Mutations in *RAI1* associated with Smith–Magenis syndrome

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Smith–Magenis syndrome (SMS) is a mental retardation syndrome associated with deletions involving chromosome 17p11.2. Persons with SMS have characteristic behavioral abnormalities, including self-injurious behaviors and sleep disturbance, and distinct craniofacial and skeletal anomalies. We identified dominant frameshift mutations leading to protein truncation in *RAI1* in three individuals who have phenotypic features consistent with SMS but do not have 17p11.2 deletions detectable by standard fluorescence *in situ* hybridization techniques.

Smith–Magenis syndrome (SMS) is a syndrome of multiple congenital anomalies and mental retardation that encompasses unusual behavioral abnormalities, sleep disturbance, distinct craniofacial and skeletal anomalies and speech delay[1–3]. Most persons with SMS have a large and common deletion of roughly 4 Mb involving chromosome 17p11.2, but many other affected individuals have smaller, atypical deletions involving 17p11.2 (refs. 4–7). Thorough evaluation of affected individuals and their associated deletions has led to several recent studies documenting and refining the SMS critical interval[4,6,8]. Our most recent evaluation of the critical interval indicates that the minimum deletion region on 17p11.2 associated with the SMS phenotype is about 950 kb and contains roughly 25 genes[7]. An abbreviated contig containing several known genes in the SMS critical interval is represented in Figure 1*a*. Although SMS has generally been regarded as a contiguous gene syndrome[1,4,6,9], we hypothesized that individuals with features consistent with SMS

Table 1 • Phenotypic comparison of individuals with SMS

	Individual SMS126 (typical deletion)	Individual HOU142-540 (small deletion)	Individual SMS129 (29-bp deletion)	Individual SMS156 (4929delC)	Individual SMS159 (1308delC)
Features common in >75%of individuals with SMS					
Craniofacial/skeletal					
Brachycephaly	–	–	+	+	+
Midface hypoplasia	+	+	–	–	–
Prognathism (relative to age)	+	+	+	+	+
Tented upper lip	+	+	+	+	+
Broad, square face	+	–	+	+	–
Brachydactyly	+	–	–	+	–
Short stature	+	+	–	–	–
Otolaryngological					
Chronic ear infections	+	+	–	+	+
Hoarse, deep voice	+	+	+	–	+
Neurological					
Mental retardation	+	+	+	+	+
Speech delay	+	+	+	–	–
Motor delay	+	–	–	–	+
Infantile hypotonia	+	–	–	–	+
Sleep disturbance	+	+	+	+	+
Behavioral					
Self hugging	+/–	+	+	+	+
Onychotillomania	+	+	+	+	+
Polyembolokoilomania	+	+	+	+	+
Head banging/face slapping	+	+	+	–	+
Hand biting	+	+	+	+	–
Attention seeking	+	+	+	+	+
Features common in 50–75% of individuals with SMS					
Hearing loss	+	+	–	+	–
Ocular abnormalities					
Myopia	+	+	+	+	–
Strabismus	+	+	+	–	+
Iris abnormalities	+	+	–	–	–
Synophrys	–	+	+	+	–
Scoliosis	+	–	+	–	+
Features common in <50% of individuals with SMS					
Cardiovascular anomalies	+	+	–	–	–
Renal anomalies	–	+	–	–	–
Seizures	–	+	–	–	+
Cleft lip or palate	–	–	–	–	–
Gender	Female	Male	Male	Female	Male
Age at evaluation (y)	41	10, 14, 15	14, 30	17, 21, 31	13, 19

Phenotypic comparison of individuals affected with SMS that had a common deletion (individual SMS126) or a smaller than normal deletion (individual HOU142-540) with affected individuals that did not have any deletions (individuals SMS129, SMS156 and SMS159). The features of individuals without deletions most closely resemble those of individual HOU142-540, who was diagnosed at the same facility. The contents of this table have been modified from the GeneReviews online database. +, the feature is present; –, the feature is not present. This study was approved by the Michigan State University Committee on Research Involving Human Subjects and informed consent was obtained from all individuals participating in this study. See Supplementary Note 1 online for additional clinical information.

but in whom no deletion could be detected may have a mutation in a single candidate gene that is primarily responsible for the phenotype.

We identified three such individuals without detectable deletions involving 17p11.2. These individuals were all residents at a facility that has diagnosed over two dozen individuals with SMS and is well known for its work in describing the clinical phenotype. All three individuals were evaluated clinically through the facility's genetics department on multiple occasions (Table 1 and Supplementary Note 1 online). Based on a strong clinical suspicion of SMS, we received samples for further analysis when initial cytogenetics and fluorescence *in situ* hybridization (FISH) studies for the 17p11.2 deletion were negative. These three individuals, SMS129, SMS156 and SMS159, have many of the typical features of SMS, including craniofacial abnormalities, sleep disturbances and characteristic behaviors, such as onychotillomania (self-mutilation of finger- and toenails), polyembolokoilomania (insertion of foreign objects into body orifices), spasmodic self-hugging and explosive aggressive episodes (Table 1). These individuals were further evaluated for sub-microscopic deletions in 17p11.2 using an extensive series of FISH probes that span the SMS critical region (see Supplementary Note 2 online). Although our FISH analyses do not eliminate the possibility of a small, cryptic deletion in 17p11.2, they do confirm that individuals SMS129, SMS156 and SMS159 do not have deletions of the SMS critical region.

Next we undertook systematic sequencing of genes in individual SMS129 beginning with three genes localized to the SMS critical interval[4,6,8]: developmentally regulated GTP binding protein 2 (*DRG2*), RAS dexamethasone-induced 1 (*RASD1*) and retinoic acid induced 1 (*RAI1*). We identified no mutations in either *RASD1* or *DRG2* but found a deletion of 29 bp in exon 3 of *RAI1* on one allele in individual SMS129 (Fig. 1*b*). This deletion was

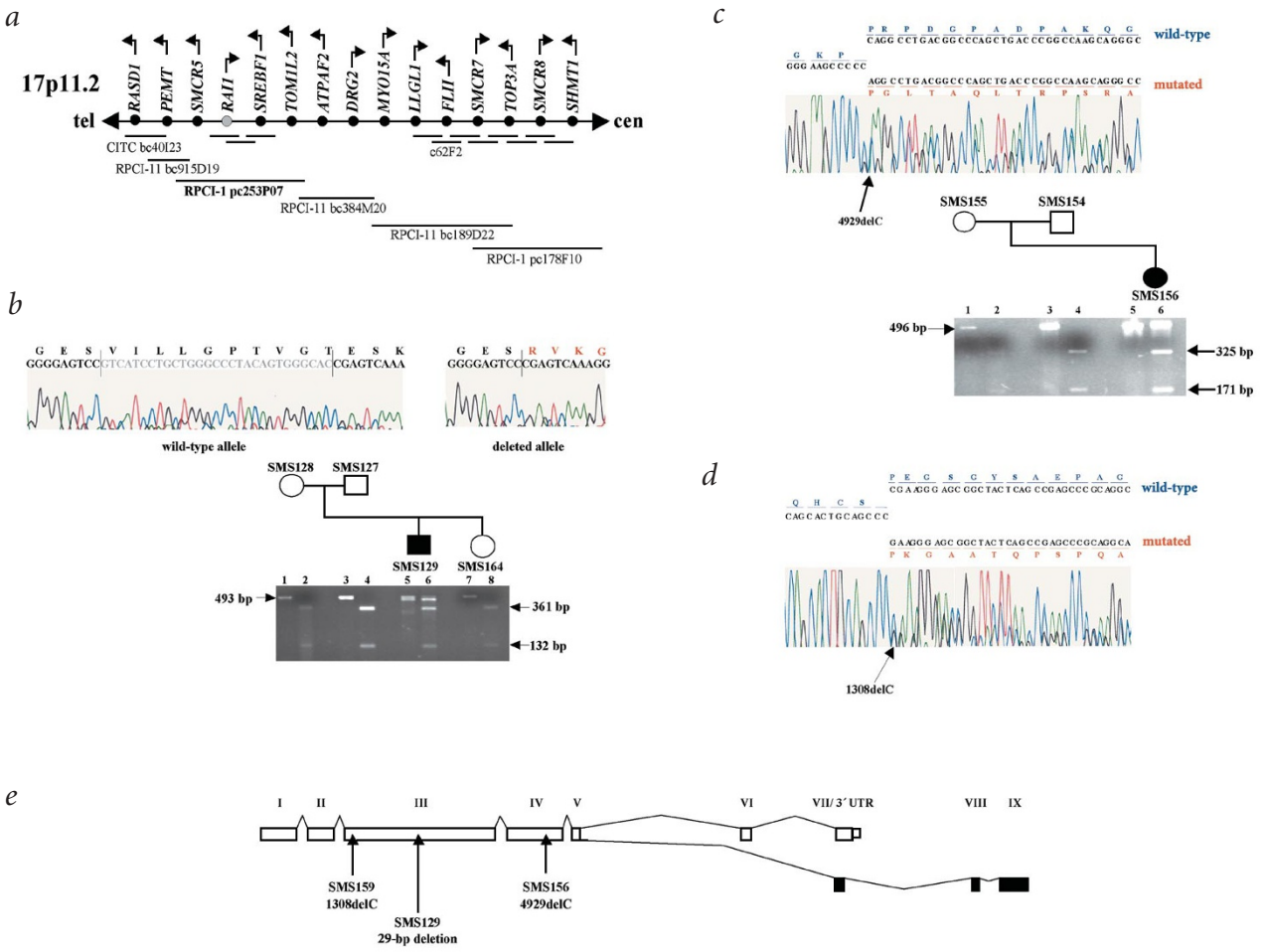

Fig. 1 Mutation analysis and genomic structure of *RAI1*. ***a***, A minimum tiling path of BACs and PACs (solid lines) for the newly refined SMS critical interval[7] and cosmids (dotted lines) used for FISH analysis are depicted. Major genes in the SMS critical region are indicated by black circles; *RAI1* is indicated by a gray circle. ***b***, The sequences of the deleted and wild-type *RAI1* alleles of individual SMS129 are shown, beginning at nt 2,622 of the *RAI1* mRNA. The 29-bp deletion, which eliminates a *Psp*OMI recognition site, is highlighted in gray between the solid lines, and four misincorporated amino acids are shown in the deleted allele. The two bands representing the deleted and non-deleted alleles in individual SMS129 were amplified by PCR and sequenced separately. The gel below the pedigree of the family of individual SMS129 shows the exon 3 PCR amplimer (lanes 1, 3, 5, 7) and the digestion of this PCR product with *Psp*OMI (lanes 2, 4, 6, 8). The doublet in the PCR product from individual SMS129 and the uncut, mutated allele evident in the digest are not present in the parents or sister of the affected individual. ***c***, The sequence tracing for the 4929delC deletion and the predicted frameshift on one *RAI1* allele in individual SMS156 are shown. A *Bgl*I recognition site is abolished by this 4929delC mutation. The results of the exon 4 PCR (lanes 1, 3, 5) and the digestion of this PCR amplimer with *Bgl*I (lanes, 2, 4, 6) are represented in the gel shown beneath the family pedigree of individual SMS156. The undigested, mutated allele in individual SMS156 is not present in the parents. ***d***, The sequence tracing for the 1308delC deletion and the predicted frameshift on one *RAI1* allele in individual SMS159 is depicted. The parents of individual SMS159 and the 200 control chromosomes analyzed by sequencing of the exon 3 PCR product do not carry this mutation (data not shown). ***e***, The genomic structure for the primary transcript of *RAI1* and a major splice variant, as determined by the UCSC genome browser, are shown. Oligonucleotide sequences and specific PCR conditions are available on request.

clearly evident as two bands resolved on a 2% agarose gel representing the deleted and non-deleted alleles (Fig. 1*b*). The deletion produces a frameshift (Fig. 1*b*) that introduces eight incorrect amino acids followed by a stop codon, truncating the protein. We predict that this deleted allele encodes a truncated and either abnormally functioning or non-functioning RAI1 protein, probably resulting in haploinsufficiency for RAI1.

We then examined individuals SMS156 and SMS159 for mutations in *RAI1*. In both individuals we identified a deletion on one allele of a single cytosine in a run of Cs (Fig. 1*c*,*d*). In individual SMS156, the deleted C occurred in a run of six Cs ending at nucleotide position 4,929 of the *RAI1* mRNA. This 4929delC on the coding strand produces a frameshift, introducing 74 incorrect amino acids and truncating the protein (Fig. 1*c*). Similarly, we found a deletion on one allele of a single cytosine in a run of four Cs ending at nucleotide position 1,308 in exon 3 of the *RAI1* (Fig. 1*d*) in individual SMS159. This deletion also causes a frameshift that incorporates 34 incorrect amino acids beginning at amino-acid position 437, followed by a stop codon and truncation of the protein. None of the parents of these individuals carried any of these mutations (Fig. 1*a–c* and data not shown), although we cannot rule out mosaicism in the germ cells. We also screened 200 control chromosomes from individuals of European descent and did not detect these mutations in this population (data not shown). The genomic structure of *RAI1* and location of the three mutations in the primary transcript are shown in Figure 1*e*. (See Supplementary Note 3 for the complete *RAI1* sequence.)

RAI1 is a novel gene whose cellular role is still unclear. *Rai1* was first identified in mouse carcinoma cells when it was upregulated following treatment with retinoic acid[10]. It is possible that *Rai1* is involved in neuronal differentiation, as *in situ* and immunocytochemistry analysis of adult mouse brain showed that the *Rai1* transcript and protein product are localized to neurons[10]. The RAI1 protein, which contains regions of sequence similarity to the transcriptional coactivator TCF20 (refs. 11,12), may also be localized to the nucleus and stimulate transcription. Our sequence analysis shows that human RAI1 contains a putative bipartite nuclear localization signal beginning at amino-acid positions 1,113 and 1,176 and predicted N-glycosylation sites. As no known DNA-binding motifs have been identified, RAI1 may interact with other DNA-binding proteins to exert its effects on transcription. These processes may be evolutionarily conserved, as the RAI1 protein has approximately 77% overall identity to the mouse Rai1 protein[12] and putative *RAI1* expressed-sequence tag (EST) homologs have been sequenced from *Rattus norvegicus*, *Bos taurus* and *Xenopus laevis*.

We suggest that SMS may be similar to previously described microdeletion syndromes in which a single gene is implicated in most of the features but other deleted genes may modify the overall phenotype[13–15]. Haploinsufficiency of *RAI1* is probably responsible for the behavioral, neurological, otolaryngological and craniofacial aspects of this syndrome, but more variable features such as heart and renal defects are probably due to hemizygosity of other genes in the 17p11.2 region. Finally, we advocate the use of genomic probes that contain *RAI1*, such as RPCI-1 253P07, for use in FISH to screen individuals who may have SMS. In individuals with strong clinical features and negative FISH analysis, mutation screening may prove informative.

Accession numbers. Human *RAI1* mRNA, GenBank AJ271790; human RAI1 protein, GenProt CAC20423; human *RAI1* genomic region, GenBank AJ271791 and NT_030843; bc915D19, GenBank AC080148; bc384M20, GenBank AC087163; pc253P07, GenBank AL354000; pc178F10, GenBank AL035367; bc189D22, GenBank AC099988; *RAI1* transcript variant KIAA1802, GenBank AB058723. Mouse *Rai1* mRNA, GenBank NM_009021; mouse Rai1 protein, NP_033047. Putative *RAI1* EST homologs from *R. norvegicus*, GenBank BU758552; *B. taurus*, GenBank BE667697; and *X. laevis*, GenBank BG813716. Human *TCF20* mRNA, GenBank XM_040067; mouse *Tcf20*, GenBank NM_013836.

URL. When putative single-nucleotide polymorphisms (SNPs) were identified, we compared these to known SNPs found in the National Center for Biotechnology Information SNP database (available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp) and SNP information from the genomic contig containing *RAI1*. Information on Parents and Researchers Interested in Smith–Magenis Syndrome is available at http://www.smithmagenis.org. The GeneReviews online database is available at http://www.genereviews.org. The University of California Santa Cruz genome browser is available at http://genome.ucsc.edu.

Note: Supplementary information is available on the Nature Genetics website.

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Competing interests statement

The authors declare that they have no competing financial interests.

Rebecca E. Slager[1], Tiffany Lynn Newton[2], Christopher N. Vlangos[1], Brenda Finucane[3] & Sarah H. Elsea[1,2]

[1]Genetics Graduate Program, S-320 Plant Biology Building and [2]Departments of Zoology and Pediatrics/Human Development, Michigan State University, East Lansing, Michigan 48823, USA. [3]Elwyn Training and Research Institute, Elwyn, Pennsylvania 19063, USA. Correspondence should be addressed to S.H.E. (e-mail: elsea@msu.edu).

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