**ORIGINAL ARTICLE**

**RAI1 variations in Smith–Magenis syndrome patients without 17p11.2 deletions**

S Girirajan, L J Elses II, K Devriendt, S H Elsea

**Background:** Smith–Magenis syndrome (SMS) (OMIM No 182290) is a mental retardation syndrome characterised by behavioural abnormalities, including self injurious behaviours, sleep disturbance, and distinct craniofacial and skeletal anomalies. It is usually associated with deletion involving 17p11.2 and is estimated to occur in 1/25 000 births. Heterozygous frameshift mutations leading to protein truncation in retinoic acid induced 1 gene (RAI1) have been identified in individuals with phenotypic features consistent with SMS. RAI1 lies within the 17p11.2 locus, but these patients did not have 17p11.2 deletions.

**Objective:** Analysis of four individuals with features consistent with SMS for variations in RAI1, using a polymerase chain reaction and sequencing strategy. None of these patients carry 17p11.2 deletions.

**Results:** Two patients had small deletions in RAI1 resulting in frameshift and premature truncation of the protein. Missense mutations were identified in the other two. Orthologs across other genomes showed that these missense mutations occurred in identically conserved regions of the gene. The mutations were de novo, as all parental samples were normal. Several polymorphisms were also observed, including new and reported SNPs. The patients’ clinical features differed from those found in 17p11.2 deletion by general absence of short stature and lack of visceral anomalies. All four patients had developmental delay, reduced motor and cognitive skills, craniofacial and behavioural anomalies, and sleep disturbance. Seizures, not previously thought to be associated with RAI1 mutations, were observed in one patient of the cohort.

**Conclusions:** Haploinsufficiency of the RAI1 gene is associated with most features of SMS, including craniofacial, behavioural, and neurological signs and symptoms.

**METHODS**

**Patients and samples**

Patients having the physical and neurobehavioural characteristics diagnostic of SMS were referred from genetics clinics from various parts of USA and Europe. The study was approved by the Michigan State University committee on research involving human subjects and the institutional review board of the Virginia Commonwealth University.

**Abbreviations:** SMS, Smith–Magenis syndrome
Written informed consent was obtained for each subject in this study. Permission to print photographs and to publish detailed patient information was obtained from the parents of each child before manuscript submission.

About 7–10 ml of blood was drawn by antecubital venepuncture following sterile procedures. When blood could not be obtained, buccal brush samples were collected. The molecular evaluation protocol followed is illustrated in fig 1.

**Fluorescent in situ hybridisation**

All patients were evaluated for 17p11.2 deletions by FISH to metaphase chromosome spreads using PAC probe RP11-253P07, representing the RAI1 locus. A commercially available nick-translation kit was used to incorporate spectrum green or spectrum orange dUTP following the manufacturer’s instructions (Vysis, Downers Grove, Illinois, USA). A 17q-tel probe (RP1-314M5) was used as a control. Metaphase chromosomes were prepared for hybridisation by incubating at 37°C in 2×SSC (NaCl/sodium citrate) for 30 minutes followed by dehydration through an ethanol series and air drying. After overnight hybridisation at 37°C, slides were washed and then counterstained using Vectashield antifade with DAPI (Vector Labs, Burlingame, California, USA).

Analyses of FISH experiments were carried out on a Zeiss Axioplan IE microscope and photographed with Axiovision MR black and white camera using Zeiss Axio-vision software, version 4.2 (Carl Zeiss, Thornwood, New York, USA).

**PCR and sequencing**

DNA from whole blood was isolated by the phenol-chloroform method. Standard laboratory optimised protocols were followed to isolate DNA from buccal smears and cell lines. Polymerase chain reaction (PCR) was carried out to amplify patient DNA using overlapping RAI1 primers covering the entire coding region (Genbank AY172136), spanning exons 3–6 (see table 1 for primers and annealing temperatures). PCR was done with 100 ng genomic DNA, 20 pmol each of forward and reverse primers, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, 200 μM dNTPs, and 1 U 72°C DNA polymerase in a total reaction volume of 25 μl. Reactions were held at 94°C for five minutes followed by 30 cycles of denaturing at 94°C for one minute, annealing at 55–62°C (see table 1) for one minute, and an extension at 72°C for one minute, followed by a final extension at 72°C for 10 minutes. Reactions were held at 4°C until use. PCR products (5 μl) were either purified by digesting with 2 U of shrimp alkaline phosphatase and 10 U

---

**Table 1**  
**RAI1 PCR primers and annealing temperatures**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size (bp)</th>
<th>Annealing temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>SHE 323: TCTGAGGCTAAAGGAAGGAGTGG</td>
<td>SHE 324: GACTTGGGATGCTGAGGAAA</td>
<td>770</td>
<td>62°C</td>
</tr>
<tr>
<td>3</td>
<td>SHE 325: AAAGGCAGAGGAGGCAAGAC</td>
<td>SHE 326: CAGTGGTGCTTTATGCTG</td>
<td>875</td>
<td>62°C</td>
</tr>
<tr>
<td>3</td>
<td>SHE 327: TGTCAGAAGGCTGTCG</td>
<td>SHE 328: GGGAAACAGTGGGAAAGC</td>
<td>777</td>
<td>60°C</td>
</tr>
<tr>
<td>3</td>
<td>SHE 329: TCTCGCTGGAGAACCACAG</td>
<td>SHE 330: AACACAGCTGAGACCCTTGA</td>
<td>904</td>
<td>59°C</td>
</tr>
<tr>
<td>3</td>
<td>SHE 331: CACTCACTGCACAGCAGGAG</td>
<td>SHE 332: ATGAAGGCCCCGACTTCCTTT</td>
<td>1000</td>
<td>60°C</td>
</tr>
<tr>
<td>3</td>
<td>SHE 333: CCAAGAAGGCTCCTCGCAACAC</td>
<td>SHE 334: CCTGGGTTTCTCTCTGAGG</td>
<td>763</td>
<td>59°C</td>
</tr>
<tr>
<td>3</td>
<td>SHE 335: AAACCGAGGCTTGCTC</td>
<td>SHE 336: CACACCTGGAGGCTTGTG</td>
<td>964</td>
<td>60°C</td>
</tr>
<tr>
<td>3</td>
<td>SHE 339: CTACCCCTGACAGCTCCTC</td>
<td>SHE 340: AGAGGCAATGGGAGGACACAG</td>
<td>887</td>
<td>60°C</td>
</tr>
<tr>
<td>4</td>
<td>SHE 341: CCAGGCTGTGATCAACCTC</td>
<td>SHE 342: GGCAAGAGGAGGGAACACT</td>
<td>420</td>
<td>60°C</td>
</tr>
<tr>
<td>5</td>
<td>RA 46: GGAAGTGAGGAGGATGGAG</td>
<td>RA 45: GGAAGTGAGGAGGATGGAG</td>
<td>310</td>
<td>62°C</td>
</tr>
<tr>
<td>6+3’ UTR</td>
<td>SHE 345: AACTGGAAGACTCCAGAGCAGTGT</td>
<td>SHE 346: GACTTGGGAAAGGGACACAC</td>
<td>816</td>
<td>55°C†</td>
</tr>
</tbody>
</table>

*Primers cover the coding region of RAI1 and are based on Genbank No AY172136.
†Amplification requires Invitrogen® 10X PCR enhancer.
PCR, polymerase chain reaction; temp, temperature; UTR, untranslated region.

---

Figure 1  Flow chart for molecular evaluation of Smith–Magenis syndrome (SMS). The recommended procedure for the diagnosis of SMS is illustrated. In suspected cases of SMS, initial evaluation should include chromosomes analysis, followed by FISH with an RAI1 containing probe. Non-deletion cases would then be referred for RAI1 sequencing analysis and any identified mutations would be confirmed de novo by investigation of parental samples.
of exonuclease I (USB Corporation, Cleveland, Ohio, USA) at
37°C for 15 minutes followed by 80°C for 15 minutes to
remove excess primers, or were gel purified using a Qiagen
gel extraction kit (Qiagen Inc, Valencia, California, USA).
Sequencing was carried out either at the Virginia
Commonwealth University Sequencing Core or at the
Michigan State University Genomics Technology Support
Facility.

Cycle sequencing was done using 10 ng/µl of the purified
PCR product, 10 pmol of each primer, and 4 µl of the ABI
Prism BigDye terminator cycle sequencing ready reaction mix
(Perkin Elmer, Applied Biosystems, Foster City, California,
USA) in a 10 µl final volume for 25 cycles. Following cycle
sequencing, the samples were purified with ethanol, pre-
cipitated, resuspended in formamide, denatured at 95°C for
five minutes, and loaded onto an ABI 3700 genetic analyser
(Applied Biosystems/Hitachi). Sequencing was initially done
with the forward primer and the presence of any DNA
variation confirmed by sequencing in the reverse direction.
Available parental samples were evaluated for all identified
mutations. The chromatograms and the sequence data were
aligned to the RAI1 mRNA database sequence at the NCBI
(GenBank AY172136) using Clustal X (version 1.83).

RESULTS

We were referred four patients with clinical symptoms of
SMS who were initially evaluated by FISH analysis at
commercial laboratories for 17p11.2 deletions but were
negative for any such deletion. All patient samples were
evaluated in this laboratory by FISH using an RAI1 specific
probe. None of these patients carried a deletion of the
17p11.2 region; thus all were further screened for variations
in the RAI1 coding region. Significant nucleotide changes
were identified in exon 3 of the gene. In this report, we
describe four novel de novo mutations that support a
diagnosis of SMS in these patients. Each of the patients
presented also carries inherited RAI1 polymorphisms; some
are documented in the NCBI SNP database and some are
reported for the first time in this study (table 3).

SMS153

SMS153 is a 19 year old woman of European descent with
developmental delays and self destructive behaviours who
was initially evaluated at the age of 14 (fig 2A). She is the
only child of a G1P1 mother with artificial insemination
was initially evaluated at the age of 14 (fig 2A). She is the
SMS153 are documented in the NCBI SNP database and some are
negative for any such deletion. All patient samples were
commercial laboratories for 17p11.2 deletions but were
SMS who were initially evaluated by FISH analysis at
We were referred four patients with clinical symptoms of
RESULTS

We were referred four patients with clinical symptoms of
SMS who were initially evaluated by FISH analysis at
commercial laboratories for 17p11.2 deletions but were
negative for any such deletion. All patient samples were
evaluated in this laboratory by FISH using an RAI1 specific
probe. None of these patients carried a deletion of the
17p11.2 region; thus all were further screened for variations
in the RAI1 coding region. Significant nucleotide changes
were identified in exon 3 of the gene. In this report, we
describe four novel de novo mutations that support a
diagnosis of SMS in these patients. Each of the patients
presented also carries inherited RAI1 polymorphisms; some
are documented in the NCBI SNP database and some are
reported for the first time in this study (table 3).

SMS153

SMS153 is a 19 year old woman of European descent with
developmental delays and self destructive behaviours who
was initially evaluated at the age of 14 (fig 2A). She is the
only child of a G1P1 mother with artificial insemination
was initially evaluated at the age of 14 (fig 2A). She is the
SMS153 are documented in the NCBI SNP database and some are
negative for any such deletion. All patient samples were
commercial laboratories for 17p11.2 deletions but were
SMS who were initially evaluated by FISH analysis at
We were referred four patients with clinical symptoms of
RESULTS

We were referred four patients with clinical symptoms of
SMS who were initially evaluated by FISH analysis at
commercial laboratories for 17p11.2 deletions but were
negative for any such deletion. All patient samples were
evaluated in this laboratory by FISH using an RAI1 specific
probe. None of these patients carried a deletion of the
17p11.2 region; thus all were further screened for variations
in the RAI1 coding region. Significant nucleotide changes
were identified in exon 3 of the gene. In this report, we
describe four novel de novo mutations that support a
diagnosis of SMS in these patients. Each of the patients
presented also carries inherited RAI1 polymorphisms; some
are documented in the NCBI SNP database and some are
reported for the first time in this study (table 3).

SMS153

SMS153 is a 19 year old woman of European descent with
developmental delays and self destructive behaviours who
was initially evaluated at the age of 14 (fig 2A). She is the
only child of a G1P1 mother with artificial insemination
was initially evaluated at the age of 14 (fig 2A). She is the
SMS153 are documented in the NCBI SNP database and some are
negative for any such deletion. All patient samples were
commercial laboratories for 17p11.2 deletions but were
SMS who were initially evaluated by FISH analysis at
Table 2 Phenotypic features of SMS patients with RAI1 mutations compared to those with 17p11.2 deletions

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Craniofacial/skeletal</td>
<td>del(17)(p11.2) (%)</td>
<td>89</td>
<td>3/3 1/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8/9</td>
<td>89</td>
</tr>
<tr>
<td>Brachycephaly</td>
<td>93</td>
<td>0/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6/9 67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midface hypoplasia</td>
<td>52</td>
<td>3/3 1/2</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>6/7 86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prognathism (relative to age)</td>
<td>73</td>
<td>3/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8/9 89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broad, square face</td>
<td>81</td>
<td>2/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8/9 89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synophrys</td>
<td>62</td>
<td>2/3 N</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3/7 43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleft lip/palate</td>
<td>9</td>
<td>0/3 0/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0/9 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>85</td>
<td>1/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7/9 78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short stature (&lt;5th centile)</td>
<td>69</td>
<td>0/3 0/2</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>1/9 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scoliosis</td>
<td>49-67</td>
<td>2/3 2/2</td>
<td>N</td>
<td>–</td>
<td>–</td>
<td>4/8 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Otolaryngologic abnormalities</td>
<td>85</td>
<td>2/3 1/2</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4/9 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic ear infections</td>
<td>68</td>
<td>1/3 0/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1/9 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hearing loss</td>
<td>80</td>
<td>2/3 N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6/7 86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological/behavioural</td>
<td>100</td>
<td>3/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9/9 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable mental retardation</td>
<td>&gt;90</td>
<td>1/3 N</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4/7 57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speech delay†</td>
<td>&gt;90</td>
<td>1/3 N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>4/7 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor delay†</td>
<td>&gt;90</td>
<td>1/3 N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4/7 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotonia</td>
<td>&gt;90</td>
<td>1/3 N</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>4/7 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizures by history</td>
<td>11 to 30</td>
<td>1/3 0/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2/9 22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>70 to 100</td>
<td>3/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7/7 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self hugging/ hand wringing‡</td>
<td>70 to 100</td>
<td>3/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9/9 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention seeking</td>
<td>80 to 100</td>
<td>3/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9/9 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self injurious behaviour‡</td>
<td>78 to 96</td>
<td>3/3 2/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9/9 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onychotillomania</td>
<td>25 to 85</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6/7 86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyembolokoilomania</td>
<td>25 to 85</td>
<td>3/3 N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7/7 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head banging/face slapping</td>
<td>71</td>
<td>2/3 N</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5/7 72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand biting/self biting</td>
<td>77</td>
<td>2/3 N</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5/7 72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular abnormalities</td>
<td>53</td>
<td>2/3 1/2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>6/9 67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myopia</td>
<td>50</td>
<td>2/3 1/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4/9 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strabismus</td>
<td>64</td>
<td>0/3 0/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1/9 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iris abnormalities</td>
<td>&gt;50</td>
<td>3/3 0/2</td>
<td>–</td>
<td>N</td>
<td>+</td>
<td>–</td>
<td>4/8 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other features</td>
<td>Structural cardiovascular anomaly</td>
<td>30</td>
<td>0/3 0/2</td>
<td>–</td>
<td>N</td>
<td>–</td>
<td>0/9 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structural renal anomaly</td>
<td>30</td>
<td>0/3 0/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0/9 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female Male Male Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at evaluation (years)</td>
<td>14 to 19 4 to 14 14 to 17 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SMS195 is a 17 year old adopted youth of northern European and Jewish ancestry (table 2). The biological parents reportedly have three normal children. Other family history was not available. He was delivered full term weighing 3.86 kg and 53.3 cm in length. Neonatal jaundice, sleep disturbance, and mildly delayed motor and cognitive milestones were the major complaints during early childhood. Medical history is notable for arthritis of the fifth digits and the knees bilaterally. He has high myopia, a loud and hoarse voice, a waddling gait, pes planus, and dry skin. Alternating diarrhoea and constipation has continued since early childhood. At 15 years, he was prepubertal at Tanner stage II, and evaluation of delayed puberty was sought. After treatment with testosterone injections for eight months he had reached Tanner stages III–IV and had developed pubic and axillary hair.
and Bi et al. mutations reported to date are indicated. Mutations in this report 1 are depicted, along with previously reported mutations from Slager mutations in Smith–Magenis syndrome 825 .

Poster frontal lobe in the precentral gyrus. She was asymmetry in the gyral sulcation pattern along the left sides. Magnetic resonance imaging showed a slight delta activity with medium to high amplitude spikes, sharps apparent abnormal sleep patterns. Her EEG showed 2.5–5 Hz sedated as a component of seizure therapy but has no umbilicus, ears, and mouth. She was frequently irritable in until they bleed, and polyembolokoilamania of rectum, hugging, twirling of objects, biting and picking of toenails motor, and cognitive skills are below the 7th centile, with an occurring 10 to 30 times a month. Language, psychosocial, involved grand mal, Jacksonian, and petit mal episodes at age 4, when seizures began. Seizures were complex and walked at 12 months, but speech was delayed and regressed from ‘‘autistic-like’’ activity. She sat at six months and of nine months, with explosive tantrums when distracted offspring. Behavioural problems were first noticed at the age 4.1 kg to a 33 year old mother with two normal, living SMS175 is an 11 year old white girl with mental retardation, progressive speech delay, stereotypic behaviour, intractable SMS195 has a heterozygous centile. Weight for height (BMI) remains above the 95th centile.

Laboratory findings include a normal karyotype and normal FISH for del(17)(p11.2). SMS195 has a heterozygous amino acid 1808 in the RAI1 protein (table 3; fig 2C; fig 3). He was adopted at three months of age; thus parental DNA samples were not available for sequencing. This mutation was not identified in more than 100 normal chromosomes.

**DISCUSSION**

We present four patients with mutations in the *RAI1* gene, each with features consistent with a diagnosis of SMS. These patients do not carry any detectable deletion involving 17p11.2 by FISH or G-banding analysis; however, all identified mutations in the *RAI1* gene are de novo. Our original study identified three such patients, 14 while work by Bi et al. involved two additional subjects, bringing the total number reported with *RAI1* mutations to nine. Even though the patients described have been evaluated by different clinicians from around the world, all have very similar features and all were initially considered to have SMS (fig 1: table 2; fig 3).

Consistent in the patients presented in this study, and in those presented previously, 14 was a definitive delay in growth appearing to be short for age. Sleep disturbances with a variety of manifestations—including difficulty getting to sleep, reduced REM sleep, frequent waking, and increased daytime sleepiness and napping—are present in all of the patients with *RAI1* mutations. None of the patients evaluated had any systemic involvement, which reinforces our previous conclusion that haploinsufficiency of *RAI1* is responsible for the behavioural, neurological, otolaryngological, and craniofacial features of the syndrome, but that more variable features hair. Treatment was discontinued because of aggressive behaviour. His abnormal behaviours are particularly significant and include sleep disturbances (hypersomnolence as an infant, moving to frequent and early awakenings and daytime napping from around age 4 to the present), reported bipolar episodes, head banging, tantrums, and aggressive and intrusive behaviours. He also has deep scarring from obsessively picking his skin. His behaviour has required several psychiatric hospital admissions, and at the time of writing placement in a residential treatment facility was being sought. He has been prescribed numerous psychiatric drugs since the age of 6. At 14, he had the developmental age of a 9 year old, with an IQ of 89.

His early childhood weight was less than the 25th centile but it has gradually increased to the 75th centile. Some gain in height was seen with hormone therapy, but his height remains below the 3rd centile while his weight is on the 75th centile. Weight for height (BMI) remains above the 95th centile. Laboratory findings include a normal karyotype and normal FISH for del(17)(p11.2). SMS195 has a heterozygous G5423A mutation causing a serine to asparagine change at amino acid 1808 in the *RAI1* protein (table 3; fig 2C; fig 3).

We present four patients with mutations in the *RAI1* gene, each with features consistent with a diagnosis of SMS. These patients do not carry any detectable deletion involving 17p11.2 by FISH or G-banding analysis; however, all identified mutations in the *RAI1* gene are de novo. Our original study identified three such patients, 14 while work by Bi et al. involved two additional subjects, bringing the total number reported with *RAI1* mutations to nine. Even though the patients described have been evaluated by different clinicians from around the world, all have very similar features and all were initially considered to have SMS (fig 1: table 2; fig 3).

Consistent in the patients presented in this study, and in those presented previously, 14 was a definitive delay in growth appearing to be short for age. Sleep disturbances with a variety of manifestations—including difficulty getting to sleep, reduced REM sleep, frequent waking, and increased daytime sleepiness and napping—are present in all of the patients with *RAI1* mutations. None of the patients evaluated had any systemic involvement, which reinforces our previous conclusion that haploinsufficiency of *RAI1* is responsible for the behavioural, neurological, otolaryngological, and craniofacial features of the syndrome, but that more variable features
RAI1 is highly conserved in mammalian species. Alignment of human RAI1 (NP_109590.3), chimp (Chr 19, uncurated sequence numbers from 17975106-18062618, UCSC Genome browser), rat (XP_220520.5), and mouse Rai1 (NP_033047.1) sequences. Human and mouse sequences are 84% identical and 88% similar, whereas the chimp sequence is 99.5% identical to human. Rat and humans share an identity of 84.4% and 88.5% in similarity. While the number of polyglutamines (asterisks) ranges from 9–18 in humans (shown here at 14 repeats), mice and rats have four CAG repeats, while chimps carry nine repeats. The polyserine repeats (dots), nuclear localisation signals (dashes), and the PHD domain (diamonds) are indicated. Sequences that are similar to the transcription factor, TCF-20 are indicated (crosses) including amino acid residues 1823–1842 that lie in the PHD domain.
such as cleft lip/palate and cardiac and renal defects are probably caused by hemizygosity of another gene or other genes in the 17p11.2 region. 14 Some features such as hypotonia, speech and motor delay, and hearing loss are observed less often or are less severe in the patients carrying RAI1 mutations than in those with 17p11.2 deletions (table 2). It is important to note that even though all patients were previously evaluated for 17p11.2 deletions by G banding techniques and FISH, we confirmed these reported findings with the RAI1 specific probe, as most commercially available probes do not include this gene. 15

These patients carry de novo nucleotide changes that were absent in the parental DNAs, although germline mosaicism cannot be ruled out. The mutations reported cause a significant change in the amino acid sequence of the RAI1 protein that probably leads to the formation of abnormal or truncated protein or processing by nonsense mediated decay. These data strengthen the hypothesis that haploinsufficiency and dosage imbalance of the RAI1 protein causes SMS. All mutations so far identified occur in exon 3 of RAI1 which codes for more than 98% of the protein, though a few polymorphisms are distributed in other exons. It is observed that the missense mutations alter amino acids in the highly conserved regions of the gene (figs 2 and 4).

Except for a few polymorphisms that occurred in the homozygous state, all others occurred in the heterozygous condition, and all but one of them have previously been reported in the SNP database (table 3). The polymorphic CAG repeat region contained 10 to 13 repeats in this group of patients. The number of CAG repeats in RAI1 has been identified or reported.

Table 3  RAI1 mutations and polymorphisms identified in four patients with Smith–Magenis syndrome

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Nucleotide change*</th>
<th>Amino acid change</th>
<th>Polymorphisms†</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMS153</td>
<td>253del(19 bp)</td>
<td>Deletion/frameshift</td>
<td>Pro664Pro[1 homozygous]</td>
</tr>
<tr>
<td>SMS188</td>
<td>380delIC</td>
<td>Frameshift</td>
<td>Gly983Ala [1 homozygous]</td>
</tr>
<tr>
<td>SMS195</td>
<td>G5423A</td>
<td>Ser1808Asn</td>
<td>Pro664Pro [homozygous]</td>
</tr>
<tr>
<td>SMS175</td>
<td>A4685G</td>
<td>Gln1562Arg</td>
<td>Pro664Pro [homozygous]</td>
</tr>
</tbody>
</table>

*DNA changes with nucleotide number starting from the ATG in the coding sequence.
†SNP database: †SNP 3803763; *SNP 11078398; ††SNP 11649804; †‡A→G change at nucleotide 5534 (a novel polymorphism not reported in the database). (CAG)0/10 represents the number of CAG repeats present on each allele.

While it is postulated that RAI1 is a transcriptional regulator involved in neuronal development, its exact biochemical and functional role remains elusive. Studies in our laboratory (data not shown) indicate that RAI1-GFP fusion constructs are localised to the nucleus, consistent with a postulated function in transcription; however, further studies are required to fully understand its role in this process. A primary focus will be the identification of the pathways RAI1 modulates in the complex developmental and behavioural processes affected in SMS.

ACKNOWLEDGEMENTS

We are grateful to Christopher N Vlangos, Rebecca E Slager, Tiffany L Newton, Catherine Barth, Barbara Szomju, the MSU Genomic Technology Support Facility, and the Massey Cancer Center Nucleic Acid Research Facility for expert technical assistance, to the Smith–Magenis syndrome families, and to Parents and Researchers Interested in SMS (PRISMS) for their persistence in support of our research. This work was funded by a grant from US National Institutes of Health (HD38534) and resources from Virginia Commonwealth University.

Authors’ affiliations

S Girirajan, Department of Human Genetics, Virginia Commonwealth University, Richmond, Virginia, USA
S H Elsea, Departments of Pediatrics and Human Genetics, Virginia Commonwealth University
L J Elsay, The Dr John T Macdonald Foundation Center for Medical Genetics, University of Miami School of Medicine, Miami, Florida, USA
K Devriendt, Department of Human Genetics, University Hospital Leuven, Leuven, Belgium

Competing interests: none declared
Young patients with colorectal cancer are genetically susceptible

Researchers are advocating genetic testing for young patients with early onset colorectal cancer, after discovering mutations that predispose to the disease, as in hereditary non-polyposis colorectal cancer (HNPPC). Testing should be done even if family histories do not conform to the Amsterdam criteria for HNPPC, they say.

High frequency microsatellite instability was evident in eight tumours from 11 patients (73%) evaluated in cohort of 16 patients aged 24 years at diagnosis. Germline mutations occurred in mismatch repair genes in six out of 14 tumours (43%) from 14 patients tested—two mutations in MLH1, three in MSH2, and one in PMS2. Half the families met the Amsterdam criteria for HNPPC. Among the others, four out of five patients tested had tumours with high frequency microsatellite instability and germline mutations were present in three. Secondary tumours occurred in seven (44%) patients in the entire cohort during follow up, three quarters in the gastrointestinal tract; and in almost three quarters the primary tumour showed high frequency microsatellite instability.

The cohort was identified from 1382 patients in the Familial Gastrointestinal Cancer Registry, Toronto, Canada, 1960–2003. Clinical and pathological reviews were reported and pedigrees drawn up from clinical data and interviews with the probands and their relatives. DNA was extracted from microdissected material from paraffin blocks of the original resected tumours to look for HNPPC-type mutations.

Case series of colorectal cancer in children and adolescents have not focused on genetic profiles of the tumours or looked for genetic susceptibility within families before.

References:

11. Maier D, Stumm G, Krup H, Henning S, Reinhardt R, Schwartz CE, Heiss NS, Potocki L, Heiss NS. inception, 73% evaluated in cohort of 16 patients aged 24 years at diagnosis. Germline mutations occurred in mismatch repair genes in six out of 14 tumours (43%) from 14 patients tested—two mutations in MLH1, three in MSH2, and one in PMS2. Half the families met the Amsterdam criteria for HNPPC. Among the others, four out of five patients tested had tumours with high frequency microsatellite instability and germline mutations were present in three. Secondary tumours occurred in seven (44%) patients in the entire cohort during follow up, three quarters in the gastrointestinal tract; and in almost three quarters the primary tumour showed high frequency microsatellite instability.

The cohort was identified from 1382 patients in the Familial Gastrointestinal Cancer Registry, Toronto, Canada, 1960–2003. Clinical and pathological reviews were reported and pedigrees drawn up from clinical data and interviews with the probands and their relatives. DNA was extracted from microdissected material from paraffin blocks of the original resected tumours to look for HNPPC-type mutations.

Case series of colorectal cancer in children and adolescents have not focused on genetic profiles of the tumours or looked for genetic susceptibility within families before.


Please visit the Journal of Medical Genetics website [www.jmedgenet.com] for a link to the full text of this article.
RAI1 variations in Smith–Magenis syndrome patients without 17p11.2 deletions

S Girirajan, L J Elsas II, K Devriendt and S H Elsea

J Med Genet 2005 42: 820-828 originally published online March 23, 2005
doi: 10.1136/jmg.2005.031211