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Molecular Analysis of the Smith-Magenis Syndrome: A Possible Contiguous-Gene Syndrome Associated with del(17)(p11.2)

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Summary

We undertook clinical evaluation (32 cases) and molecular evaluation (31 cases) of unrelated patients affected with Smith-Magenis syndrome (SMS) associated with an interstitial deletion of band p11.2 of chromosome 17. Patients were evaluated both clinically and electrophysiologically for peripheral neuropathy, since markers showing close linkage to one form of Charcot-Marie-Tooth disease (CMT1A) map to this chromosomal region. The common clinical findings were broad flat midface with brachycephaly, broad nasal bridge, brachydactyly, speech delay, and hoarse, deep voice. Fifty-five percent of the patients showed clinical signs (e.g., decreased or absent deep tendon reflexes, pes planus or pes cavus, decreased sensitivity to pain, and decreased leg muscle mass) suggestive of peripheral neuropathy. However, unlike patients with CMT1A, these patients demonstrated normal nerve conduction velocities. Self-destructive behaviors, primarily onychotillomania and polyembolokoilamania, were observed in 67% of the patients, and significant symptoms of sleep disturbance were observed in 62%. The absence of REM sleep was demonstrated by polysomnography in two patients. Southern analysis indicated that most patients were deleted for five 17p11.2 markers—FG1 (D17S446), 1516 (D17S258), pYNM67-R5 (D17S29), pA10-41 (D17S71), and pS6.1-HB2 (D17S445)—thus defining a region which appears to be critical to SMS. The deletion was determined to be of paternal origin in nine patients and of maternal origin in six patients. The apparent random parental origin of deletion documented in 15 patients suggests that genomic imprinting does not play a role in the expression of the SMS clinical phenotype. Our findings suggest that SMS is likely a contiguous-gene deletion syndrome which comprises characteristic clinical features, developmental delay, clinical signs of peripheral neuropathy, abnormal sleep function, and specific behavioral anomalies.

Introduction

Contiguous-gene syndromes are recognizable syndromes and comprise microdeletion and microduplication syndromes (Schmickel 1986; Ledbetter and Cavenee 1989). Specific features of these syndromes may occur individually in families, as phenotypes segregating in a Mendelian fashion. The complex phenotypic abnormalities may result from DNA rearrangements involving several contiguous genes (Schmickel 1986). These syndromes are typically described as clinical entities, prior to establishment of a chromosomal etiology. The cytogenetic abnormality is consistently small and difficult or impossible to detect by routine methods. Some patients with the complete clinical phenotype demonstrate no visible cytogenetic abnormality even after high-resolution analysis.

Contiguous-gene deletion syndromes include retinoblastoma with mental retardation (MR) (del 13q14); Wilms tumor, aniridia, genital abnormalities, and re-

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tardation (WAGR) (del 11p13); Langer-Gideon syndrome (del 8q24); Prader-Willi and Angelman syndromes (del 15q11); α-thalassemia and MR (del 16p13.3); Miller-Dieker syndrome (del 17p13); and DiGeorge syndrome (del 22q11) (Schmickel 1986; Ledbetter and Cavenee 1989). In addition, other syndromes associated with terminal deletions of chromosomes, such as Wolf-Hirschhorn syndrome (del 4p16) (Ivens et al. 1990) and cri-du-chat syndrome (del 5p16) (Overhauser et al. 1989), likely represent phenotypes associated with DNA rearrangements involving contiguous genes. Male patients with multiple X-linked disorders that are due to deletion of contiguous genes in the Xp21 region and that lead to various combinations of Duchenne muscular dystrophy, chronic granulomatous disease, McCleod phenotype, retinitis pigmentosa, glycerol kinase deficiency, congenital adrenal hypoplasia, ornithine transcarbamoylase deficiency, and various degrees of MR have been described (Francke et al. 1987). Similarly, deletions of the Xp22 region have been demonstrated to have combinations of steroid sulfatase deficiency, Kallman syndrome, and MR (Ballabio et al. 1989).

Smith-Magenis syndrome (SMS) is a clinically recognizable multiple congenital anomaly/MR syndrome due to an interstitial deletion of chromosome 17p11.2. The disorder was first described by Smith et al. in 1982, and the spectrum of clinical features was delineated in 1986 by Smith et al. and Stratton et al. To date, a total of 27 patients have been reported (Patil and Bartley 1984; Smith et al. 1986; Stratton et al. 1986; Popp et al. 1987; Lockwood et al. 1988; Colley et al. 1990; Hamill et al. 1990). Recently, DNA markers linked to the gene for Charcot-Marie-Tooth disease type 1A (CMT1A) were mapped to 17p11.2 (Raeymaekers et al. 1989; Vance et al. 1989, 1991; Chance et al. 1990; McAlpine et al. 1990; Middleton-Price et al. 1990; Patel et al. 1990*a*, 1990*b*). A somatic-cell hybrid panel (vanTuinen et al. 1987) was used to map these linked markers to 17p11.2 by virtue of absence of Southern hybridization to a hybrid constructed from a del(17)(p11.2) patient (Patel et al. 1990*a*, 1990*b*). Because of the mapping of the CMT1A gene to this region of chromosome 17, we evaluated 32 SMS patients to determine whether they had evidence of peripheral neuropathy consistent with CMT disease. In addition, we evaluated other clinical findings in order to define the common and variable features of the syndrome. A molecular analysis of the deletion in these SMS patients was performed using proximal 17p DNA markers and analysis for DNA polymorphisms. To determine whether genomic imprinting plays a role in the SMS phenotype, as has been discovered to be the case for chromosome 15 in the Prader-Willi and Angelman microdeletion syndromes (Nicholls et al. 1989; Williams et al. 1990), we studied the parental origin of deletion in SMS patients.

Subjects and Methods

Subjects

Of the thirty-two SMS patients evaluated, 22 were ascertained by the cytogenetic laboratories and genetic clinics at Baylor College of Medicine, Denver Children's Hospital, Oregon Health Sciences University, and the University of Arizona at Tucson, and 10 patients were ascertained by other genetic centers. Most patients were ascertained for dysmorphic features and/or developmental delay, and chromosome analysis demonstrated del(17)(p11.2). Six previously reported and 26 newly identified patients were evaluated. Twenty of them were examined by two authors of the present paper (F. Greenberg and J. R. Lupski). The clinical evaluations were done by using standardized forms which included demographic, anthropometric, morphologic, developmental, behavioral, sleep-habit, and neurologic findings. Nerve-conduction studies were performed through the local Muscular Dystrophy Association (MDA) clinics. Data on each of the patients were tabulated and entered into a data base file by using a dBase III Plus® program. Data on chromosome analysis, including high-resolution banding patterns for chromosome 17, were available for all patients. Thirty-one of the 32 patients reported here had a deletion of proximal 17p, while one patient (93-360) had a translocation with one breakpoint in 17p11.2. Thirty-one of the 32 patients were analyzed by molecular methods.

DNA Probes

The probes used in the present study, their chromosomal location, the restriction enzyme displaying polymorphisms, expected allele sizes, and source and/or reference are listed in table 1. Probe cH3 is a cosmid identified from a library constructed from flow-sorted human chromosome 17 by using FG-1 (Guzzetta et al. 1991) (D17S446) as a hybridization probe. A 900-bp *Taq*I fragment containing a $(GT)_{19}$ sequence was identified from cH3 and cloned into pTZ19R (pRM7-GT), and the nucleotide sequence was obtained by the dideoxy method using Sequenase® (U.S. Biochemi-

Table 1

DNA Markers

Marker (locus)	Location	RFLP	Allele Size (kb)	Reference
LEW301 (D17S58)	17cen-p11.2	*Taq*I	4.5/3.1	Barker et al. 1987
		*Bgl*II	10.0/8.0	Barker et al. 1987
FG1 (D17S446)	17p11.2	*Apa*I	12.0/7.5	Guzzetta et al. 1990
pYNM67-R5 (D17S29)	17p11.2	*Taq*I	3.4/2.0+1.3	Ray et al. 1990
		*Bgl*II	8.1/6.7	Ray et al. 1990
c1516 (D17S258)	17p11.2	*Hin*dIII	22.0/12+10	Patel et al. 1990*a*
p1516-R4 (D17S258)	17p11.2	*Msp*I	3.3/2.4	Franco et al. 1990
pA10-41 (D17S71)	17p11.2	*Msp*I	2.4/1.9	Barker et al. 1987
		*Pvu*II	3.2/3.0	Barker et al. 1987
pS6.1-HB2 (D17S445)	17p11.2	*Msp*I	1.7/1.3	Present study
			1.1/1.0	Patel et al. 1990*b*
		*Sst*I	12.2/7.1	Patel et al. 1990*b*
VAW409R1 (D17S122)	17p11.2-p12	*Msp*I	5.3/2.7+2.6	Wright et al. 1990
VAW409R3 (D17S122)	17p11.2-p12	*Msp*I	2.8/2.7/1.9	Wright et al. 1990
VAW412R3 (D17S125)	17p11.2-p12	*Msp*I	10.5/5.4	Wright et al. 1990
EW401 (D17S61)	17p11.2-p12	*Msp*I	5.2/4.4	Wright et al. 1990
c1517 (D17S259)	17p11.2-p12	*Msp*I	6.2/4.0/2.4	Patel et al. 1990*a*
VAW410R1 (D17S123)	17p11.2-p12	*Bgl*II	2.1/2.0	Wright et al. 1990
		*Taq*I	10.0/9.4	Wright et al. 1990
EW405 (D17S121)	17p11.2-p12	*Msp*I	2.0/1.5	Wright et al. 1990
VAW411R2 (D17S124)	17p11.2-p12	*Msp*I	10.5/6.1	Wright et al. 1990
		*Bgl*II	11.0/10.7	Wright et al. 1990
EW403 (D17S63)	17p11.2-p12	*Msp*I	13.5/6.8	Wright et al. 1990
EW503 (D17S67)	17p11.2-p12	*Msp*I	6.9/5.7	Wright et al. 1990
EW502 (D17S66)	17p11.2-p12	*Bgl*II	2.2/1.4	Wright et al. 1990

cals). Flanking PCR primers were synthesized by standard methods and used to analyze $(GT)_n$ polymorphisms as described (Weber and May 1989). The sequence of the priming oligodeoxynucleotide on the GT strand of pRM7-GT is 5′-ATTATTTATTTTG-ATGTCTGAACAC-3′, while that of the priming oligodeoxynucleotide on the CA strand of pRM7-GT is 5′-CTTGGTGAAACGCTGTCTGTAC-3′. The latter primer has homology to the *Alu* repeat sequence.

Southern Analysis and Densitometry

Southern analysis was performed as described elsewhere (Patel et al. 1990*a;* Franco et al. 1991). Equal amounts (5 μg) of digested genomic DNA were included in each lane to ensure reproducibility of densitometric signal. All probes were labeled by the random hexanucleotide priming method (Feinberg and Vogelstein 1983). If a marker was not fully informative for RFLPs, the copy number was determined by dosage analysis of signals obtained by simultaneous hybridization of the experimental marker and the marker DR47, representing a single-copy sequence on chromosome 9. The intensity of the bands in each lane was quantified using an LKB 2400 Gel Ultrascan XL laser densitometer as described elsewhere (Franco et al. 1991). The analysis of segregation patterns for alleles at the VNTR locus YNH24 (D2S44) (Nakamura et al. 1987) demonstrated no instances of false paternity.

PCR and GT Repeat Polymorphism Screening

The unique sequence primer from the GT strand of marker RM7-GT was end labeled at 37°C in a 15-μl reaction volume containing 1.2 μM primer, 100 μCi [γ ^{32}P]ATP at 6,000 Ci/mmol, 1 × One Phor-All Plus buffer (Pharmacia), and 10 units polynucleotide kinase (Pharmacia). The T4 polynucleotide kinase was heat inactivated by incubating the reaction mixture at 65°C for 10 min. The end-labeled primer resulting from the kinase reaction was used directly in the PCR reaction, without separating the unincorporated nucleotides (0.40 μl/reaction). PCR was performed using standard conditions in a 25-μl reaction volume. The reaction mixture contained 1 μM of each oligodeoxynucleotide primer, 250 μM each of dATP,

dCTP, dGTP, and dTTP, 2.5 μl 10 × PCR buffer (500 mM KCl, 120 mM Tris HCl [pH 8.0], 1.5 mM $MgCl_2$, and 0.01% gelatin), 0.63 units of AmpliTaq (Cetus) DNA polymerase, and 0.4 μl end-labeled primer, as stated above. The amplification conditions were an initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C denaturation (1 min), 55°C annealing (1 min) and 72°C extension (2 min) in an automated thermal cycler (Perkin Elmer—Cetus). Reaction products (1.5 μl) were mixed with 2 μl formamide stop solution (U.S. Biochemicals) and electrophoresed in a 6% polyacrylamide DNA sequencing gel at 40 W for 3.5 h. Gels were dried and autoradiographed for 2–12 h by exposing them to Kodak XAR-5 film with either one or two intensifying screens at −70°C.

Results

Clinical Spectrum of SMS

In the present series of 32 patients, there were 14 males and 18 females. The age range was 1 mo–72 years, with a mean of 15 years and a median of 10 years. The mean maternal age was 26 years, and the mean paternal age was 30 years. The percentages of findings for the most common physical features of the 26 newly ascertained SMS patients we examined are shown in table 2 and are compared with those of the 27 previously reported patients (Patil and Bartley 1984; Smith et al. 1986; Stratton et al. 1986; Popp et al. 1987; Lockwood et al. 1988; Colley et al. 1990; Hamill et al. 1990).

The most common physical findings were brachycephaly with flat midface, broad nasal bridge, brachydactyly, and short stature (usually 2–3 SDs below the mean for age). Clinical symptoms included failure to thrive in infancy and limitation of movement at the elbow, which in some cases was documented to be associated with radioulnar synostosis. Common but less consistent physical abnormalities included prominent forehead, synophrys, prognathism, posteriorly rotated ears, low-set ears, and/or other ear anomalies. Abnormalities seen in a smaller percentage of patients were congenital heart defects (primarily ventricular or atrial septal defects) in 31% and cleft lip and palate in 7%. Less common findings included cutaneous syndactyly of the fingers or toes, microcornea, iris coloboma, and craniosynostosis. Another unusual and striking feature noted in the older children, adolescents, and adults was a hoarse, deep voice, which was noted in 82% of the patients in the present study.

Of the 32 patients evaluated, pes planus or pes cavus was noted in 48%, and 24% had scoliosis. A total of 17 (55%) of 31 patients had clinical signs, including significantly decreased or absent deep-tendon reflexes and insensitivity to pain, which suggested peripheral neuropathy. A summary of the presumed neuropathic changes and nerve-conduction studies in these patients is shown in table 3. On the basis of previous psychometric testing (in most cases), the SMS patients showed varying degrees of MR, with the majority fall-

Table 2

SMS (del 17p11.2) Physical Features

Physical Feature	% of Reported Patients[a] (*N* = 21)	Proportion of Reevaluated Patients (*N* = 6)	Proportion (%) New Patients (*N* = 26)	% of Total Patients (*N* = 53)
Flat midface	95	6/6	24/26 (92)	94
Brachycephaly	86	5/6	21/26 (81)	83
Prominent forehead	81	1/6	16/26 (62)	64
Broad nasal bridge	76	4/6	23/26 (88)	81
Prominent jaw	38	4/6	15/26 (58)	51
Ear abnormalities	67	5/6	17/26 (65)	68
Brachydactyly	81	5/6	21/26 (81)	81
Limitation at elbow	NE	3/6	5/22 (23)	29
Pes planus/cavus	NE	3/6	11/23 (48)	61
Scoliosis	NE	3/6	4/23 (17)	24
Congenital heart defect	38	1/6	6/21 (29)	31

[a] NE = not specifically examined for in other studies.

Table 3

Clinical Signs Suggestive of Peripheral Neuropathy and Peroneal NCV in 19 SMS Patients

Patient (Age in years)	Status of[a]				Peroneal Nerve-Conduction[b] Velocity (m/s)
	Pes Cavus or Pes Planus	Scoliosis	Decreased or Absent DTR	Insensitivity to Pain	
55-200 (8)	+	+	+	+	R39.4 and L45.9
56-203[c] (4)	+	–	–	–	62.5
57-206 (19)	+	+	ND	ND	ND
64-239 (10)	+	–	+	–	ND
65-241 (16)	+	–	+	+	51
66-244 (20)	–	–	+	–	ND
67-246 (21)	+	–	+	–	55
68-248 (6)	–	–	+	–	58
69-251 (12)	+	–	+	+	59
71-255 (14)	+	–	–	+	ND
75-266 (4)	–	–	–	+	50
78-280 (2)	–	–	+	+	68.7
79-283[c] (18)	+	+	–	–	65
94-362 (72)	–	+	+	–	44
95-363 (40)	+	+	–	+	51
96-364 (26)	–	–	+	–	49
100-389 (2)	+	+	ND	ND	ND
112-474 (35)	–	+	ND	+	ND
112-475 (20)	+	+	–	ND	ND

[a] + = Present; – = absent; ND = not determined.

[b] Normal value, expressed as mean ± SD in 120 nerves from 60 patients who were 16–86 (mean 41) years and who had no apparent disease of the peripheral nerves with site of stimulation below the knee, was 48.3 ± 3.9 (Kimura 1989).

[c] Had no significant signs of peripheral neuropathy, but peroneal nerve-conduction velocity was measured.

ing within the moderate range. Other neurobehavioral abnormalities included infantile hypotonia, seizures, developmental delay with speech delay greater than motor delay, conductive hearing loss, and hyperactivity (table 4). Sixty-two percent of patients had symptoms of a sleep disorder which manifested as difficulty falling asleep, difficulty staying asleep, and frequent awakening during the night. Both one previously reported patient (patient 2 in Stratton et al. 1986) and one of the present study's patients (64-239) had absence of REM sleep, documented by polysomnography. Self-destructive behavior was noted in 67% of patients. This behavior consisted of head banging, wrist biting, onychotillomania (pulling out fingernails and toenails) and polyembolokoilamania (insertion of foreign bodies into body orifices).

Several of the physical and behavioral findings either appeared to be more noticeable with increasing age or demonstrated an age-dependent penetrance; these included frontal prominence, prognathism, brachydactyly, and the hoarse voice. In addition, facial features appeared to coarsen somewhat with age. Although onychotillomania was uncommon under 5–6 years of age, in some patients self-destructive behaviors such as head banging and wrist biting were noted as early as the second year of life.

Five patients were of particular note. Patient 55-200, a 9-year-old severely mentally retarded boy with cleft lip and palate and congenital heart defect (patient 2 in Smith et al. 1986), had clinical evidence of a peripheral neuropathy including decreased deep-tendon reflexes in the arms, absent reflexes in the legs, a stork-leg deformity of the legs, and prominent pes cavus. Nerve-conduction studies showed a velocity of 39.4 m/s in the right common peroneal nerve and 45.9 m/s in the left (table 3). The right and left peroneal nerve had increased distal latencies (R = 4.02 milliseconds; L = 5.12 milliseconds) and decreased base-to-peak amplitude (R = 1.5 K; L = 2.5 K). Sural sensory-nerve responses were absent bilaterally. Patient 92-357 had del(17)(p11.2p12)—a deletion involving 17p11.2—but did not have a phenotype similar to those of the other patients, and the only consistent findings were broad nasal bridge, short stature,

Table 4

SMS (del 17p11.2) Behavioral/Functional Features

Behavioral/Functional Features	% of Reported Patients[a] (*N* = 21)	Proportion of Reevaluated Patient (*N* = 6)	Proportion (%) of New Patients (*N* = 26)	% of Total Patients (*N* = 53)
Infantile hypotonia	71	2/5	8/15 (53)	66
Seizures	32	1/5	5/24 (21)	30
Short stature/FTT	61	5/6	21/23 (91)	78
Speech delay	92	4/4	21/22 (95)	98
Conductive hearing loss	58	4/5	12/18 (67)	67
Hoarse, deep voice	62	4/5	15/18 (65)	74
Hyperactivity	92	5/5	15/23 (65)	82
Sleep disorder	31	1/3	15/23 (65)	51
Possible peripheral neuropathy	NE	4/5	13/26 (50)	55
Self-destructive behavior	73	5/6	15/24 (63)	70

[a] NE = not specifically examined for in earlier studies (i.e., Smith et al. 1982, 1986; Patil and Bartley 1984; Stratton et al. 1986; Popp et al. 1987; Lockwood et al. 1988; Colley et al. 1990; Hamill et al. 1990).

speech delay, hoarse voice, and hyperactivity. Patient 93-360 had an apparently balanced translocation—46,XY,t(2:17)(p25.3;11.1), with a breakpoint in the Smith-Magenis region—involving chromosome 2 and chromosome 17. He had relatively mild physical findings similar to those seen in SMS patients and had some behavioral problems, including hyperactivity without self-destructive behavior. Patient 94-362 (patient 8 in Smith et al. 1986), 72 years of age at the time of the present study, was noteworthy for being the oldest patient in our study. Patient 112-474 was clinically diagnosed as having SMS and subsequently was confirmed, by high-resolution cytogenetic analysis, to have del(17)(p11.2), demonstrating that SMS is a clinically recognizable syndrome.

SMS Is Associated with Deletion of Proximal 17p DNA Markers

All the patients reported here had cytogenetic evidence of a DNA rearrangement involving 17p11. Previous cytogenetic analysis revealed del(17)(p11.2) in all but three patients. Patient 93-360 had translocation t(2;17)(p25.3p11.1) with one breakpoint in 17p11, while two patients, 92-357 and 100-389, had deletions that appeared to extend distally [del(17)(p11.2p12)] by cytogenetic analysis. In order both to define the deletion interval common to most SMS patients and to identify patients who had novel deletion intervals, genomic DNA isolated either from peripheral lymphocytes or from Epstein-Barr virus–transformed lymphoblastoid cell lines was examined by Southern analysis using several proximal 17p DNA markers as probes. Each DNA marker displays one or more RFLPs (table 1) after digestion of genomic DNA with the appropriate enzyme(s). RFLP analysis was then used to determine deletion status, with parental DNA being used as a control when it was available. The data are tabulated in figure 1. A plus sign indicates the presence of the DNA marker, while a minus sign indicates deletion of that DNA marker in that patient. A boldface plus or minus sign indicates a fully informative RFLP analysis, while the plain plus and minus symbols indicate deletion status determined by measuring the dosage of an allele by using densitometry.

Five DNA markers—FG-1, pYNM67-R5, c1516, pA10-41, and pS6.1-HB2—were deleted in almost all SMS patients. These markers appear to define a region critical to the SMS phenotype. Analysis of the deletion status of these and additional 17p markers in individual SMS patients revealed the following: (1) Patient 92-357 was deleted for the four markers pA10-41, pS6.1-HB2, EW401, and EW405 but not for FG-1, pYNM67-R5, or c1516, indicating a more distal deletion when compared with the other patients. Thus, these data suggest that FG-1, pYNM67-R5, and c1516 are proximal markers in the critical SMS region, that pA10-41 and pS6.1-HB2 are distal in the critical SMS region, and that EW401 and EW405 are distal to the SMS critical region. (2) Except for patient 92–357, the majority of the patients were not deleted

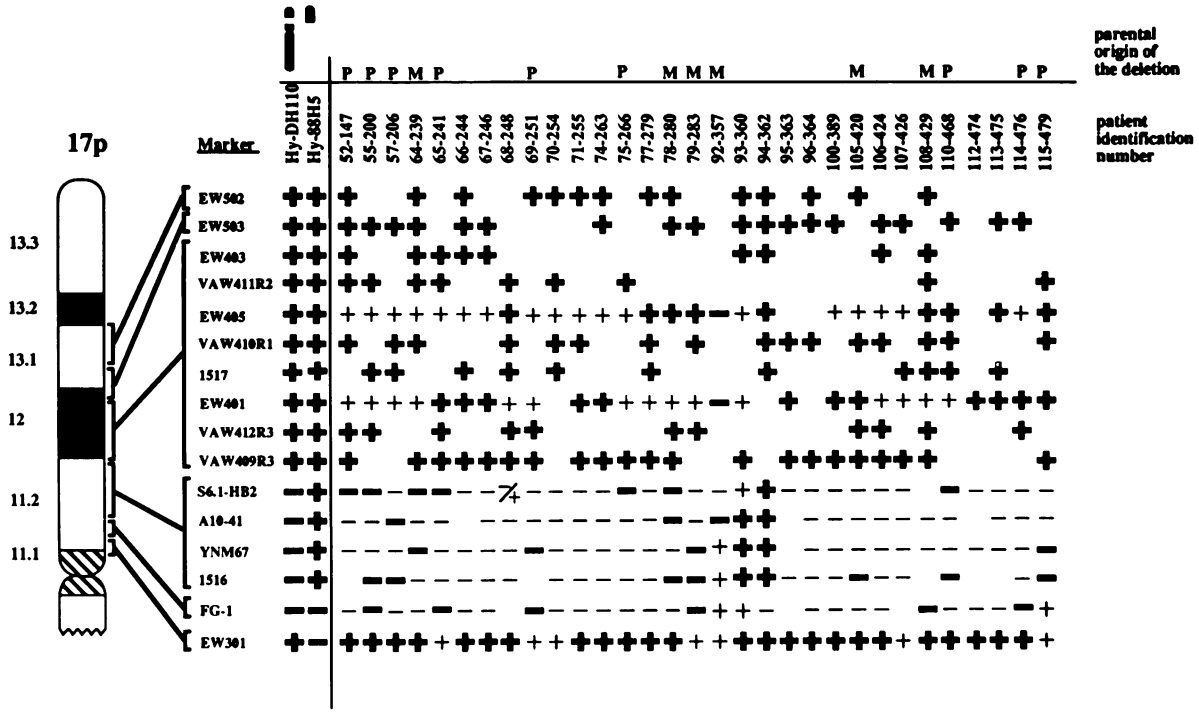

Figure 1 Deletion status of 17p DNA markers in SMS patients. An idiogram illustrating both the short arm of chromosome 17 and the relative position of individual markers is shown on the left. Marker order, from VAW409R3 distal, is taken from the published genetic map of Wright et al. (1990), except for 1517, whose precise position with respect to flanking markers is not known. The order of markers proximal to VAW409R3 is from the present study and is based on the deletion analysis of SMS patients, especially 92-357, and of hybrids DH110-D1 and MH22-6. Within the deletion interval encompassed by 1516, YNM67, A10-41, and S6.1HB2, the relative order of 1516 with respect to YNM67 and of A10-41 with respect to S6.1-HB2 cannot be determined from this analysis. Informative analysis for marker FG-1 (D17S446) was determined by $(GT)_n$ polymorphism analysis as in fig. 3. A blank space within the region encompassed by DNA markers EW301–S6.1-HB2 represents data which were not informative. For most markers distal to VAW409R3, except for EW401 and EW405 where patient 92-357 was deleted by a fully informative analysis, only fully informative heterozygous individuals are listed. If more than one marker was used at a specific locus (e.g., c1516 and p1516-R4 at D17S258; VAW409R1 and VAW409R3 at D17S122) or if more than one polymorphism was recognized by a single probe (e.g., EW301, pYNM67-R5, pA10-41, and pS6.1-HB2), a cumulative deletion status was scored. The first two columns illustrate the results obtained with the 17p DNA markers by using human chromosome 17–retaining somatic-cell hybrids. MH22-6 retains an intact human chromosome 17 as its only human complement, while 88H5 retains the distal portion of 17p with a breakpoint in the SMS region. The other 31 columns are from individual SMS patients. The – / + designation at the S6.1-HB2 locus for patient 68-248 reflects an apparent deletion, by densitometry, with one polymorphism but not with the other. Further studies, using somatic-cell hybrids, are in progress.

for EW301, EW401, EW402, EW403, EW404, EW405, VAW409R1, VAW409R3, VAW410R1, VAW411R2, VAW412R3, EW502, and EW503. (3) Patient 93-360 (with a 2;17 translocation) was found not to be deleted for any of the markers studied. (4) Patient 94-362, the oldest patient, appeared to be deleted for only one DNA probe, FG-1.

Parental Origin of Deletion

Since SMS is purportedly caused by a de novo deletion of chromosome 17 in a parental gamete, we sought to determine whether the deletion occurred preferentially in the paternal or maternal gamete. Parental origin of the deletion was determined by following the inheritance of polymorphic alleles, as shown in figures 2 and 3. Examples of fully informative Southern analyses of patients and parents are shown in figure 2, while similar fully informative $(GT)_n$ polymorphism analyses are shown in figure 3. The parental origin of the deletion could be determined in 15 patients. The deletion was paternally derived in nine individuals and was maternally derived in six individuals. Within this group of 15 patients, there appeared to be no significant clinical differences between indi-

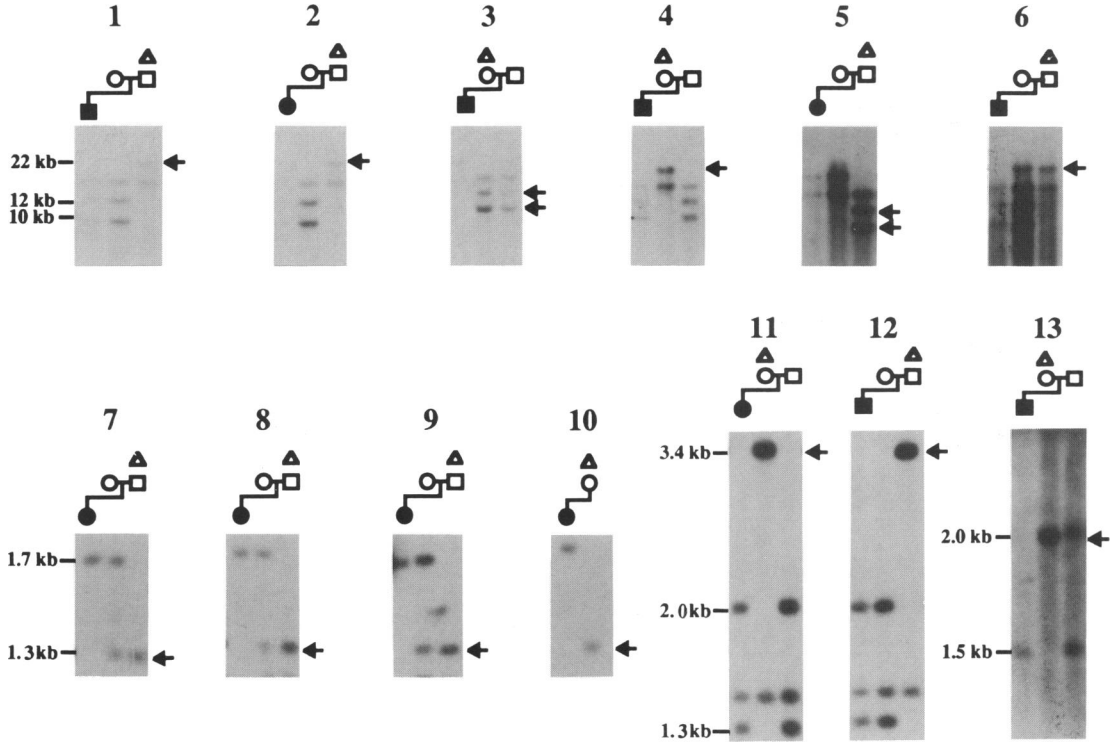

Figure 2 Parental origin of deletion in SMS patients. Genomic DNA from 13 SMS patients and their parent(s) was digested with the indicated restriction endonuclease and subjected to Southern analysis with the indicated probe. Panels 1–6, *Hin*dIII-digested DNA from patients 55-200, 57-206, 79-283, 105-420, 110-468, and 115-479 respectively, that was hybridized to 1516. Panels 7–10, *Msp*I-digested DNA from patients 75-266, 65-241, 52-147, and 78-280, respectively, that was hybridized to pS6.1-HB2. Panels 11 and 12, *Taq*I-digested DNA from patients 64-239 and 69-251, respectively, that was hybridized to pYNM67-R5. Panel 13, *Msp*I-digested DNA from patient 92-357 that was hybridized to EW405. The pedigree structure is shown above each autoradiograph. A triangle above a symbol identifies the parent who was the origin of the deletion. The sizes of the alleles are shown on the left of each blot. The arrow depicts the deleted allele in the SMS patient. The blots shown are the results obtained using a DNA marker that gave a fully informative analysis.

viduals with paternally derived and individuals with maternally derived deletions.

Discussion

SMS, associated with an interstitial deletion of the short arm of chromosome 17, was first described in 1982. Although the number of patients reported is relatively small, there are likely many unreported patients who will be ascertained with improvement in techniques for high-resolution cytogenetic banding. In Harris County, Texas, over a 2-year time period, we have detected four infants with this deletion, suggesting a minimum birth prevalence of approximately 1/25,000. Thus, SMS may be more common than cri-du-chat syndrome (del 5p16) (Niehbuhr 1978), which has an estimated frequency of 1/50,000, and about as common as Prader-Willi syndrome (del 15q12) (Burd et al. 1990). On the basis of the frequency of SMS, our preliminary investigations indicate that this syndrome may be a relatively common cause of MR, because of deletion of a specific chromosomal region.

As determined in the present study, clinical findings in SMS patients were dysmorphic features including brachycephaly, broad nasal bridge, mild synophrys, posteriorly rotated or low-set ears, prognathism, and brachydactyly. Clinical symptoms of the patients included failure to thrive in infancy, short stature, infantile hypotonia, developmental delay, and subsequent MR with speech and language delay greater than motor delay. Variable features included cleft lip and/or palate, congenital heart defect, microcornea, and craniosynostosis. Self-destructive behavior, particularly onychotillomania (pulling out fingernails or toenails) was common in older individuals. Other self-

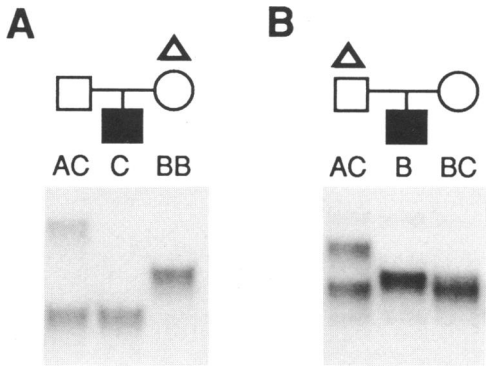

Figure 3 Parental origin determined by $(GT)_n$ polymorphism at the D17S446 locus. The $(GT)_n$ polymorphism associated with the D17S446 locus is shown for patients 108-429 and 114-476 and their parents. Note that patient 108-429 has not inherited one of the maternal alleles at this locus, while patient 114-476 has not inherited one of the paternal alleles at this locus.

destructive behaviors, such as head banging, wrist biting, and polyembolokoilamania (insertion of foreign bodies into various body orifices) were less specific for this disorder. About two-thirds of patients had sleep disturbance, and two patients studied by polysomnography had absence of REM sleep.

Onychotillomania due to picking or manipulation of the nails is a condition which has been reported (*a*) in association with either delusion of infestation or depressive neurosis or (*b*) as an isolated finding (Sait et al. 1985; Colver 1987). SMS patients have more severe manifestations of onychotillomania in that they have been observed to extract the entire nail from the nail bed. The severe expression is probably related to what has been observed in many SMS patients: relative insensitivity to pain. This insensitivity to pain may be a consequence of peripheral neuropathy, altered emotional response to pain, or both. This type of onychotillomania may be relatively specific to this disorder.

Absence of REM sleep is a rare disorder, and its effects are uncertain (Hobson 1990). Although 62% of patients in the present study had clinical histories of sleep disorders, thus far only two patients have had formal sleep evaluations; and both of these patients were found to have absence of REM sleep, without any exposures to medication. The behavioral abnormalities in SMS patients may be related to decreased REM sleep. The observation of the absence of REM sleep in SMS patients suggests that this may be due to a loss of a gene, involved in sleep function, that maps to 17p11.2. The association between absence of REM sleep and CMT1A has previously suggested the possibility that a gene associated with REM sleep is in proximity to the CMT1A locus (Tandan et al. 1990).

RFLP analysis with polymorphic proximal 17p DNA markers demonstrated five markers deleted in the majority of SMS patients; these five markers are FG-1 (D17S446), pYNM67-R5 (D17S29), c1516 (D17S258), A10-41 (D17S71), and pS6.1-HB2 (D17S445), which define a region critical to SMS. Patient 92-357 was found to have a deletion which had one breakpoint within the critical SMS region and which extended telomerically, to involve DNA markers EW401 (D17S61) and EW405 (D17S121). Results obtained with this patient enabled us to order some of the proximal 17p markers (fig. 1). Indeed, his phenotype had some overlapping SMS features, as well as some unique features which likely result from genes which map within the telomeric extension of his deletion. The translocation t(2;17)(p25.3;p11.1) patient 93-360, although not deleted for any of the proximal 17p markers studied thus far, displayed subtle clinical features of SMS, most notably the distinct behavioral disturbances. This suggests that he may have a submicroscopic deletion within the region—but that it is not encompassed by any of the markers used in the present study. Alternatively, the phenotype may result from a position effect secondary to juxtaposition of 17p11.2 genes to a different environment, or the translocation may interrupt a single critical gene in this region. By densitometric analysis, patient 94-362 appeared to be deleted for only one proximal 17p marker; studies using somatic-cell hybrids to confirm this finding are in progress. It is interesting that she is the longest-lived patient and had less severe clinical problems, lending support to our hypothesis that the extent of hemizygosity in this patient may be lower than that in other SMS patients, although we cannot rule out a cryptic translocation of some proximal 17p material in this patient.

The DNA markers deleted in SMS patients are linked to CMT1A (Raeymaekers et al. 1989; Vance et al. 1989; Chance et al. 1990; McAlpine et al. 1990; Middleton-Price et al. 1990; Patel et al. 1990*a*, 1990*b*). CMT1A is the most commonly inherited peripheral neuropathy characterized clinically by (*a*) absence of deep-tendon reflexes, (*b*) distal muscle wasting resulting in either pes cavus or pes planus and in a claw-hand deformity, and (*c*) distal sensory neuropathy (Lupski et al. 1991*a*). CMT1A is characterized electrophysiologically by decreased nerve-conduction

velocity (Kimura 1989; Lupski et al. 1991*a*). SMS patients demonstrate clinical signs suggestive of a peripheral neuropathy, but their peroneal motor-nerve-conduction velocities were normal, except for patient 55-200. Recently, we have demonstrated that CMT1A is completely linked and associated with a large DNA duplication in proximal 17p, a duplication which appears to encompass VAW409R3, VAW-412R3, and EW401 (Lupski et al. 1991*b*). These markers border the SMS deletion region. It is interesting that patient 92-357 is deleted for EW401, one of the markers apparently duplicated in CMT1A, and yet displays no clinical signs of peripheral neuropathy.

In a number of human genetic disorders, the phenotypic expression of the disease may depend on paternal or maternal inheritance of the mutation (Hall 1990). It has been hypothesized that genomic imprinting is an epigenetic process that marks the paternal or maternal chromosomes involved in such parental effects. Genomic imprinting has been implicated in Prader-Willi and Angelman syndromes, both caused by cytogenetically indistinguishable deletions of bands q11-q13 of chromosome 15. Molecular studies appear to indicate that, while Angelman syndrome is due to a deletion of the maternal allele, Prader-Willi syndrome is caused by a deletion of the paternal allele (Nicholls et al. 1989; Williams et al. 1990). The extent to which imprinting effects on the human genome may be discerned through the study of the parental origin of the deleted segment in microdeletion syndromes remains to be determined. We analyzed 15 SMS pedigrees by analysis of DNA polymorphisms associated with 17p11 markers which were fully informative for parental origin of the deletion. Nine of the deletions were of paternal origin, while six were of maternal origin. The clinical phenotype was similar, regardless of parental origin of the deletion. Although further clinical studies are needed to investigate this hypothesis, the variability of the SMS phenotype does not appear to be associated with parental origin of deletion. These results are similar to those in a series of Miller-Dieker syndrome patients who have deletion of distal 17p (Dobyns et al. 1991).

In conclusion, in addition to the characteristic previously described features, SMS appears to be a contiguous-gene microdeletion syndrome which is associated with del(17)(p11.2) and which can include clinical signs of peripheral neuropathy, self-destructive behavior, and sleep disorders or absence of REM sleep. A DNA rearrangement leading to the deletion of several contiguous genes in 17p11.2 is likely the molecular mechanism underlying de novo del(17)(p11.2). It is interesting that other DNA rearrangements including duplications (Magenis et al. 1986) and translocations (Schrander-Stumpel et al. 1990) have been reported for the 17p11.2 region. A complete physical map of 17p11.2 may be developed by utilizing the present study's patients to construct a deletion mapping panel—and then correlating this with yeast artificial chromosome contigs and also with a pulsed-field gel electrophoretic restriction map. This should enable identification of deletion breakpoints in individual patients and is a means to examine mechanisms of DNA rearrangements in man. It will also enable both delineation of specific genes which map to this region and correlation of the genotype with the phenotype in individual SMS patients.

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