article

Genotype–phenotype correlation in Smith-Magenis syndrome: Evidence that multiple genes in 17p11.2 contribute to the clinical spectrum

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Purpose: Smith-Magenis syndrome (SMS) is a complex disorder that includes mental retardation, craniofacial and skeletal anomalies, and behavioral abnormalities. We report the molecular and genotype–phenotype analyses of 31 patients with SMS who carry 17p11.2 deletions or mutations in the *RAI1* gene. **Methods:** Patients with SMS were evaluated by fluorescence in situ hybridization and/or sequencing of *RAI1* to identify 17p11.2 deletions or intragenic mutations, respectively, and were compared for 30 characteristic features of this disorder by the Fisher exact test. **Results:** In our cohort, 8 of 31 individuals carried a common 3.5 Mb deletion, whereas 10 of 31 individuals carried smaller deletions, two individuals carried larger deletions, and one individual carried an atypical 17p11.2 deletion. Ten patients with nondeletion harbored a heterozygous mutation in *RAI1*. Phenotypic comparison between patients with deletions and patients with *RAI1* mutations show that 21 of 30 SMS features are the result of haploinsufficiency of *RAI1*, whereas cardiac anomalies, speech and motor delay, hypotonia, short stature, and hearing loss are associated with 17p11.2 deletions rather than *RAI1* mutations ($P<.05$). Further, patients with smaller deletions show features similar to those with *RAI1* mutations. **Conclusion:** Although *RAI1* is the primary gene responsible for most features of SMS, other genes within 17p11.2 contribute to the variable features and overall severity of the syndrome. ***Genet Med*** **2006:8(7):417–427.**

Key Words: *Smith-Magenis syndrome, 17p11.2 deletion, RAI1, genotype–phenotype, mental retardation*

Smith-Magenis syndrome (SMS) (Online Mendelian Inheritance in Man [OMIM] 182290) is a multiple congenital anomalies and mental retardation syndrome associated with either an interstitial deletion involving 17p11.2 (including *RAI1*) or a mutation in the *RAI1* gene.[1–4] The SMS phenotype includes distinctive craniofacial and skeletal features, global developmental delay, cognitive impairment, and mild to moderate mental retardation.[5,6] Behavioral abnormalities include significant sleep disturbances with inverted circadian rhythm of melatonin and maladaptive and self-injurious behaviors.[6–9] Facial features consist of a broad, square-shaped face with brachycephaly, midface hypoplasia, tented upper lip, and micrognathia in early infancy progressing to prognathism with age.[2] Up-slanting palpebral fissures, deep-set eyes, short full-tipped nose, and downturned corners of the mouth are also seen in the majority of patients with SMS. Hoarse deep voice, hearing loss, and other otolaryngologic problems such as vocal cord nodules and polyps are common.[3,10,11] Infancy and childhood are associated with failure to thrive, hypotonia, and feeding difficulties.

Neurobehavioral abnormalities in SMS become more pronounced with age and are characterized by hyperactivity, temper tantrums, attention-seeking, self-hugging, polyembolokoilamania (insertion of objects into bodily orifices), and onychotillomania (pulling out fingernails and toenails).[9] Less frequently seen features include cleft lip/palate, renal/urinary tract abnormalities, thyroid dysfunction, and seizures. Congenital cardiac abnormalities such as valvular defects, septal anomalies, and tetralogy of Fallot identifiable by echocardiographic changes have also been reported.[1,3,12] Comprehensive eye evaluations reveal a high frequency of myopia, iris anomalies, strabismus, microcornea, and, rarely, retinal detachment.[13,14] Hypercholesterolemia has been reported in approximately 70% of patients with SMS.[15]

Chromosomal deletions of 17p11.2 associated with SMS result from nonhomologous mechanisms in addition to nonallelic homologous recombination mediated either by SMS-re-

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peat clusters or low-copy repeats during maternal or paternal gametogenesis.[16,17] Approximately 70% of patients with 17p11.2 deletions have a "common" ~3.5 Mb deletion, whereas the remaining 30% have larger or smaller deletions.[18] Recently, the minimal overlapping region of deletion common to all patients with SMS carrying 17p11.2 deletions was reduced to ~650 kb by analyzing patients with unusual 17p11.2 deletions.[19,20] Further, we identified intragenic mutations in the gene encoding the retinoic acid-induced 1 protein (*RAI1*) in patients with SMS without a 17p11.2 deletion.[4,21] Although comprehensive reports of clinical features associated with SMS deletions have been described,[3,6,22] a thorough phenotypic analysis based on correlation with 17p11.2 deletion size or *RAI1* mutations has not been reported.

To further evaluate the role that *RAI1* plays in the SMS phenotype, we evaluated and compared the individual features of 31 patients with SMS carrying either a 17p11.2 microdeletion, including common and unusual deletions, to those patients with mutations in the *RAI1* gene. Results indicate that although *RAI1* clearly is responsible for most features of SMS, other genes residing within 17p11.2 likely contribute to more variable features and severity of the syndrome.

METHODS

Patient ascertainment and samples

Thirty-one patients (16 males and 15 females; median age at evaluation 9 years, ranging from 10 days to 41 years old) with signs and symptoms consistent with SMS were referred through genetic clinics from various parts of North America, Europe, and Australia.[4,18,19,21] The Michigan State University Committee on Research Involving Human Subjects and the Virginia Commonwealth University Institutional Review Board approved this study. Written informed consent was obtained for each participating individual and/or their parents enrolled in this study. A standard clinical questionnaire was requested for all subjects with SMS who were enrolled in this study. Clinical evaluations including physical examination; development and cognitive skill assessments; ear, nose, and throat, and audiologic assessments; and ophthalmologic examinations were reviewed. Clinical laboratory reports of immunologic tests, lipid profile, renal ultrasound, echocardiography, thyroid function tests, and polysomnography were available on selected patients. Clinically acquired anthropometric data, including height and weight, were available on most individuals, whereas data on others were based on parental reports. Short stature was determined on the basis of relative heights of the parents and siblings or heights <5th centile by measurement for that age. History of chronic ear infections was considered if the child was on tympanostomy (pressure equalizing) tubes with or without consequential hearing loss. Approximately 7 to 10 mL of blood was drawn by antecubital venipuncture in all patients and available parents by using sterile techniques. Metaphase chromosomes and DNA were isolated from blood samples using standard methods.

17p11.2 deletion analyses by fluorescent in situ hybridization

Fluorescent in situ hybridization (FISH) probes were chosen on the basis of chromosome 17p11.2 mapping information from our previously published SMS contig of large-insert bacterial artificial chromosomes, P1 artificial chromosomes, and cosmids.[23] FISH was performed as previously described.[18] SMS129, SMS153, SMS156, SMS159, SMS175, SMS188, SMS195, SMS201, SMS278, and SMS300 were not deleted for 17p11.2 probes.[4,21]

RAI1 mutation analyses

Polymerase chain reaction was performed to amplify patient DNA using overlapping primers covering the entire *RAI1* coding region spanning exons 3 to 6. Sequencing was performed with both forward and reverse primers to cover the entire amplimer. A detailed protocol of polymerase chain reaction and sequencing analyses was previously reported.[21] Primer sequences are available from the authors on request. Reactions for both the strands were repeated if any sequence variation was identified by the initial sequencing. Available parental samples were also sequenced for all identified mutations to confirm mutation was de novo. The chromatograms and sequence data were aligned to the *RAI1* mRNA database sequence at the National Center for Biotechnology Information (GenBank AY172136 and NM_030665) using Clustal X, version 1.83 (IGBMC, Strasbourg, France) or 4Peaks software (www.mekentosj.com).

Statistical analysis

Statistical analysis was performed with the JMP statistical software, version 5.1 (SAS Institute, Cary, NC). Comparison of proportions in two-way contingency tables was performed with the Fisher exact test using the *Fit Y by X* tool. All reported *P* values are two-tailed. The significance level was set at 5% (.05).

RESULTS

Clinical and molecular features of patients with 17p11.2 deletions

We evaluated 31 patients referred to us with phenotypic features consistent with SMS. These patients were initially analyzed for the 17p11.2 deletion by FISH (including the *RAI1* locus, RP1-253P07); all negative samples were then screened for mutations in *RAI1*. Samples that were previously evaluated using Vysis (Abbott Molecular Inc, Des Plaines, IL), Cytocell (Cytocell Technologies, Cambridge, UK), or Qbiogene (Qbiogene Inc, Irvine, CA) FISH probes, or by G-banding techniques were reevaluated with the *RAI1*-specific probe in our laboratory.[19]

Initial evaluation by FISH on metaphase chromosomes showed 17p11.2 deletions in 21 of 31 patients. These deletions were further analyzed by FISH using probes from the SMS region to determine the extent of the deletion in each case (Fig. 1). By using a collection of FISH probes mapping along chromosome 17p11.2, we determined that 8 of the 21 deletions were consistent with the 3.5 Mb SMS common deletion medi-

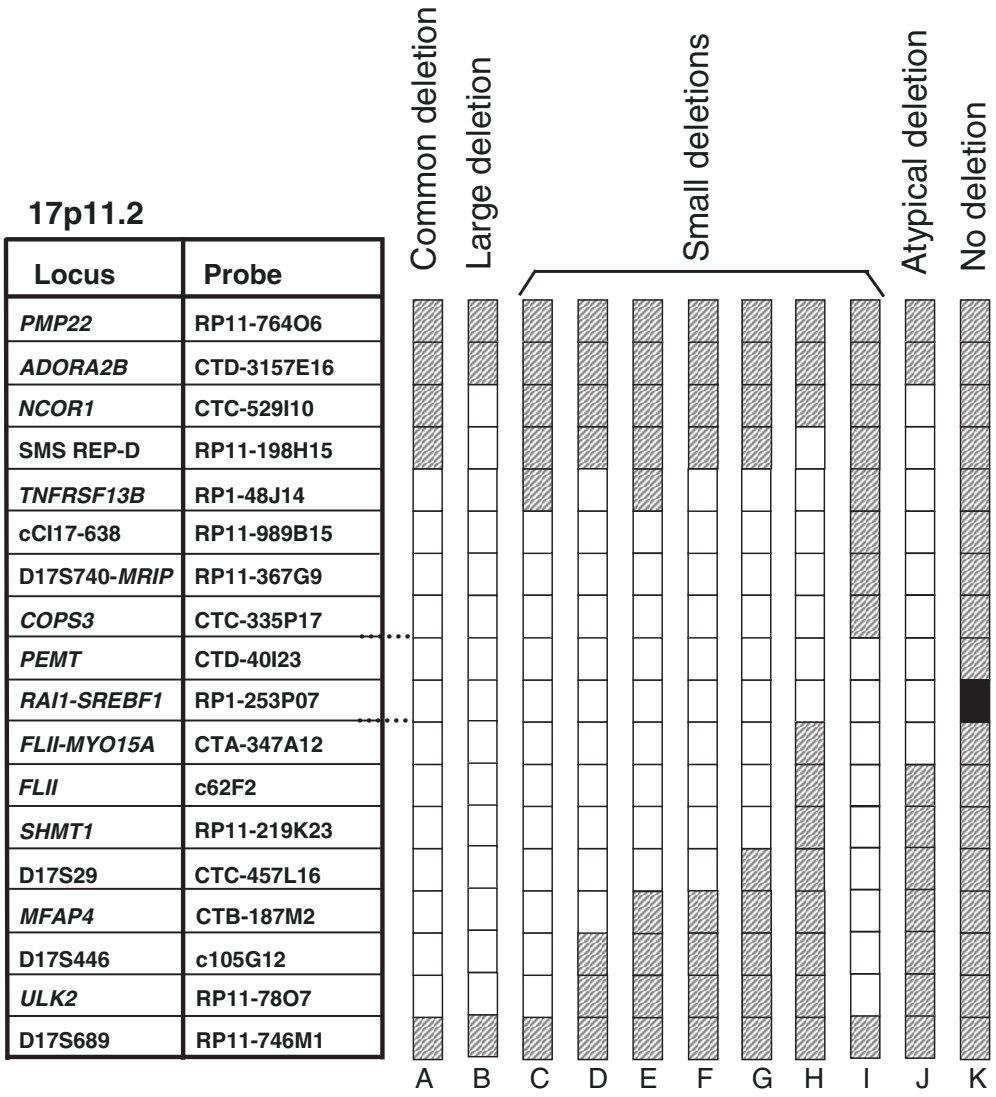

Fig. 1. 17p11.2 deletions identified in patients with Smith-Magenis syndrome. Deletion mapping using FISH probes representative of the loci in the region is shown.[4,18,19,21] The order of the clones is according to Lucas et al.[23] and confirmed by www.genome.ucsc.edu. 17p11.2 regions that are not deleted (hatched boxes); deletion of a particular probe/locus (open boxes); and *RAI1* (black box). (A) Common SMS deletion (~3.5 Mb) seen in 8 individuals (SMS102, SMS105, SMS108, SMS111, SMS118, SMS123, SMS126, and SMS131); (B) Large 17p11.2 deletion (SMS112, SMS116); C–J are unusual deletions: (C) SMS217; (D) SMS162, SMS167, and SMS170; (E) SMS109 and SMS143; (F) SMS146; (G) SMS149; (H) M2359; (I) SMS135; (J) SMS182; and (K) Patients not deleted for any 17p11.2 probe (10 cases, see Methods).

ated by SMS repeat sequences.[16,24,25] The common SMS deletion is inclusive of the 17p11.2 region from *TNFRSF13B* to *ULK2* (Fig. 1). Some deletions described here (SMS109, SMS116, SMS126, SMS135, SMS149, SMS182, and M2359) have been reported.[18,19]

Two patients, SMS112 and SMS116, have slightly larger deletions distally, spanning from *ADORA2B* (CTD-3157E16) to *ULK2* (RP11-78O7) (Fig. 1). SMS112 has severe mental retardation, intractable seizures, muscle weakness, pes planus, and abnormal gait, and was diagnosed with acute lymphocytic leukemia at 2.5 years of age (Tables 1 and 2). Although some signs of obsessive compulsive features are seen, aggressive behaviors are not apparent because she is severely affected to the extent that she is currently nonambulatory. SMS116 has hypothyroidism with increased thyroid-stimulating hormone levels and shortness of breath caused by a minimal infrahilar atelectasis of the right lung. SMS116 has distinctive facial features with puffiness of the eyes, high arched palate, and dental anomalies (Tables 1 and 2). Similar features and a more severe phenotype are also observed in one patient with an atypical deletion, SMS182 (Fig. 1, J; Tables 1 and 2).

Of the remaining 11 patients who have unusual deletions, 10 patients carry smaller 17p11.2 deletions and 1 patient carries an atypical deletion. SMS109, SMS143, SMS146, SMS149, SMS162, SMS167, SMS170, SMS217, SMS135, and M2359 have small deletions that map within the common deletion breakpoints (Fig. 1). An atypical deletion was identified in SMS182, where the deletion extends distally beyond the common deletion breakpoint (Fig. 1). The proximal breakpoint for SMS182 maps within the region between *FLII* and *LLGL1*

Table 1
Phenotypic comparison of Smith-Magenis syndrome patients with common, large, atypical, and small 17p11.2 deletions

Common features	17p11.2 deletion[a]	Common deletion[b,18]		Large deletion[b]		Atypical deletion[19]		Small deletion[b,18,19]	
	(%)[2,3,6,9,15,22,37,38]	Frequency	(%)	Frequency	(%)	Frequency	(%)	Frequency	(%)
Craniofacial/skeletal									
Brachycephaly	89	7/7	100	2/2	100	1/1	100	9/9	100
Midface hypoplasia	93	7/8	87.5	2/2	100	1/1	100	8/9	88.8
Prognathism (relative to age)	52	2/2	100	N	N	N	N	4/4	100
Tented upper lip	73	7/8	87.5	2/2	100	1/1	100	8/9	88.8
Broad, square face	81	8/9	88.8	2/2	100	1/1	100	7/9	77.7
Synophrys	62	2/9	22.2	N	N	1/1	100	3/9	33.3
Cleft lip/palate	9	0/9	0	0/2	0	0/1	0	0/9	0
Brachydactyly	85	7/9	77.7	2/2	100	1/1	100	8/8	100
Short stature	69	7/8	87.5	2/2	100	1/1	100	6/9	66.6
Scoliosis[39]	49–67	3/7	42.8	1/2	50	N	N	3/7	42.4
Otolaryngologic abnormalities									
Chronic ear infections	85	7/8	87.5	2/2	100	1/1	100	7/8	87.5
Hearing loss	68	5/7	71.4	2/2	100	1/1	100	5/8	62.5
Hoarse, deep voice	80	7/7	100	2/2	100	N	N	7/7	100
Neurologic/behavioral									
Variable mental retardation	100	8/9	88.8	2/2	100	1/1	100	9/9	100
Speech delay[39]	>90	8/8	100	2/2	100	1/1	100	9/9	100
Motor delay[39]	>90	8/8	100	2/2	100	1/1	100	9/9	100
Hypotonia	>90	9/9	100	2/2	100	1/1	100	9/9	100
Seizures by history	11–30	2/8	25	1/2	50	1/1	100	0/8	0
Sleep disturbance	70–100	8/8	100	2/2	100	1/1	100	9/9	100
Self-hugging/hand-wringing[40]	70–100	3/6	50	N	N	1/1	100	6/9	66.6
Attention-seeking	80–100	7/7	100	N	N	N	N	6/7	85.7
Self-injurious behaviors[40]	78–96	8/8	100	1/2	50	1/1	100	8/9	88.8
Onychotillomania	25–85	3/8	37.5	N	N	N	N	5/8	62.5
Polyembolokoilamania	25–85	5/8	62.5	1/2	50	N	N	6/8	75
Head-banging/face-slapping	71	6/8	75	1/2	50	1/1	100	6/9	66.6
Hand/self-biting/skin-picking	77	7/8	87.5	1/2	50	1/1	100	7/9	77.7
Ocular abnormalities									
Myopia	53	4/9	44.4	2/2	100	0/1	0	5/9	55.5
Strabismus	50	7/9	77.7	2/2	100	1/1	100	7/9	77.7
Other features									
Cardiovascular abnormalities	30	4/8	50	1/2	50	1/1	100	2/9	22.2
Renal/urinary tract abnormality	30	1/9	11.1	0/2	0	1/1	100	1/9	11.1

N, not evaluated.
[a]Percentages based on data from approximately 100 patients described previously and the Smith-Magenis syndrome GeneReviews 2005 (www.geneclinics.org).
[b]Patients reported in this study.

(CTA-347A12), and the deletion extends distally to 17p12 near the *ADORA2B* gene (Fig. 1).[19] Deletion data from SMS149, SMS135, M2359, and SMS182, and data from one patient reported in Schoumans et al. helped refine the smallest region of overlap among 17p11.2 deletions in SMS to 650 kb.[18–20] Table 1 compares SMS features in patients with common, large, small, and atypical deletions. Although SMS-specific features were consistently seen in all patients with deletions (Table 1 and Fig. 2), there were nonspecific features seen in some individuals. A summary of nonspecific clinical features in each patient with common, small, atypical, or large deletions is provided in Table 2.

Clinical and molecular analysis of patients with *RAI1* mutations

Ten patients were not deleted for the *RAI1* probe, RP1-253P07, and were further analyzed for mutations in the *RAI1* gene by sequencing (Tables 3 and 4). Seven of these patients have been described in previous reports.[4,21] In this study, we report three additional de novo mutations in the *RAI1* gene that cause SMS (Fig. 2, *top*). Detailed clinical and molecular information for these individuals is described in Figure 2 (*top*) and Tables 3 and 4. In addition, a number of polymorphisms were identified in the *RAI1* gene (Table 4). Each of these polymorphisms occurs in the normal population and is not thought to play a role in the SMS phenotype at this time.

SMS201 is a 12-year-old male (Fig. 2I). Early history included neonatal jaundice, poor suck, and gag reflex in addition to typical SMS features listed in Table 3. Biochemical tests revealed normal cholesterol levels at age 7 years. Magnetic resonance imaging of the brain showed calcification in the left occipital and posterior parietal cortex. Plagiocephaly resulting from the premature fusion of right lambdoid suture, with facial asymmetry with the right side fuller than the left, was noted. He has a heterozygous deletion of a cytosine at nucleotide position 1119 in the *RAI1* gene (Fig. 2, *top left*; Table 4). This deletion causes a frameshift starting at amino acid 373 leading to misincorporation of 65 amino acids and a premature stop codon. This mutation was not seen in parental samples, and it has not been observed in more than 100 normal chromosomes.

Table 2
Nonspecific clinical features in patients with 17p11.2 deletion[a]

Common deletions	
SMS102	Fetal bradycardia
	Enlarged liver and pancreas
	Hypertelorism, thick ear lobes
	Trichotillomania
SMS105	Bronchial stenosis
	Laryngeal cleft, laryngomalacia
	Sinusitis
	Palpable liver, liver fibrosis
	Hypoglycemia
	Myopathy
	Cardiomegaly
	Macular degeneration/optic nerve hypoplasia
	Micrognathia
	Enlarged liver and pancreas
	Hypertelorism, thick ear lobes
	Trichotillomania
SMS108	Dry skin
	Gastroesophageal reflux
	Sleep apnea with breathing difficulties
SMS111	Cephalohematoma at birth
	Cranial asymmetry and scoliosis of skull
	Dental abnormalities
SMS118	Heart valvular abnormalities
SMS123	Tracheomalacia
	Constipation
	Undescended testis
SMS126	Elevated 7-dehydrocholesterol
	Retinal detachment
	Glaucoma
	VSD with subvalvular pulmonic stenosis
	Muscular weakness
SMS131	Decreased IgA levels
	Sinusitis
	Tremors at birth
	Abnormal gait
	Constipation
Small deletions	
SMS109	Oligohydramnios
	Pulmonic stenosis
	Trichotillomania
	Loose bowel movements
SMS143	Flat feet
	Waddling gait
SMS146	2-3 toe syndactyly
	Abnormal gait
	Constipation
SMS149	Flat feet
	Endocardial cushion defect
	Undescended testis
	Frequent urinary tract infections
	Wolfflin Kruckman spots in irides
	Cerebellar hypoplasia
	Enlarged cisterna magna
SMS135	Recurrent pneumonitis
	Pulmonary infiltrates
	Obstructive sleep apnea
	Hypopnea
	Micrognathia
Atypical deletion	
SMS182	Undescended testis
	Complex partial seizures
	Gastroesophageal reflux
	Hypothyroidism
	Hypercholesterolemia
	Chronic constipation
	Cyanotic spells attributable to apnea
	Asthma
	Elevated IgG levels
Large deletions	
SMS112	Flat feet
	Abnormal gait
	Myopathy
	Decreased ambulation
	Acute lymphocytic leukemia
SMS116	Gastroesophageal reflux
	Decreased IgA levels
	Sinusitis
	Constipation
	Elevated TSH, hypothyroidism
	2–3 toe syndactyly
	Dental anomalies
	Periorbital fullness

VSD, ventricular septal defect; Ig, immunoglobulin; TSH, thyroid-stimulating hormone; SMS, Smith-Magenis syndrome.
[a]Nonspecific clinical features are seen in some of patients with SMS: Patients not listed here exhibit only the characteristic SMS features and/or have not been fully evaluated for other manifestations.

SMS278 is a 13-year-old girl with brachydactyly, sleep disturbance, and prognathism in addition to other features consistent with SMS (Table 3; Fig. 2G). She also exhibits self-injurious behaviors such as nail-pulling and skin picking to the point of bleeding. SMS278 has a cytosine deletion at nucleotide 4649 that leads to misincorporation of 36 amino acids before being terminated by a stop codon (Fig. 2, *top right*; Table 4). Parental samples did not carry this single base pair deletion nor did more than 100 control chromosomes.

SMS300 is a 5-year-old female born at 33 weeks gestation. She was reported as hirsute as a neonate. She had mild developmental delay, walked at 2 years of age, and spoke her first words at 3.5 years. She was uninterested and passive and slept only 3 hours each night. Her behavior was described as "demanding," and she picked at her gums and around her fingernails. As a toddler, she would poke her genitalia with sticks and toys, but this has now subsided. In addition to typical SMS features, she has 2-3 toe syndactyly, mild clinodactyly of fifth fingers, and lax joints. On sequencing the *RAI1* gene, SMS300 showed a heterozygous deletion of four bases, GCCG, starting from nucleotide 4933 that leads to misincorporation of 35 amino acids before premature termination by a stop codon. Neither parent carried this DNA change (data not shown; Table 4). This intragenic deletion was not seen in our sequencing analysis of more than 100 control chromosomes.

Genotype–phenotype analyses

We compared 30 characteristics commonly associated with SMS in individuals with 17p11.2 deletions to those individuals with *RAI1* mutations. All features were compared and scored as present, absent, not relevant for age, or data not available. Some features often associated with SMS, such as hypercholesterolemia, could not be evaluated in our study because of in-

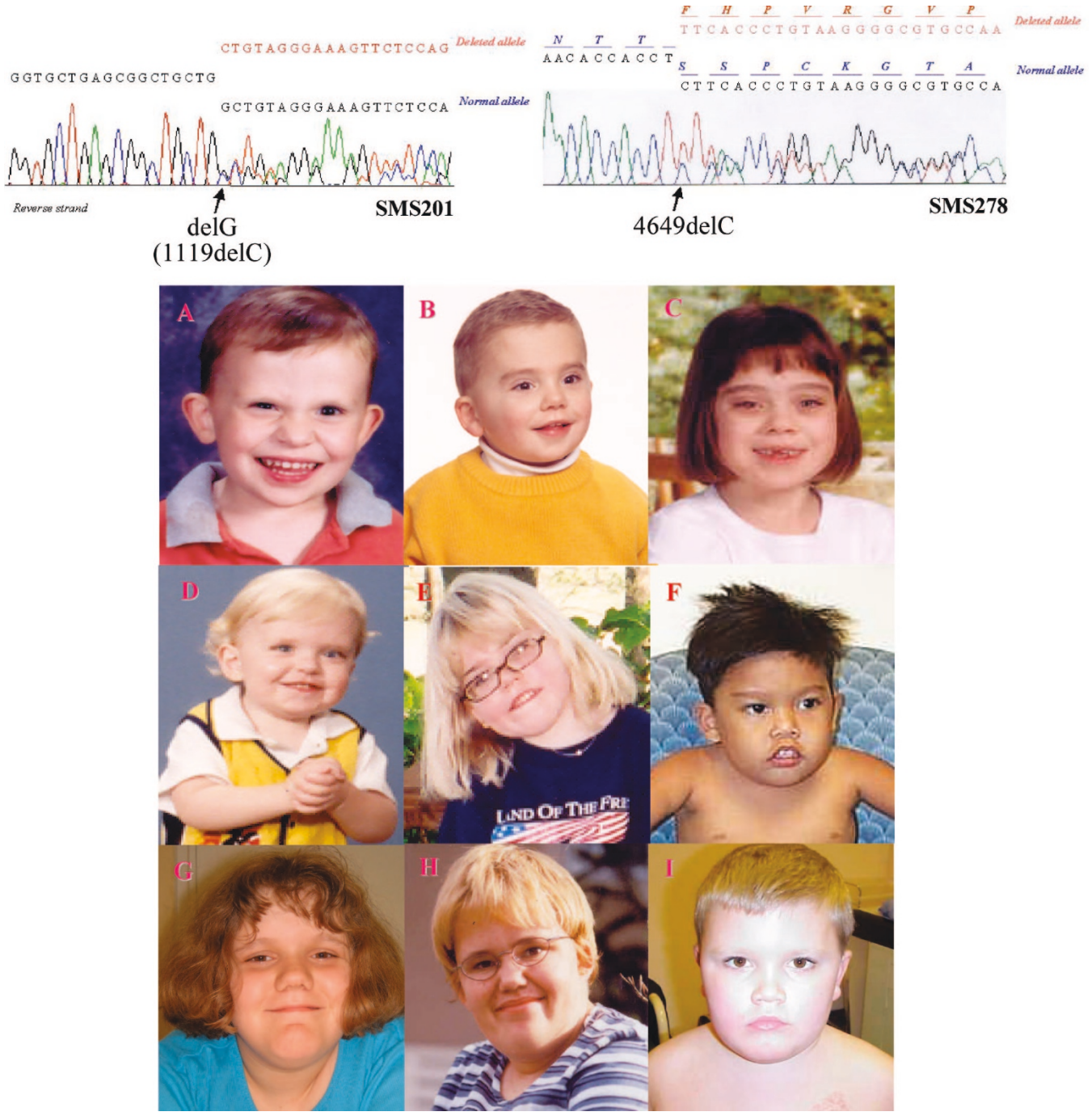

Fig. 2. *Top:* Representative chromatograms of *RAI1* sequencing. SMS201 (*left*) has a deletion of cytosine 1119, just distal to the polyglutamine tract in exon 3 that results in a frameshift leading to misincorporation of 65 amino acids ultimately terminated by a stop codon. Reverse strand sequence is shown, as the forward sequence analysis is obstructed by polymorphic (CAG) repeats. SMS278 (*right*) carries a deletion of cytosine 4649 in exon 3 that leads to misincorporation of 36 amino acids and a premature stop codon. Sequence for the normal (*black*) and deleted (*red*) alleles are shown. (*Bottom:*) Patients with SMS who have common or unusual 17p11.2 deletions or *RAI1* mutations . A–C show individuals with common deletions: (A) SMS111 at 5 y; (B) SMS123 at 3 y. (C) SMS167 at 8.5 y (has a deletion slightly smaller that common deletion, see also Fig. 1.) D–F show individuals with unusual 17p11.2 deletions: (D) SMS143 at 2 y; (E) SMS109 at 8 y; (F) SMS135 at 3 y 8 mon. G–I show individuals with *RAI1* mutations: (G) SMS278 at 13 y; (H) SMS153 at 15 years; (I) SMS201 at 6 y.

sufficient data. Results indicate that most (21/30) phenotypic features of SMS are consistent in both deletion and *RAI1* mutation cases (Table 3); however, some key differences exist, as shown in Figure 3, including the prevalence of speech and motor delays, cardiac anomalies, short stature, hypotonia, and hearing loss. Renal and genitourinary tract anomalies, although not present in *RAI1* mutation cases, were not significantly different between the two groups (Table 3).

Short stature is more pronounced in the patients with deletion (80%), and this frequency is significantly different from that in patients with mutations in *RAI1* (9%) ($z = 3.61$, $P = .0003$) (Fig. 3). Most patients (16/20) with deletions have heights <5th centile

Table 3
Phenotypic comparison between Smith-Magenis syndrome patients with 17p11.2 deletion and *RAI1* mutation

Common features	17p11.2 deletion[a]	17p11.2 deletion[b]		*RAI1* mutation[b,4,21,41]		Fisher exact test
	(%)[2,3,6,9,15,22,37,38]	Frequency	(%)	Frequency	(%)	*P* value
Craniofacial/skeletal						
Brachycephaly	89	19/19	100	9/11	81.8	.12
Midface hypoplasia	93	18/20	90	8/11	72.7	.31
Prognathism (relative to age)	52	6/6	100	8/9	88.8	1
Tented upper lip	73	18/20	90	11/12	91.6	1
Broad, square face	81	18/21	85.7	10/11	90.9	1
Synophrys	62	6/19	31.5	3/9	33.3	1
Cleft lip/palate	9	0/21	0	0/12	0	1
Brachydactyly	85	18/20	90	10/12	83.3	.6
Short stature	69	16/20	80	1/11	9	.0003[c]
Scoliosis[39]	49–67	7/17	41.1	4/11	36.3	1
Otolaryngologic abnormalities						
Chronic ear infections	85	17/19	89.4	6/11	54.5	.068
Hearing loss	68	13/19	68.4	1/10	10	.005[c]
Hoarse, deep voice	80	16/16	100	8/8	100	1
Neurologic/behavioral						
Variable mental retardation	100	20/21	95.2	12/12	100	1
Speech delay[39]	>90	20/20	100	7/10	70	.029[c]
Motor delay[39]	>90	20/20	100	7/10	70	.029[c]
Hypotonia	>90	21/21	100	5/9	61	.0045[c]
Seizures by history	11–30	4/19	21	2/12	16.6	1
Sleep disturbance	70–100	20/20	100	12/12	100	1
Self-hugging/hand-wringing[40]	70–100	10/18	55.5	11/12	100	.049[c]
Attention-seeking	80–100	13/16	81.25	12/12	100	.24
Self-injurious behaviors[40]	78–96	18/20	90	12/12	100	.52
Onychotillomania	25–85	8/20	40	8/10	80	.057
Polyembolokoilamania	25–85	12/19	63.1	9/10	90	.2
Head-banging/face-slapping	71	14/20	70	6/10	60	1
Hand-biting/self-biting	77	16/20	80	7/10	70	1
Ocular abnormalities						
Myopia	53	11/21	52.3	6/10	60	1
Strabismus	50	17/21	80.9	4/10	40	.055
Other features						
Cardiovascular abnormalities	30	8/20	40	0/11	0	.027[c]
Renal/urinary tract abnormality	30	3/20	15	0/11	0	.53

[a]Percentages based on data from approximately 100 patients described previously and the Smith-Magenis syndrome GeneReviews 2005 (www.geneclinics.org).
[b]Patients reported in this study.
[c]Significant *P* values.

and weights between <5th centile to <75th centile, depending on the age at evaluation. Similarly, head circumference for age in patients with deletions ranges from <3rd centile to <50th centile. Typically, the growth curves in these patients are initially <5th centile but gradually increase to the 10th to 25th centile with age (data not shown). In contrast, 8/9 patients with *RAI1* mutations have heights at or above the 75th centile, and 7/9 of these individuals are obese (weight >97th centile), suggesting that patients with overgrowth phenotypes should be evaluated for mutations in *RAI1*.

Speech delay ($P = .029, z = 2.18$) and motor delay ($P = .029$, $z = 2.18$) contribute to a more severe phenotype in patients with 17p11.2 deletion compared with those with *RAI1* mutations (Fig. 3). Similarly, hypotonia during infancy is seen at a higher frequency in patients with deletion (100%) compared to patients with mutation (61%) ($P = .0045, z = 2.84$) (Fig. 3). Hearing loss in patients with a 17p11.2 deletion (68.4%) occurred in higher proportion compared with those with *RAI1* mutations (10%) ($P = .005, z = 2.80$) (Fig. 3). No patient with an *RAI1* mutation ($P = .027$) has any observable cardiovascular abnormalities. Comparatively, 40% of the patients with deletion have either a structural or a functional congenital cardiac defect. Similarly, 15% of all patients with a 17p11.2 deletion have a renal or urinary tract abnormality, whereas none of the patients with mutations exhibit this feature (0/11) (Table 3).

Chronic ear infections ($P = .068$) and strabismus ($P = .055$) were also more frequent in patients with deletion; however, these data were not statistically significant. Frequencies of other SMS features such as frontal bossing (8/19 and 3/9), muscular weakness (5/19 and 3/9), and sinusitis (3/19 and 2/9) in individuals with 17p11.2 deletions and those with mutations, respectively, were not significantly different.

DISCUSSION

We evaluated molecular and clinical data for 31 patients with SMS who carry either a 17p11.2 deletion or a mutation in the *RAI1* gene. We then analyzed 30 distinctive SMS features in individuals with deletions and compared them with individu-

Table 4
Mutations and polymorphisms in the *RAI1* gene

	Nucleotide change	Amino acid change	Remarks
Mutations	253del19[21]	Misincorporation of 60 amino acids	Deletion
	1119delC	Misincorporation of 65 amino acids	Deletion *(this report)*
	1449delC[4]	Misincorporation of 34 amino acids	Deletion
	2773del29[4]	Misincorporation of 8 amino acids	Deletion
	C2878T[41]	Arg960Stop	Nonsense mutation
	3103insC[41]	Misincorporation of 30 amino acids	Insertion
	3801delC[21]	Misincorporation of 46 amino acids	Deletion
	4649delC	Misincorporation of 36 amino acids	Deletion (*this report*)
	A4685G[21]	Gln1562Arg	Missense mutation
	4933delGCCG	Misincorporation of 35 amino acids	Deletion (*this report*)
	G5423A[21]	Ser1808Asn	Missense mutation
	5265delC[4]	Misincorporation of 74 amino acids	Deletion
Polymorphisms	G269C [21,41]	Gly90Ala	SNPrs3803763
	C493A[21,41]	Pro165Thr	SNPrs11649804
	G837A[21,41]	Gln279Gln	SNPrs11078398
	G1992A[21,41]	Pro664Pro	SNPrs8067439
	G5334A[21]	Arg1778Arg	
	T5601C[21,41]	Ile1867Ile	SNPrs3818717
	PolyQ (CAG/CAA)[21,41]	9–15 repeats, no expansions seen	

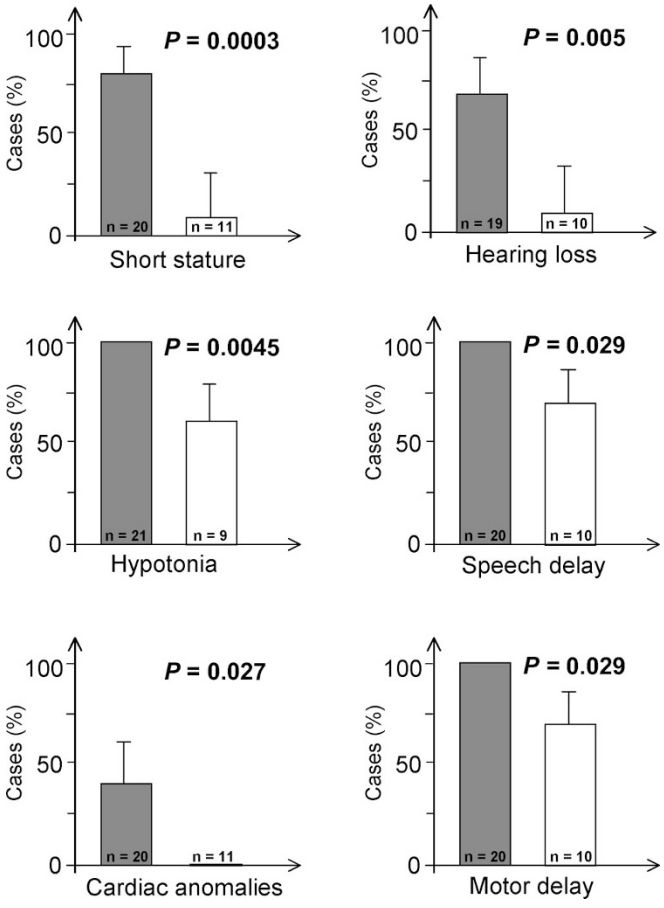

Fig. 3. Histograms representing significant phenotypic differences between *RAI1* and 17p11.2 deletion cases: 17p11.2 deletion data (*gray bar*) and *RAI1* mutation data (*white bar*). Proportions were compared using the Fisher exact test. *P* values are two-tailed. *P* values less than .05 are significant, and 95% confidence levels for the two groups are indicated. Also refer to Table 3.

als with mutations. The results presented in this study indicate that sleep disturbance, mental retardation, and neurobehavioral features are consistent in all patients evaluated (Tables 1 and 3). However, short stature, hearing loss, speech and motor delay, hypotonia, and cardiovascular anomalies are associated with 17p11.2 deletions rather than with *RAI1* mutations (Table 3, Fig. 3). Our analysis indicates that *RAI1* has little or no role in features such as cardiac and renal anomalies in SMS. A minor role can be attributed to *RAI1* in hearing loss, hypotonia, and speech and motor delay because the incidence of these features is substantially higher than in the general population. On the other hand, given that the only patient with short stature in the *RAI1* cohort has growth hormone deficiency,[21] the incidence of short stature in individuals with *RAI1* mutation is actually lower than the population incidence (5%), which would suggest that mutations in *RAI1* can lead to overgrowth phenotypes.

Analysis of phenotypic features correlating with small, large, and atypical deletions showed that specific regions of 17p11.2 likely contribute to certain more variable features, thus implicating genes in these associated regions (Fig. 4). Our data suggest that the chromosomal region contributing to short stature is located between *SREBF1* and D17S29 ($P = .006$) (Fig. 4). This implies that dosage of gene(s) mapping to 17p11.2 in addition to *RAI1* may affect growth.

Hearing loss in SMS is variable and may be mild to moderate (conductive) or severe (sensorineural). Mild to moderate loss is often associated with chronic middle ear infections, whereas severe hearing loss is more likely the result of a genetic defect.[2,3,10] Data show that the SMS region contributing to hearing loss lies in the region encompassing *LLGL1* and *FLCN* ($P = .046$) (Fig. 4). Profound sensorineural hearing loss has been reported in patients with SMS whose deletions unmask recessive mutations in *MYO15A*, which is located within this genomic region.[11] Thus, it is possible that mutations or poly-

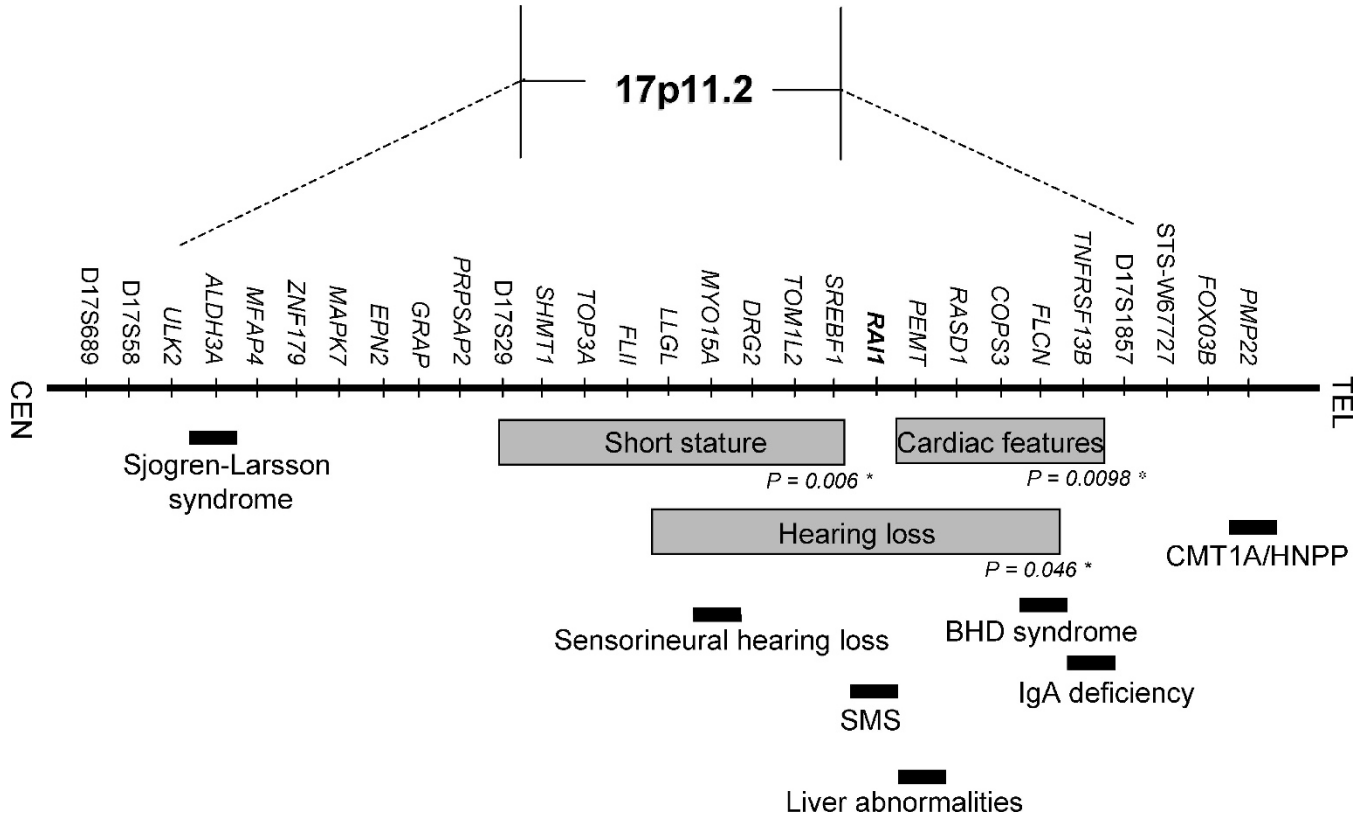

Fig. 4. Contribution of genes/regions on chromosome 17p11.2 toward the variable features in Smith-Magenis syndrome (SMS). The contributory genes/regions (*gray phenotypic blocks*) were deduced by comparing the deletion breakpoints of common, large, small, and atypical deletions and correlating them with the patient's SMS features (Fig. 1). *P* values were obtained by comparing the presence or absence of a feature in patients with small deletions and *RAI1* mutations (short stature, 7/20; hearing loss, 6/18; and cardiac features, 2/21) with those with common, large, and atypical deletions (short stature, 10/11; hearing loss, 8/10; and cardiac features, 6/11) by using the Fisher exact test. *Significant values were set at $P < .05$. Information about a known gene from published sources (*black bars*), including immunoglobulin-A deficiency (*TNFRSF13B*),[31] Birt-Hogg-Dube syndrome (*FLCN*),[28,29] Sjogren-Larsson syndrome (*ALDH3A*),[32] sensorineural hearing loss (*MYO15A*),[34] fatty liver and liver abnormalities (*PEMT*),[33] Charcot-Marie-Tooth disease (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP),[35,36] and the core SMS features (*RAI1*).[4,21,41]

morphisms in the remaining copy of *MYO15A* contribute to hearing loss in some individuals with SMS. In our study, approximately 10% of individuals with *RAI1* mutations exhibit hearing loss of unknown cause (Table 3). This may be secondary to chronic ear infections necessitating pressure equalizing tubes in most of these patients. Further analysis of the cause of hearing loss in SMS is required to more fully understand the potential genetic contribution.

In our patient cohort, self-injurious behaviors are seen as early as 12 to 18 months, similar to that reported by Greenberg et al.[2] The incidence of these behavioral features is lower in patients with large and atypical deletions (whose deletions extend to 17p12), likely because of the severity of the phenotype seen in these individuals, including severe mental retardation and significant motor delays requiring full-time care. Individuals with large and atypical deletions (extending distally) are usually more severely affected than those with small deletions. Supporting this, Yamamoto et al.[26] described a patient with SMS carrying a very large deletion with severe cardiac, pulmonary, and renal anomalies leading to infantile lethality. In addition, Natacci et al.[27] described a patient with a large 17p11.2 deletion presenting with SMS and Joubert syndrome who had profound mental retardation, severe language delay, and retinal detachment along with cerebellar vermis hypoplasia. Our analysis further suggests that genes distal to *RAI1* may contribute to the cardiac phenotype ($P = .0098$) (Fig. 4).

It is interesting to note that individuals with smaller deletions have features that are similar to those with mutations. For example, patients with smaller 17p11.2 deletions do not have cardiac or renal anomalies and their phenotypes closely resemble those of *RAI1* cases. Schoumans et al.[20] reported one patient with an unusual but small deletion encompassing *RAI1* with no cardiac/renal anomalies, lending support to our findings. Similarly, patients with small deletions have a lower incidence of short stature compared with those with the common or large 17p11.2 deletions (Table 1).

Certain features common to SMS are age-dependent and thus were not fully evaluated. A hoarse, deep voice is evident in individuals only after the development of speech and is not completely documented in all of our patients with SMS. Similarly, scoliosis seen in older children and prognathism seen in postpubertal patients cannot be completely evaluated because most of our patients are prepubertal. Likewise, cholesterol levels (typically elevated in SMS) were not mea-

sured in most of our patients.[15] Cleft lip and/or cleft palate, reported previously[1,3] in patients with SMS deletion, were not found in our study cohort.

All SMS patients with a 17p11.2 deletion are deleted for *RAI1*. Mutations in *RAI1* likely result in a truncated and/or nonfunctional protein resulting in haploinsufficiency.[4,21] The presence of common SMS features in both patients with deletion or intragenic mutation strongly suggests that these features are the result of functional abrogation of RAI1. Our study shows that 21 of 30 distinct SMS features are solely the result of *RAI1* mutations. Other genes in the deletion interval likely account for the variable features in SMS. Some genes in the SMS deletion interval are already implicated in various disorders (Fig. 4). Further, hemizygosity caused by heterozygous 17p11.2 deletion can lead to the unmasking of autosomal recessive alleles in the region, leading to the possibility of an individual actually having two distinct genetic disorders. The Birt-Hogg-Dube syndrome gene, folliculin (*FLCN*), maps within the SMS region, and dominant mutations cause benign skin tumors, renal tumors, and primary spontaneous pneumothorax.[28,29] The function of folliculin (*FLCN*) is still unknown, and further studies are required to decipher its role in tumors and SMS (if any). Similarly, decreased immunoglobulin-A levels reported in the literature,[1,3] and in some individuals with deletions in this study (Table 2), can also be caused by mutations in the *TNFRSF13B* gene that encodes transmembrane activator and calcium modulator and cyclophilin ligand interactor (Fig. 4).[30,31] The *ALDH3A* locus maps proximal to *RAI1* within the SMS common deletion breakpoints, and mutations in this fatty aldehyde dehydrogenase gene cause a neurocutaneous disease called Sjögren-Larsson syndrome.[32] Patients with SMS who carry deletions have a higher incidence of dry skin (11/19) than those with mutations (1/9, $P = .2$) suggesting a possible role of *ALDH3A* in causing this feature (Fig. 4). Fatty liver and ensuing liver abnormalities seen in some SMS cases (Table 2) may be attributed to alterations in the phosphatidylethanolamine N-methyltransferase (*PEMT*) gene. Polymorphisms in *PEMT* are associated with nonalcoholic fatty liver disease (Fig. 4),[33] and further study is required to determine whether PEMT has a role in liver abnormalities seen in some SMS cases. Further studies on each of these genes in patients with SMS who carry a 17p11.2 deletion will be required to determine whether any or all of these genes contribute to the variability of the syndrome.

The incidence of SMS is reported to be 1 in 25,000,[3] but with increasing awareness and proper diagnosis, the incidence is expected to be higher. Many of our patients were initially evaluated for Fragile X, DiGeorge, Down, or Prader-Willi syndromes, and the diagnosis of SMS was only considered much later. Although we conclude that haploinsufficiency of *RAI1* is responsible for most of the SMS features, the involvement of other genes cannot be ruled out because severity of the phenotype increases with increased deletion size. It is also possible that other genes elsewhere in the genome might play a role in altering the functional availability of RAI1 for downstream effects. Further investigation of additional genes in the region is required to determine the role they play in modification of the features and/or severity of the SMS phenotype.

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