ORIGINAL ARTICLE

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

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J Med Genet 2005;**42**:820–828. doi: 10.1136/jmg.2005.031211

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Received 1 February 2005
Revised version received 16 March 2005
Accepted for publication 21 March 2005

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.

Smith–Magenis syndrome (SMS) is a multiple congenital anomalies and mental retardation syndrome usually associated with an interstitial deletion involving chromosome 17p11.2.[1] It is characterised by distinctive craniofacial anomalies,[2] delayed milestones, cognitive impairment, hearing loss,[3] myopia, and mild to severe mental retardation.[4] Behavioural abnormalities include significant sleep disturbances and maladaptive and self injurious behaviours.[5–7] The facial appearance is characterised by a broad square shaped face, brachycephaly, midface hypoplasia, and micrognathia in early infancy, progressing to relative prognathism with age. Upslanting palpebral fissures, deep set eyes, short full tipped nose, tented upper lip, and downturned corners of the mouth are normally seen in the majority of SMS patients. Infancy and childhood are associated with hypotonia, sleep disturbances with inverted melatonin circadian rhythm, failure to thrive, and feeding difficulties. Neurobehavioural abnormalities become more pronounced with age and are characterised by hyperactivity, temper tantrums, attention seeking, self hugging, polyembolokoilomania (insertion of objects into bodily orifices), and onychotillomania (picking and pulling out of finger and toe nails). In addition, about 70% of SMS patients have a raised serum cholesterol.[8]

The incidence of this disorder has been estimated to be 1:25 000 births[9] although with more awareness and proper diagnosis, the incidence is expected to be higher. About 75% of SMS patients with 17p11.2 deletions have a common deletion spanning ~3.5 Mb, but the size of the deletion is variable from ~1.5 Mb to ~9 Mb.[10 11] Deletions associated with SMS are reported to result from unequal crossing over mediated by SMS repeat clusters.[12] We refined the minimum overlapping region common to all SMS patients carrying a 17p11.2 deletions to ~700 kb by analysing patients with unusual 17p11.2 deletions using fluorescent in situ hybridisation (FISH).[13]

Further, studies in our laboratory showed that a gene encoding the retinoic acid induced protein 1 (*RAI1*) carried small intragenic deletions in patients who had no FISH detectable deletion in the 17p11.2 region.[14] *RAI1* (GenBank AY172136, AJ271790; OMIM*607642) maps to the central portion of the SMS critical interval, adjacent to *SREBF1*. It is formed by six exons generating an ~8.5 Kb mRNA and a 1906 amino acid protein. The *RAI1* protein contains a bipartite nuclear localisation signal, polyglutamine and polyserine tracts, and a PHD/zinc finger domain at the C-terminus.[15] Murine *Rai1* (originally reported as *GT1*) is upregulated in mouse embryonal carcinoma cells following retinoic acid treatment to induce neuronal differentiation.[16]

In this paper, we describe the sequencing analysis of *RAI1* in four non-deletion patients with SMS and compare their phenotypic data with 17p11.2 deletion patients. A comprehensive study of the structure of this gene in humans and its conserved sequences in mouse, rat, and chimp genomes is also provided to facilitate the analysis of *RAI1* mutations in SMS.

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

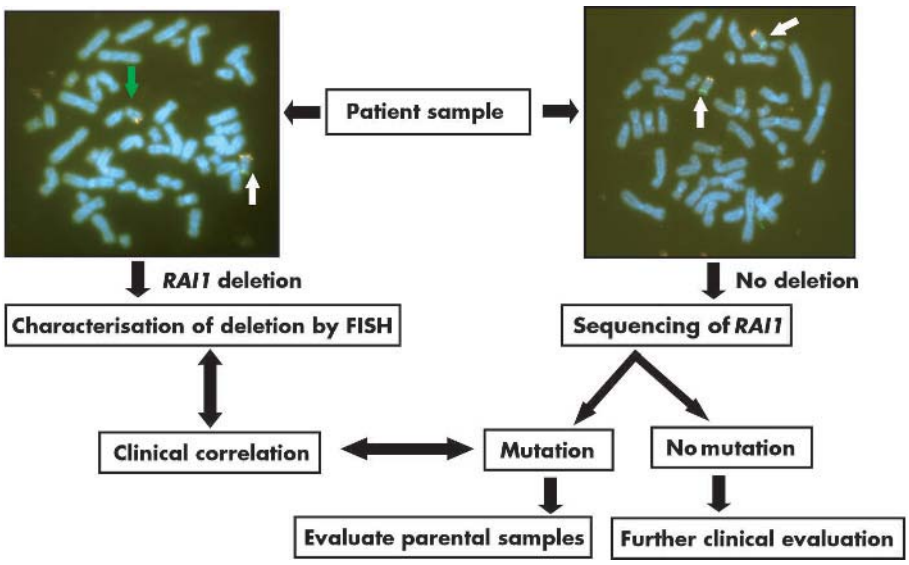

Figure 1 Flow chart for molecular evaluation of Smith–Magenis syndrome (SMS). The recommended procedure for the diagnosis of SMS is illustrated. In suspected cases of SMS, initial evaluation should include chromosomes analysis, followed by FISH with an *RAI1* containing probe. Non-deletion cases would then be referred for *RAI1* sequencing analysis and any identified mutations would be confirmed de novo by investigation of parental samples.

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℃ in 2×SSC (NaCl/sodium citrate) for 30 minutes followed by dehydration through an ethanol series and air drying. After overnight hybridisation at 37℃, 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 Axion 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 $MgCl_2$, 200 μM dNTPs, and 1 U *Taq* DNA polymerase in a total reaction volume of 25 μl. Reactions were held at 94℃ for five minutes followed by 30 cycles of denaturing at 94℃ for one minute, annealing at 55–62° (see table 1) for one minute, and an extension at 72℃ for one minute, followed by a final extension at 72℃ for 10 minutes. Reactions were held at 4℃ 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*

Exon	Forward primer	Reverse primer	Product size (bp)	Annealing temp
3	SHE 323: TCTGAGGCAAAAGGAAGTGG	SHE 324: GACTGGGAATGCTGAGGAAA	770	62℃
3	SHE 325: AAAGGCAGAAGCTGCAGAAC	SHE 326: CAGTGCTGGCTTTATGCTG	875	62℃
3	SHE 327: TGTCAAGAACCTCGTGTCCA	SHE 328: GGGAAACAGTCAAAAGCTGC	777	60℃
3	SHE 329: TCTCGCTGGAGAACCACAG	SHE 330: AACCAGCTCTGGACCTTTGA	904	59℃
3	SHE 331: CACTCATCTGCACCAAGGAG	SHE 332: ATGAAGGCCGACTTCCTTTT	1000	60℃
3	SHE 333: CCAAGAAGCTCCTCGACAAC	SHE 334: TTCCGGCTCCTCTTCTTAGG	763	59℃
3	SHE 335: AAACCGAGGCCTTCACATC	SHE 336: CACACTCGGGACCTTTGAGT	964	60℃
3	SHE 339: CTACCCTGAACACTGCCTCC	SHE 340: AGAGGCAATGGAGACAGGAA	887	60℃
4	SHE 341: CCAGGCTGGTATCAAACTCC	SHE 342: GGCAAGAGAGGAGGGAAACT	420	60℃
5	RA 46: GGAGTGGAGTGGAGTGTGGAGG	RA 45: GGACTGTGAAGGAGGTGCGAGG	310	62℃
6+3′ UTR	SHE 345: ACTGTGAAGTCCGAGGTCGT	SHE 346: GACTGGAAAGGGGACAAACA	816	55℃†

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

of exonuclease I (USB Corporation, Cleveland, Ohio, USA) at 37℃ for 15 minutes followed by 80℃ 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, precipitated, resuspended in formamide, denatured at 95℃ 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 G_1P_1 mother with artificial insemination (known healthy sperm donor). SMS153 was born at 42 weeks after an induced labour followed by caesarean section. Birth weight was 4.3 kg and she was noted to have floppy muscle tone, upslanting palpebral fissures, and midface hypoplasia as a neonate. She was initially diagnosed as having Down's syndrome, but all laboratory studies were negative for trisomy 21.

Some developmental milestones were normal, but she was significantly delayed in motor skills and language development. Long tantrums, attention seeking, and repetitive behaviours began at ~18 months of age. Enuresis was a problem until the age of 12. Tonsils and adenoids were removed in early childhood because of frequent ear infections. She was thoroughly evaluated for pseudohypoparathyroidism, given her history of mental retardation and brachydactyly of the fourth metacarpophalangeal joint, but there were no significant biochemical findings. At 15 years she had a developmental age of 8 to 10 years, with IQ of 67 (Wechsler scale). Her facial and behavioural features are consistent with SMS (table 2). She has a waddling gait, loud and hoarse voice, decreased sensitivity to pain, and short fingers and hands. She has low set ears, clino-brachydactyly of the fifth fingers bilaterally, and prognathism. This patient has significant sleep disturbance (including frequent napping and multiple night awakenings), which has improved to some extent with melatonin supplements. Skin picking, onychotillomania (toenails), and polyembolokoilomania (mouth) are constant issues for the family. Overeating and weight management have always been difficult. At age 19, her weight is above the 97th centile, her height is around the 75th centile, and her body mass index (BMI) is above the 97th centile at 34. She has carried a variety of psychiatric diagnoses throughout life, including attention deficit disorder, obsessive-compulsive disorder, pervasive developmental delay (not otherwise specified), and more recently has exhibited bipolar episodes. She has taken a variety of drugs to manage her behaviours.

Additional laboratory studies include a normal karyotype, negative fragile X studies, normal FISH for del(22)(q11.2), and normal FISH for del(17)(p11.2). SMS153 has a heterozygous deletion of 19 bp starting at nucleotide 253 in exon 3 of *RAI1* that causes a frame shift mutation leading to misincorporation of 60 amino acids followed by a stop codon (table 3; fig 2A; fig 3). This change was not seen in her mother's DNA nor was it identified in more than 100 normal chromosomes.

SMS188

SMS188 is a 14 year old boy of European descent who was evaluated in the genetics clinic between the ages of 4 and 14 years (table 2). He is the third child of healthy unrelated parents. He also has three healthy younger half siblings. Family history is negative with regard to mental handicap or congenital malformations. Pregnancy was normal, but he was noted even before birth to be a very active baby. As an infant, he was very restless, cried excessively, and slept poorly. He could walk at 18 months and spoke his first words at a normal age. Starting from infancy, his behaviour was remarkable. Sleep was severely disturbed—typically, he went to sleep around 23.00 h and woke up around 03.30 h. There was head banging and occasionally self mutilation. He destroyed his toys and the furniture in his bedroom. From the age of 5, residential care in a school for children with developmental delay and behavioural difficulties was necessary. His intelligence at the age of 9.5 years was evaluated by WISCR and showed a full scale IQ of 73, a verbal IQ of 85, and a performance IQ of 65.

On clinical examination at age 4 years 10 months, his weight was 20.7 kg (75th centile), his length was 109.5 cm (25th to 50th centile), and his head circumference was 52.5 cm (75th centile). Facial features included a brachycephaly, midface hypoplasia, a tented upper lip, and a broad, square face. He had epicanthic folds and an internal strabismus of the left eye. His hands were broad and short with a transverse palmar crease on the right hand. He was hyperactive, constantly moving around, and on excitation, he clapped his hands. On several occasions, foreign bodies needed to be removed from his ears or nose. He also had a hoarse voice. At the age of 12 years 10 months, his weight was 51.8 kg (75th to 90th centile), his length was 156.9 cm (75th to 90th centile), and his head circumference was 55 cm. Puberty progressed normally. The sleep disturbances were slightly improved, but he remained very active and his behaviour was uncontrolled, including sexual obsessions.

Laboratory findings include a normal karyotype and normal FISH for 17p11.2 deletion. SMS188 carries a deletion of a single cytosine in exon 3 at nucleotide position 3801 on one allele of *RAI1* (table 3; fig 2B; fig 3). This deletion results in a frameshift starting at amino acid 1267, leading to misincorporation of 46 amino acids and a downstream stop codon. Neither parents nor siblings carry this DNA variation, nor has it been observed in more than 100 normal chromosomes.

Table 2 Phenotypic features of SMS patients with *RAI1* mutations compared to those with 17p11.2 deletions

	Mutation identified								
Common features	**del(17)(p11.2) (%)***	**Slager *et al* (2003)[14] *RAI1* mutations**	**Bi *et al* (2004)[17] *RAI1* mutations**	**SMS153 253del 19 bp**	**SMS188 3801delC**	**SMS195 Ser1808Asn**	**SMS175 Gln1562Arg**	**Total**	**Common features (%) *RAI1* mutations**
Craniofacial/skeletal									
Brachycephaly	89	3/3	1/2	+	+	+	+	8/9	89
Midface hypoplasia	93	0/3	2/2	+	+	+	+	6/9	67
Prognathism (relative to age)	52	3/3	1/2	+	+	N	N	6/7	86
Tented upper lip	73	3/3	2/2	+	+	−	+	8/9	89
Broad, square face	81	2/3	2/2	+	+	+	+	8/9	89
Synophrys	62	2/3	N	−	+	−	−	3/7	43
Cleft lip/palate	9	0/3	0/2	−	−	−	−	0/9	0
Brachydactyly	85	1/3	2/2	+	+	+	+	7/9	78
Short stature ($<5^{th}$ centile)	69	0/3	0/2	−	−	+	−	1/9	11
Scoliosis†	49–67	2/3	2/2	N	−	−	−	4/8	50
Otolaryngologic abnormalities									
Chronic ear infections	85	2/3	1/2	+	−	−	−	4/9	44
Hearing loss	68	1/3	0/2	−	−	−	−	1/9	11
Hoarse, deep voice	80	2/3	N	+	+	+	+	6/7	86
Neurological/behavioural									
Variable mental retardation	100	3/3	2/2	+	+	+	+	9/9	100
Speech delay†	>90	1/3	N	+	−	+	+	4/7	57
Motor delay†	>90	1/3	N	+	+	+	−	4/7	57
Hypotonia	>90	1/3	N	+	−	+	+	4/7	57
Seizures by history	11 to 30	1/3	0/2	−	−	−	+	2/9	22
Sleep disturbance	70 to 100	3/3	2/2	+	+	+	+	7/7	100
Self hugging/hand wringing‡	70 to 100	3/3	2/2	+	+	+	+	9/9	100
Attention seeking	80 to 100	3/3	2/2	+	+	+	+	9/9	100
Self injurious behaviours‡	78 to 96	3/3	2/2	+	+	+	+	9/9	100
Onychotillomania	25 to 85	3/3	N	+	+	−	+	6/7	86
Polyembolokoilomania	25 to 85	3/3	N	+	+	+	+	7/7	100
Head banging/face slapping	71	2/3	N	−	+	+	+	5/7	72
Hand biting/self biting	77	2/3	N	−	+	+	+	5/7	72
Ocular abnormalities									
Myopia	53	2/3	1/2	+	+	+	−	6/9	67
Strabismus	50	2/3	1/2	−	+	−	−	4/9	44
Iris abnormalities	64	0/3	0/2	−	−	+	−	1/9	11
Other features									
Raised cholesterol	>50	3/3	0/2	−	N	+	−	4/8	50
Structural cardiovascular anomaly	30	0/3	0/2	−	−	−	−	0/9	0
Structural renal anomaly	30	0/3	0/2	−	−	−	−	0/9	0
Sex				Female	Male	Male	Female		
Age at evaluation (years)				14 to 19	4 to 14	14 to 17	11		

*Percentages are based on the following references: Smith *et al* 1998,[4] Dykens *et al* 1998,[7] Smith *et al* 2002,[8] Greenberg *et al* 1991,[9] Chen *et al* 1996,[18] Greenberg *et al* 1996,[19] Finucane *et al* 1994,[20] and the Smith-Magenis syndrome GeneReview 2003 (www.geneclinics.org).
†Smith and Gropman, 2005.[21]
‡Finucane *et al*, 2001.[22]
+, Presence of clinical feature; −, absence of clinical feature; N, information is not available or child too young to evaluate.

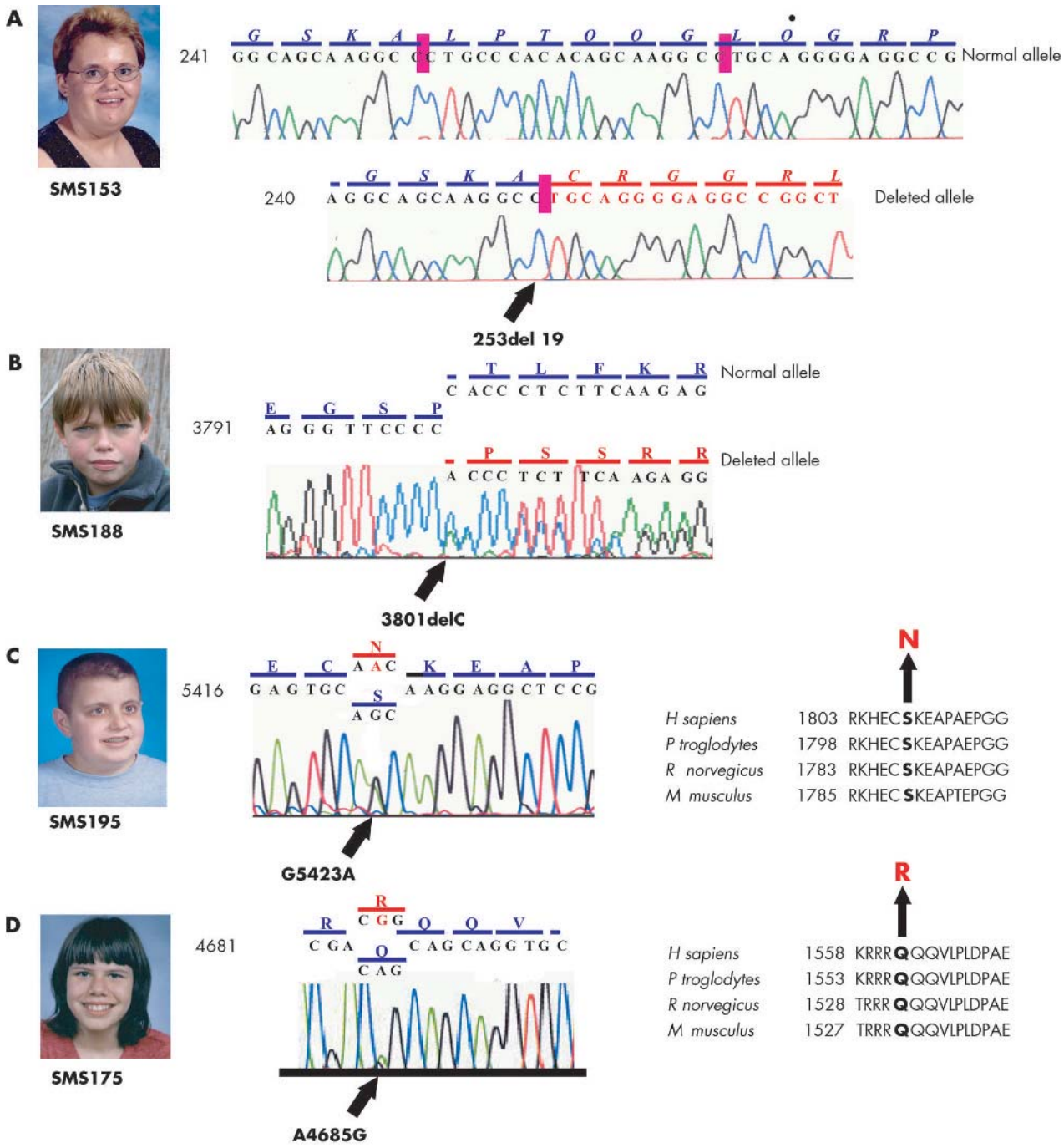

Figure 2 Subjects with Smith–Magenis syndrome who have *RAI1* mutations. (A) Photograph of SMS153, aged 16 years. Electropherograms of both normal and mutant alleles are shown. The mutant allele contains a 19 bp deletion starting at nucleotide position 253. This mutation causes a frameshift mutation and truncation of the protein. (B) Photograph of SMS188, aged 12 years. Electropherogram shows 3801delC on one *RAI1* allele, causing a frameshift mutation. (C) Photograph of SMS195, aged 14 years. Electropherogram shows a heterozygous transition mutation, G5423A, which causes a missense mutation, Ser1808Asn. (D) SMS175, aged 11 years. Electropherogram shows a heterozygous transition mutation, A4685G, causing a missense mutation, Gln1562Arg. Also shown for (C) and (D) are the conserved sequences across human, chimp, rat, and mouse at the regions of the missense mutations, showing that each mutation is located in highly conserved region of *RAI1*. This study was approved by the Virginia Commonwealth University Institutional Review Board and informed consent was obtained for all subjects and permission granted for the reproduction of photographs.

SMS195

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

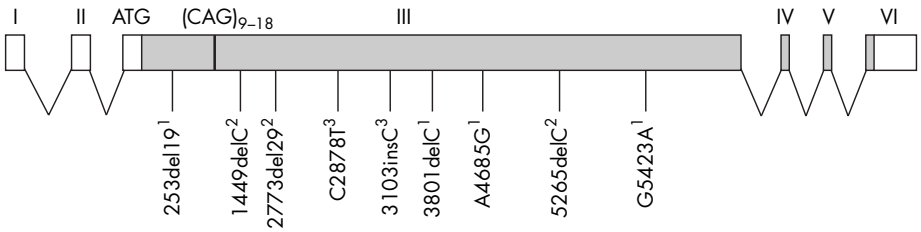

Figure 3 Summary of mutations in *RAI1* associated with Smith–Magenis syndrome. The primary *RAI1* genomic structure with six exons is shown. All mutations reported to date are indicated. Mutations in this report[1] are depicted, along with previously reported mutations from Slager *et al* (2003)[2], and Bi *et al* (2004)[3] (references 14 and 17). The numbering of nucleotides is based on Genbank AY172136. The unfilled boxes represent non-coding regions while the filled boxes represent the *RAI1* coding region.

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). 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.

SMS175

SMS175 is an 11 year old white girl with mental retardation, progressive speech delay, stereotypic behaviour, intractable complex seizures, and facial dysmorphisms.

She was delivered vaginally at 42 weeks' gestation weighing 4.1 kg to a 33 year old mother with two normal, living offspring. Behavioural problems were first noticed at the age of nine months, with explosive tantrums when distracted from ''autistic-like'' activity. She sat at six months and walked at 12 months, but speech was delayed and regressed at age 4, when seizures began. Seizures were complex and involved grand mal, Jacksonian, and petit mal episodes occurring 10 to 30 times a month. Language, psychosocial, motor, and cognitive skills are below the 7th centile, with an IQ of 57 (Wechsler scale). Her behaviour includes self hugging, twirling of objects, biting and picking of toenails until they bleed, and polyembolokoilamania of rectum, umbilicus, ears, and mouth. She was frequently irritable in infancy, often with sleep disturbance. She is currently sedated as a component of seizure therapy but has no apparent abnormal sleep patterns. Her EEG showed 2.5–5 Hz delta activity with medium to high amplitude spikes, sharps and polyspikes seen over the right central parietal, right occipital and bifrontal regions, and at times diffusely over both sides. Magnetic resonance imaging showed a slight asymmetry in the gyral sulcation pattern along the left posterior frontal lobe in the precentral gyrus. She was diagnosed with microcephaly as an infant, with a head circumference below the 5th centile, but head growth gradually improved to the normal range. Height and weight remained at the 25th centile throughout early childhood, with gradual increases over time.

On recent physical examination she was on the 75th centile for weight and height and the 50th centile for head circumference. She had a flat occiput and a round face, like her mother's. There was no midfacial hypoplasia (IC = 3.3 cm, OC = 6 cm, IP = 5.5 cm). She had full lips with tenting of the upper lip. There were multiple small, non-pigmented papillae on her tongue. She was Tanner grade II–III in sexual development. She had mild acne of the face, two 2×3 cm café-au-lait spots on the trunk, and eczema behind the ears. She showed self stimulating and exclusionary concentration on colouring or twirling objects, with oppositional behaviour, including biting, if interrupted.

Laboratory data include a normal 46,XX karyotype with no evidence of 17p11.2 deletion. Total fasting cholesterol was 3.68 mmol/l (142 mg/dl) and triglyceride 1.75 mmol/l (155 mg/dl). Her *RAI1* gene has a heterozygous adenine to guanine transition at nucleotide 4685, producing a missense substitution of an arginine for a glutamine at amino acid 1562 (table 3; fig 2D; fig 3). This Q1562R mutation was not seen in either parent's DNA, nor has it been observed in more than 100 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*[17] 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 during early childhood, with weight and height for age gradually increasing to over the 50th centile in all the patients evaluated, with the exception of SMS195 who appears 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

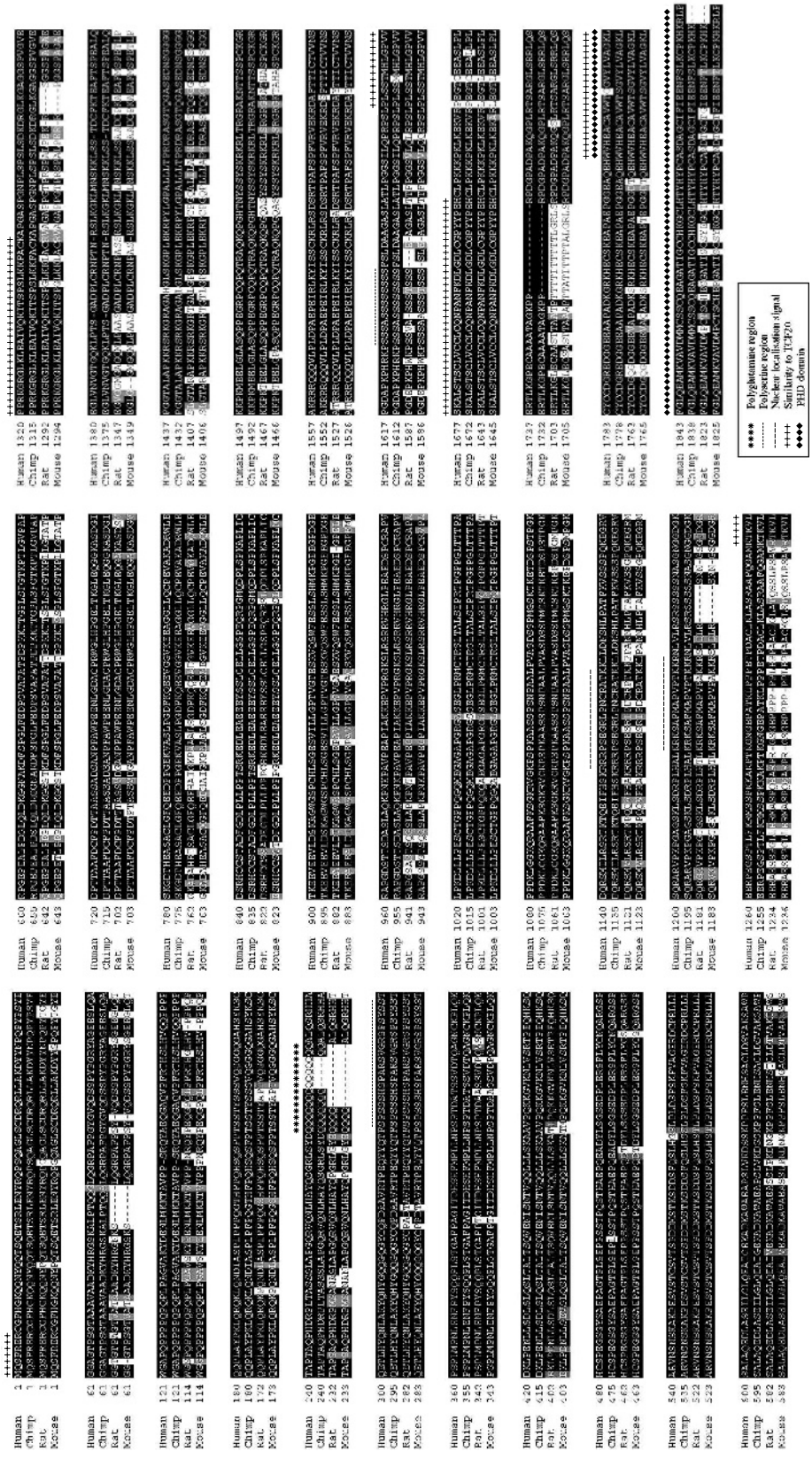

Figure 4 RAI1 is highly conserved in mammalian species. Alignment of human RAI1 (NP_109590.3), chimp (Chr 19, uncurated sequence numbers from 17975106-18062618, UCSC Genome browser), rat (XP_220520.5), and mouse Rai1 (NP_033047.1) sequences. Human and mouse sequences are 84% identical and 88% similar, 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.

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

Patient ID	Nucleotide change*	Amino acid change	Polymorphisms†
SMS153	253del(19 bp)	Deletion/frameshift	Pro664Pro‡ (homozygous) Ile1867Ile§ (heterozygous) $(CAG)_{13}$
SMS188	3801delC	Frameshift	Gly90Ala** (heterozygous) Pro165Thr†† (heterozygous) Gln279Gln¶ (heterozygous) Pro664Pro (heterozygous) Arg1778Arg‡‡ (heterozygous) Ile1867Ile (heterozygous) $(CAG)_{10/11}$
SMS195	G5423A	Ser1808Asn	Pro664Pro (homozygous) Ile1867Ile (heterozygous) $(CAG)_{10/11}$
SMS175	A4685G	Gln1562Arg	Gln279Gln (heterozygous) Pro664Pro (heterozygous) Ile1867Ile (heterozygous) $(CAG)_{9/10}$

*DNA changes with nucleotide number starting from the ATG in the coding sequence.
†NCBI SNP database: ‡SNP 8067439; §SNP 3818717; ¶SNP 11078398; **SNP 3803763; ††SNP 11649804; ‡‡G→A change at nucleotide 5334 (a novel polymorphism not reported in the database).
$(CAG)_X$ represents the number of CAG repeats present on each allele.

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.[13]

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 implicated in modulation of the susceptibility to schizophrenia, and one study showed correlation with the age of onset in spinocerebellar ataxia type 2 (SCA2).[23 24] The role of the polyglutamine repeat in any alteration of RAI1 function is unknown but may have its effects on normal neuronal development or function. Additional studies are required to fully understand any potential interaction. No polyglutamine expansion in RAI1 has been identified or reported.

RAI1 encodes a 1906 amino acid protein with a predicted molecular weight of 203 kDa (fig 4). The ~8.0 kb mRNA is expressed in all tissues studied so far and at high levels in the heart and neuronal structures.[14 15] This gene/protein is highly conserved through mammalian evolution (fig 4). The polyadenylation signal spans nucleotides 7685 to 7641, and the gene has a large 3′-UTR. The polyserine signal at the carboxy terminal end (residues 1628 to 1639) is composed of [S]3A[S]8, with stretches of three and eight serines interrupted by an alanine (fig 4). This polyserine signal is similar to that found in the *DRPLA* gene and the *Drosophila* hairless gene, both of which are involved in neuronal development.[25–27] These stretches of polyglutamine and polyserine have also been shown to be involved in transcription.[28] RAI1 has an extended PHD domain (residues 1823 to 1903) similar to that in the trithorax family of nuclear proteins which is involved in chromatin remodelling and transcriptional regulation (fig 4).[29–31] Further, amino acid sequence motifs representing four domains of the RAI1 protein are similar to the transcription factor stromelysin-1 platelet derived growth factor responsive element binding protein, TCF20 (residues 1 to 6, 1315 to 1347, 1665 to 1710, and 1823 to 1842; see fig 4).[25 32]

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.

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Competing interests: none declared

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ECHO

Young patients with colorectal cancer are genetically susceptible

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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 (HNPCC). Testing should be done even if family histories do not conform to the Amsterdam criteria for HNPCC, 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 HNPCC. Among the others, four out of five patiants 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 reports were reviewed 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 HNPCC-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.

▲ Durno C, *et al*. *Gut* 2005;**54**:1146–1150.