM67-R5 (D17S29), c1516 (D17S258), and A10-41 (D17S71). Of them, 6 patients were deleted at one or more loci, and 20 were uninformative at all four loci tested.

Microsatellite Genotyping to Determine Deletion Status

Two microsatellite markers previously mapped to 17p11.2, FG1(D17S446) and OS2-GA3 (D17S71), were used for deletion analysis. In addition, the relative physical location of a number of recently identified microsatellite markers (Gyapay et al. 1994; Gerken et al. 1995) likely to be located within the 17p11-pter region based on the genetic map was determined using a panel of somatic cell hybrids as described elsewhere (Elsea et al. 1995). This was done in order to determine whether the DNA markers were located within 17p11.2 and, particularly, to determine whether they mapped within the SMS deletion interval. Chevillard et al. (1993) had previously localized the microsatellite marker AFM243ta1 (D17S805) to the distal end of 17p11.2 in the region flanking the Charcot-Marie-Tooth disease type 1A (CMT1A) locus. Our analysis by PCR using the hybrids schematically shown in figure 2 clearly indicated that the hybrid Hy357-2D retained this microsatellite locus, while the hybrid 88H5 did not (data not shown). This allowed us to map this marker to the proximal interval of the SMS common deletion region. The microsatellite marker UT159 (D17S620) (Gerken et al. 1995) was present in both hybrids Hy357-2D and 88H5 (data not shown) and could thus be localized in the interval shared by these two hybrids, which is a part of the SMS common deletion region.

DNA samples from all 62 SMS patients were subjected to microsatellite genotyping analysis at the AFM234ta1 (D17S805) and UT159 (D17S620) loci, and only the 34 new patients were genotyped at the FG1 (D17S446) and OS2- GA3 (D17S71) loci. Of them, 24 patients were informative at one or more of these loci, and 38 were not informative at any of the loci tested.

Parental Origin

When analysis of DNA from probands and their parent(s) at the polymorphic loci indicated above was informative for deletion status, information on the parental

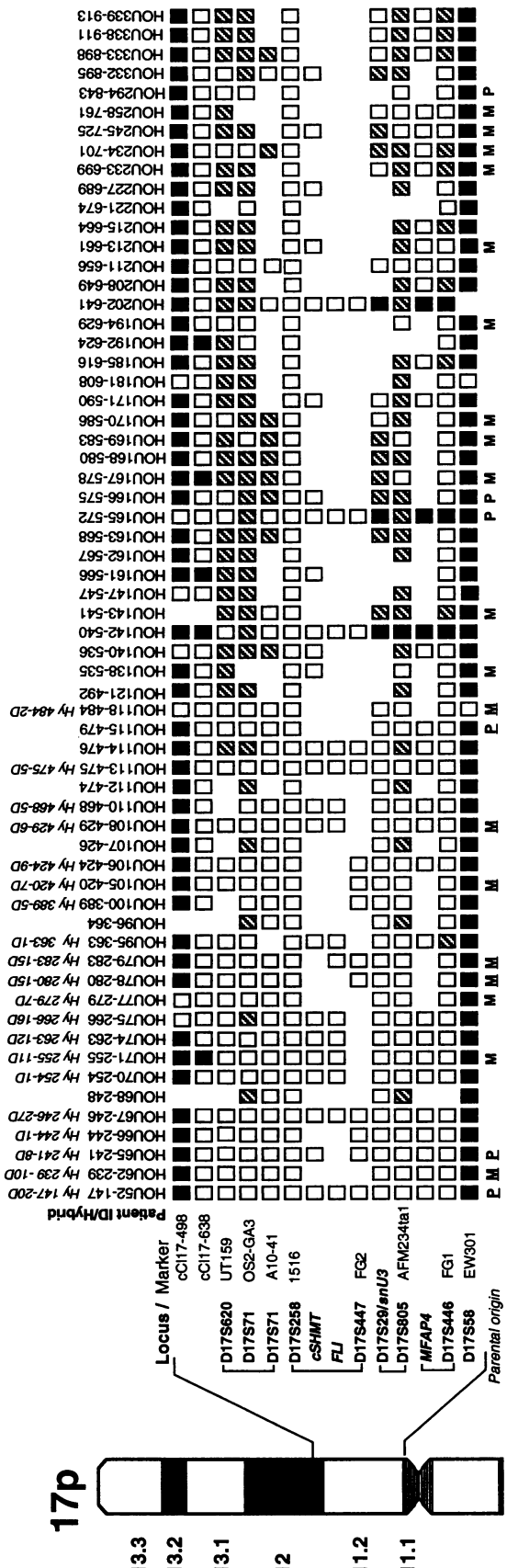

Figure 1 Schematic representation of deletion status of 17p markers in SMS patients. An idiogram illustrating the short arm of chromosome 17 and the relative position of individual markers is shown on the *left*. Marker order is based on presence or absence of the marker in key hybrids and/or deleted chromosomes 17 of key SMS patients. Brackets to the *left* indicate that relative order of that set of markers is unknown. The order of cCI17-638 and cCI17-468 was inferred from the current analyses. The methods used for determining deletion status at each locus are indicated in table 1. In some cases, more than one method (e.g., FISH and RFLP or hybrid analysis) were used to confirm deletion status. Hybrids retaining a deleted chromosome 17 from a patient, when available, are indicated above the patient identification number. Filled boxes indicate that the marker is not deleted; open boxes indicate that the marker is deleted; hatched boxes indicate that the marker is not informative; and empty spaces indicate that the deletion status was not determined. In most cases, the deletion status denoted by an empty space can be inferred by noting the status of other markers that lie either distal or proximal to the marker. Maternal and paternal origin of deletion are indicated by M and P, respectively. Underlined M and P refer to data obtained in the previous studies as indicated in Results. Cell lines for HOU68- 248, 96-364, and 143-541 could not be revived, and, hence, a complete analysis was not possible.

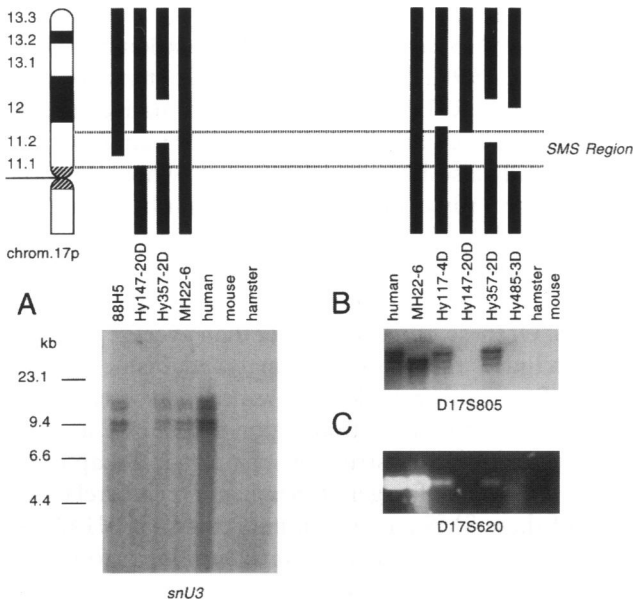

Figure 2 Subregional localization of human *snU3*, D17S805, and D17S620, by use of a human chromosome 17 somatic cell hybrid mapping panel. *A*, Mapping of the *snU3* gene. Genomic DNA from hybrids and controls was digested with the restriction enzyme *Eco*RI and hybridized with a 4.5-kb *Hin*dIII/*Eco*RI fragment of the human *snU3* gene (Yuan and Reddy 1989). The hybrid Hy147-20D derived from a typical SMS patient does not retain any of the four *Eco*RI fragments, while the hybrid Hy357-2D retains all four fragments, thus indicating that this gene cluster maps within the proximal half of 17p11.2 . *B*, Mapping of D17S805 locus. PCR was conducted on DNAs from hybrids and controls by use of one radiolabeled primer and one unlabeled primer followed by electrophoresis on a sequencing gel and autoradiography. The marker D17S805 is retained in Hy357-2D and absent in Hy147-20D, which maps it within the proximal region of 17p11.2. *C*, Mapping of D17S620 (UT159) locus. PCR was conducted on DNAs from hybrids and controls by use of unlabeled primers followed by electrophoresis of the PCR products in a 2% agarose gel. The marker maps within the same interval as D17S805 shown in *B*. The portions of chromosome 17p retained in each hybrid are shown at the *top*.

origin of the deletion could be gained. In this study, parental origin of the deletion could be ascertained in 17 SMS patients for whom DNA samples were available from at least one of the parents. Of the 17 informative patients, 13 had a deletion that was of maternal origin, and 4 had a deletion of paternal origin. By combining these data with those reported previously by Greenberg et al. (1991) and Zori et al. (1993), parental origin has been determined for 33 patients, with 13 having a deletion of paternal origin and 20 of maternal origin.

FISH

Analysis of deletion status in patients can be greatly facilitated by FISH. Patients for whom neither somatic cell hybrids nor samples of parental DNA were available to conduct Southern analysis or microsatellite genotyping were used for FISH studies to evaluate the deletion status. In addition, all patients who were uninformative by RFLP and microsatellite genotyping analysis were evaluated by FISH. Thus, a total of 53 patients were analyzed using FISH either to determine the deletion status or to complement the data obtained by analysis of microsatellite markers, RFLP markers, or hybrids. The probes used in this study were cosmids mapping to the proximal 17p region as indicated in table 1. A patient was considered deleted for a marker if a control signal was seen on the long arm of both chromosome 17 homologues with a signal from the test probe on the short arm of only one of the chromosome 17 homologues. The results obtained are summarized in figure 1. In order to define a common deletion region among the available patients, we began screening with two proximal markers, EW301 (D17S58) and FG1(D17S446), and two distal markers, cCI17-638 and cCI17-498. Most of the SMS patients were found to be deleted for either the FG1(D17S446) or *MFAP4* loci but not for EW301 (D17S58) at the proximal end and similarly were deleted for marker cCI17-638 but not for marker cCI17-498 at the distal end (fig. 1).

The results of the cumulative genotyping, FISH, and hybrid analyses are summarized in figure 1. When a proximal and a distal marker were found deleted, the markers between these flanking markers were not exhaustively analyzed. The most distal and proximal markers that are not deleted in patients HOU118-484 and 181-608 are not known. The cumulative analysis identified a common deletion interval in 52/62 patients (84%), as described above. In addition, 10 patients with molecularly distinct deletions were noted. Patients HOU118-484 (Zori et al. 1993), 181-608, and 147-547 had the largest deletions. Patients HOU71-251, 161-566, 167-578, and 192-624 are not deleted for marker cCI17-638 and, hence, have breakpoints defining a different interval distally. Conversely, HOU165-572 is not deleted for several loci proximally but is deleted for marker cCI17-498 distally. Finally, HOU142-540 and 202-641 appear to have smaller deletions, with the deletion in HOU142-540 being the smallest deletion identified to date.

Mapping of the snU3 *Gene*

One of the four genes mapped so far within the SMS deletion region is that encoding the small nuclear RNA, *snU3*. Recently Chevillard et al. (1993) mapped this gene distal to the locus D17S71, close to the CMT1A locus. Using a 4.5-kb *Hin*dIII/*Eco*RI fragment of a human *snU3* gene subclone HU3-3H as a probe for Southern analysis of the hybrid panel, we mapped the *snU3* gene to the proximal region of 17p11.2 between the markers FG1 (D17S446) and c1516 (D17S258) (fig. 2). Our conclusions are based on the results obtained on

two critical hybrids, 88H5 and Hy357-2D. Of these, 88H5 retains most of 17p11.2, including the marker YNM67 (locus D17S29), but is deleted for markers FG1 (D17S446) and EW301 (D17S58) in the proximal region (Guzzetta et al. 1992). Hy357-2D retains a deleted chromosome 17 with a proximal breakpoint between c1516 (D17S258) and A10-41 (D17S71) and a distal breakpoint between S6.1-HB2 (D17S445) and VAW411 (D17S124) (Guzzetta et al. 1992). Hy147-20D retains a deleted chromosome 17 with its proximal breakpoint between EW301 (D17S58) and FG1 (D17S446) and distal breakpoint between markers S6.1-HB2 (D17S445) and VAW409 (D17S122). Southern analysis of human genomic DNA with the cloned *snU3* RNA gene probe revealed four *Eco*RI fragments of 8.8, 9.4, 15, and 16.5 kb. Presence of these four bands in hybrid MH22-6 but absence of these bands in Hy147-20D confirmed the location of this gene cluster within the SMS region on 17p11.2. Furthermore, presence of all four bands in Hy357-2D and 88H5 unequivocally confirms its location proximal to D17S258 and distal to D17S446.

In addition, we isolated a cosmid, c94A12, by screening a chromosome 17 cosmid library (Kallioniemi et al. 1994) by using as a probe a PCR-amplified segment of the *snU3* gene described by Chevillard et al. (1993). Of the four genomic *Eco*RI fragments recognized by the *snU3* gene subclone HU3-3H, only the 9.4-kb band was present in the cosmid c94A12. We screened 23 SMS patients by FISH using cosmid c94A12 as a probe to confirm the results obtained from the hybrid panel. Of 23 patients tested, 20 were found to be deleted for this probe. Three patients, HOU142-540, HOU165-572, and HOU202-641, who did not show deletion for this probe, carry a different breakpoint in the proximal region of 17p11.2 between the markers YNM67(D17S29) and FG2(D17S447) (see fig. 1). This further substantiates our findings that the *snU3* gene cluster is located proximally on chromosome 17p11.2 in the vicinity of locus D17S29.

Discussion

Our data reveal that the majority of SMS patients appear to carry a common deletion region between markers EW301 (D17S58) and cCI17-498. The proximal deletion breakpoint is located between markers EW301 (D17S58) and FG1 (D17S446) within 17p11.2, as reported elsewhere (Greenberg et al. 1991; Guzzetta et al. 1992). The breakpoint at the telomeric end had so far been localized between markers S6.1-HB2 (D17S445), located within the SMS deletion region, and VAW409 (D17S122), located within the CMT1A locus (Greenberg et al. 1991; Guzzetta et al. 1992). Here, we define the distal breakpoint as located between markers cCI17-638 and cCI17-498, which in turn lies proximal to the CMT1A locus (data not shown), thereby narrowing the SMS deletion region. The marker S6.1-HB2 (Patel et al. 1990*a*) was not used in this study, because the associated RFLP was largely uninformative, and the locus was particularly refractory to FISH analysis.

This study also identified 10 patients whose deletions were molecularly distinct from the majority of the SMS patients evaluated. This group of 10 patients is clinically largely indistinguishable from the patients with the larger deletions and manifest the typical facial features of SMS. Of these, three had larger than average deletions that included the most proximal and distal markers tested. Seven other patients revealed new breakpoints within the common deletion region of 17p11.2 and will be very useful for construction of a physical map of the region as well as for genotype:phenotype correlations. Two of these SMS patients in particular, HOU142-540 and 202-641, will greatly enhance our efforts to further define the minimum critical region for SMS. Work is in progress to build a YAC contig for this region in order to delineate more precisely the critical interval and to identify gene(s).

To date, no patients showing the SMS phenotype in the absence of a cytogenetically detectable deletion on chromosome 17p11.2 have been identified. A typical SMS patient is estimated to harbor a deletion of ~5 Mb (B. Trask and P. I. Patel, unpublished data), but patients harboring a deletion of <2 Mb and showing subtle or variant phenotypes may go undetected unless investigated at the molecular level. Likewise, patients carrying mosaic genotypes will pose similar diagnostic problems. Patients HOU142-540 and 202-641 were both initially diagnosed as mosaic for del(17)(p11.2) (Finucane et al. 1993*b*; Juyal et al. 1995*b*). Subsequent molecular analysis by FISH ruled out mosaicism in each case (Juyal et al. 1995*a*, 1995*b*). As demonstrated in this study, each of these patients indeed carries a small deletion, which may have just been at the limits of the resolution of routine cytogenetic analysis, thus leading to the diagnosis of mosaicism. All of these features could result in misdiagnosis or incomplete assessment of SMS patients. The unfamiliarity with the syndrome and lack of expertise or facilities to conduct FISH analysis may also contribute to the lower reporting of this genetic disorder.

A recent clinical study of 27 SMS patients revealed a broad range of clinical manifestations. There was wide variability in both the type of clinical manifestation as well as the extent of clinical severity (Greenberg et al. 1995). The phenotypic variability and severity in SMS may correspond to the degree of deletion, because more genes may be involved with a larger deletion. The variability may also be attributable to unknown genetic and/or environmental factors. Although a number of cytogenetic and molecular studies on SMS patients have been reported, to date, it has proved somewhat difficult to

establish the clinical parameters defining the syndrome; in some cases, this may be attributed to biases of ascertainment and subjectivities of clinical and cytogenetic diagnoses. This may also lead to underdiagnosis of SMS.

The emergence of a common deletion interval in the majority of SMS suggests that a common mechanism may be operative in the generation of the deletions. The mechanism(s) whereby large DNA deletions, such as those causing microdeletion syndromes, occur are largely unknown at the nucleotide level. Low-copy-repeat sequences have been implicated as predisposing to abnormal pairing and unequal crossing over and producing deletion events in several disorders, including X-linked ichthyosis (Ballabio et al. 1990; Yen et al. 1990; Li et al. 1992) and hereditary neuropathy with liability to pressure palsies (Chance et al. 1994). The human genome contains "sites of instability" or "hot spots" for crossing over, breakage, and rearrangement, and these regions are shown to be prone to errors such as deletions and translocations (Chandley 1991). The presence of such hot spots in 17p11.2 could explain the relatively high frequency of SMS as a de novo chromosomal deletion.

Although more of the de novo deletions observed in the SMS patients are maternal in origin, there seems to be no difference in the phenotypic severity between these patients and those with paternally derived deletions. Variation in the genetic background could determine the phenotypic expression of the deletion by epistatic variation due to interaction with other genes, the study of which would require families with more than one offspring carrying the deletion.

In order to understand the pathogenesis of SMS, it will be necessary to identify those genes from this region that will contribute to a haploinsufficient phenotype. In view of the large size of the critical region, it is unclear how many genes are involved in SMS. The usual methodological approach to this type of problem is the comparison of deletion intervals in a large number of patients in order to construct a deletion map and determine the shortest region of overlap (SRO). In Rubinstein-Taybi syndrome, associated with microdeletions in 16p13.3, a single gene encoding the transcriptional coactivator, CREB binding protein (CBP), appears to be responsible for most if not all of the pathological conditions (Petrij et al. 1995). Patients with point mutations in the CBP gene and the Rubinstein-Taybi syndrome phenotype have provided strong evidence supporting the role of CBP in this syndrome. In the case of SMS, the large size of the interstitial deletion and nonavailability of closely spaced markers within the deletion region have hindered the refinement of an SRO. Our current studies have shown that the *snU3* and *MFAP4* loci are not deleted in three SMS patients, thus suggesting that these genes are not critical to the SMS phenotype. Clinical and molecular analyses of additional SMS patients are necessary. Further studies of the SMS critical region and the genes contained within will provide clarification of their significance and contribution to the phenotype supporting the proposal of SMS being a true contiguous gene syndrome.

Acknowledgments

We gratefully acknowledge the patients and their families for participating in the study. We are indebted to the many clinicians and cytogeneticists who referred patients for this study. We are most grateful to Dr. Yusuke Nakamura for making the anonymous markers cCI17-498 and cCI17-638 available through the Japanese Cancer Research Resources Bank. We thank Dr. Massimo Pandolfo for critical review of the manuscript and many helpful discussions. We gratefully acknowledge the following individuals for referring patients, previously uncharacterized, who were used in this study: A. Bankier, M.D.; R. D. Blackston, M.D.; R. J. Hagerman, M.D.; I. Kondo, M.D., Ph.D.; L. G. Leichtman, M.D.; A. E. Lin, M.D.; J. Siegel-Bartelt, M.D.; A. Van Erem, M.D., Ph.D.; and D. L. Van Dyke, Ph.D. We thank Dr. Ram Reddy for providing subclones of the human *snU3* gene. We thank Joy Gumin, Raymond Schoener-Scott, and Sarn Jiralerspong for technical assistance. This research was supported by National Institute of Health (NIH) grant HD28458 (to P.I.P.), the Texas Children's Hospital General Clinical Research Center grant (NIH grant M01 RR-00188), and Baylor Mental Retardation Research and Genome Center grants.

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