Clinical Report

Smith–Magenis Syndrome and Moyamoya Disease in a Patient With del(17)(p11.2p13.1)

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Chromosomal rearrangements causing microdeletions and microduplications are a major cause of congenital malformation and mental retardation. Because they are not visible by routine chromosome analysis, high resolution whole-genome technologies are required for the detection and diagnosis of small chromosomal abnormalities. Recently, array-comparative genomic hybridization (aCGH) and multiplex ligation-dependent probe amplification (MLPA) have been useful tools for the identification and mapping of deletions and duplications at higher resolution and throughput. Smith–Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation syndrome caused by deletion or mutation of the retinoic acid induced 1 (RAI1) gene and is often associated with a chromosome 17p11.2 deletion. We report here on the clinical and molecular analysis of a 10-year-old girl with SMS and moyamoya disease (occlusion of the circle of Willis). We have employed a combination of aCGH, FISH, and MLPA to characterize a ~6.3 Mb deletion spanning chromosome region 17p11.2–p13.1 in this patient, with the proximal breakpoint within the RAI1 gene. Further, investigation of the genomic architecture at the breakpoint intervals of this large deletion documented the presence of palindromic repeat elements that could potentially form recombination substrates leading to unequal crossover.

Key words: Smith–Magenis syndrome; MLPA; array-CGH; 17p11.2 deletion; moyamoya; cerebrovascular abnormalities

INTRODUCTION

Deletions and duplications in the human genome are a frequent cause of disease ranging from complex mental retardation disorders to cancer. Many options are available for the detection of these copy number abnormalities offering varying degrees of throughput and resolution. High throughput detection of copy number variations across the human genome has recently improved with the use of array-comparative genomic hybridization (aCGH), which uses an array of thousands of bacterial artificial chromosome (BAC) clones each containing a segment of the human genome [Pinkel et al., 1998; Vissers et al., 2003]. Investigation of deletions and duplications of individual loci can be achieved directly on patient chromosomes using fluorescent in situ hybridization (FISH). Since the resolution of FISH or aCGH is limited to 150–300 kb, further delineation of breakpoints near the gene of interest requires higher resolution techniques [Trask, 2002]. Multiplex ligation-dependent probe amplification...
(MLPA) has been useful in identifying deletions and duplications of susceptible loci at a higher resolution than FISH and aCGH [Schouten et al., 2002].

Smith–Magenis syndrome (SMS, OMIM # 182290), a congenital disorder with mental retardation, behavioral abnormalities, and multiple anomalies is due to a 17p11.2 deletion encompassing RAI1 or mutations in the RAI1 gene [Smith et al., 1986; Greenberg et al., 1991, 1996; Slager et al., 2003; Girirajan et al., 2005]. While RAI1 is responsible for the SMS phenotype, systemic manifestations and severity and variability of SMS are attributed to other genes in 17p11.2 [Girirajan et al., 2006]. Here, we report the clinical and molecular analysis of a 10-year-old girl with a large interstitial deletion involving chromosome arm 17p and phenotypes of SMS and moyamoya disease (OMIM % 252350, 607151, and 608796). While FISH studies using commercial SMS and RAI1-specific probes were normal, using a combination of FISH, MLPA, and aCGH, we were able to determine the extent of the chromosomal abnormality, identifying an ~6.3 Mb deletion of chromosome 17p in this patient. Further, bioinformatic analysis of the deletion breakpoint demonstrated the presence of palindromic AT-rich repeats that can lead to recombination events. This is an excellent example of the utility of multiple cytogenetic and molecular techniques to map a large deletion at a higher resolution that otherwise would have been difficult using a single technique and may have resulted in a misdiagnosis.

CLINICAL REPORT

The patient is a girl born to healthy nonconsanguineous parents of Chinese descent (Fig. 1). After a term, uncomplicated pregnancy and delivery, her birth weight was 2,640 g (<10th centile). She was first evaluated in the genetics clinic at age 5 months because of concerns of developmental delay, recurrent vomiting, and failure to thrive. She had a short neck, short palpebral fissures with bilateral epicanthal folds, small mouth with down-turned corners, and low set ears (anthropometric data are included in Table I). She had marked truncal hypotonia with poor head control, a single left palmar crease, and one large café au lait spot on her left arm. Results of initial evaluations, including TORCH screen and thyroid function tests, were normal. An echocardiogram showed a small ventricular septal defect. An abdominal ultrasound showed normal kidneys, liver, and spleen. By age 2 years, the patient had variable appetite with some episodes of choking. She also had sleep difficulties and episodes of head banging when upset, characteristic of SMS. She started walking independently at age 3 years but had an ataxic gait.

The patient was diagnosed with moyamoya disease at age 5 years. A brain MRI showed multiple areas of old ischemic lesions involving the frontal lobes and the left parietal lobe, as well as an abnormal white matter signal in the periventricular area due to ischemic changes (Fig. 1D, E).
The occlusion of both supraglenoid internal carotid arteries was consistent with the diagnosis of moyamoya disease. She was treated with aspirin. A SPECT scan indicated perfusion defects in the right frontal lobe and the left parieto-occipital lobe. Angiogram studies confirmed the diagnosis of moyamoya disease and demonstrated paucity of perfusion; therefore, she underwent pial synangiosis for “brain in jeopardy” with good clinical outcome (Fig. 1F). The parents deny any history of seizures. Further, following craniotomy for the management of moyamoya, there was a steady progression of her disabilities, consistent with significant cognitive delays.

Significant language and developmental delay was apparent by 7 years of age. Chromosome analysis at age 8 years from cultured peripheral blood lymphocytes by GTG banding method at 500 band resolution showed an interstitial deletion of the short arm of chromosome 17: 46,XX,del(17)(p11.2p13.1). Initial evaluation by fluorescent in situ hybridization (FISH) was done using commercially available FISH probes—LIS1 (Miller–Dieker syndrome), SMS Vysis probe (Abbott Molecular Inc., Des Plaines, IL), and SMS (D17S29) Oncor probe (Qbiogene Inc., Irvine, CA). All FISH studies were normal, suggesting the deletion was distal to the SMS region. Parental chromosomes were normal.

The patient, now a 10-year-old (Fig. 1A,B), is able to eat finger foods; however, her parents always feed her. She has developed no words, and her communication is via grunts with some use of sign language. Her receptive language is better, and she understands two-step commands. She does not have the very social-friendly personality seen in SMS. She does not like crowds and gets very nervous in crowded environments. Her sleep is very disorganized and disrupted. She wakes up in the middle of the night and may lay awake for most of the night. She takes multiple 10 min naps during the day, but in general her sleep is always in short periods. She received a treatment trial of melatonin with partial improvement of her sleeping patterns. The parents deny history of self-hugging behavior but describe that she has some degree of hand-wringer. She is attention-seeking, in particular by making noises and hitting objects, which has led to some hypertrophy of the tips of the toes from repeated trauma (Fig. 1C). She does not have tendency to put objects in her bodily orifices, but she does put her hands in her mouth. When she is angry, she has some face-slapping behavior, but the parents deny any hand or self-biting or skin-picking behaviors. The parents are nonconsanguineous, and deny any history of moyamoya disease, developmental delay, or congenital anomalies in any family members.

Physical examination showed two depressions in the temporal and occipital regions corresponding to the areas of the craniotomy for management of moyamoya disease. She also had mid-face hypoplasia, hypertelorism, and bilateral epicanthal folds, which are in part due to her Chinese ethnic background (Fig. 1). Her palpebral fissures were upslanted and narrow. She had strabismus. No other ophthalmologic abnormalities were documented in this patient. The forehead was prominent with high anterior hairline. The nose was normally formed with a prominent bridge, hypoplastic nares, and a short columella. Her philtrum was short, and the upperlip was thick and tented. The mouth had no lesions, the chin was pointed, and there was no evidence of clefting. Ears were posteriorly angulated and normally formed. Cardiovascular and abdominal findings were normal. The hands were wrinkled and irritated from constant saliva exposure. Her feet had very significant callus and enlargement of the second, third, fourth, and fifth toes possibly due to the constant banging of the feet against the floor and the walls which the patient does when she is seeking attention (Fig. 1C). Neurologically, she had significant developmental delay and an ataxic gait with a wide based walk. Deep tendon reflexes were brisk.

**MATERIALS AND METHODS**

**Patient Samples**

Research samples were collected at the Hospital for Sick Children, University of Toronto in accordance with IRB approved protocols of the Virginia Commonwealth University. Peripheral blood samples were collected, and metaphase chromosomes and DNA were prepared using standard cytogenetic methods.

**Fluorescent In situ Hybridization**

In order to delineate the chromosome 17 deletion, probes were chosen from our previously published SMS contig map of BAC and PAC clones, [Lucas et al., 2001] and also from UCSC genome browser (May 2004 version). The FISH probes with their

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**TABLE I. Anthropometric Data for the Patient**

<table>
<thead>
<tr>
<th>Age</th>
<th>Head circumference (cm)</th>
<th>Weight (kg)</th>
<th>Length/height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 months</td>
<td>39.7 cm</td>
<td>4.95 kg</td>
<td>54.5 cm</td>
</tr>
<tr>
<td>at 25th centile</td>
<td>&lt;3rd centile</td>
<td>&lt;3rd centile</td>
<td>76 cm</td>
</tr>
<tr>
<td>2 years</td>
<td>45.5 cm</td>
<td>8.3 kg</td>
<td>104 cm</td>
</tr>
<tr>
<td>&gt;25th centile</td>
<td>&lt;3rd centile</td>
<td>at 3rd centile</td>
<td>76 cm</td>
</tr>
<tr>
<td>7 years</td>
<td>47 cm</td>
<td>12.77 kg</td>
<td>113 cm</td>
</tr>
<tr>
<td>&lt;10th centile</td>
<td>&lt;3rd centile</td>
<td>&lt;10th centile</td>
<td>76 cm</td>
</tr>
<tr>
<td>10 years</td>
<td>48.5 cm</td>
<td>17.2 kg</td>
<td>113 cm</td>
</tr>
<tr>
<td>&lt;10th centile</td>
<td>&lt;10th centile</td>
<td>&lt;3rd centile</td>
<td>76 cm</td>
</tr>
</tbody>
</table>

*Growth centiles are based on Chinese growth charts Chang et al. [1965].*
representative loci are listed from centromeric to telomeric as follows: RP11-347A12 (FLII), RP1-253P07 (RAI1), CTD-4023 (PEMT), CTC-335P17 (COPS3), RP11-367G9 (D17S740/M-RIP), RP1-48J14 (TNFRSF13B), RP11-198H15 (SMS REP-D), and RP11-764O6 (PMP22) (Fig. 2). FISH was performed as previously described [Vlangos et al., 2003].

**Multiplex Ligation-Dependent Probe Amplification (MLPA)**

MLPA analysis was carried out using the standard protocol of MRC-Holland [Schouten et al., 2002] using two commercially available MLPA kits that cover chromosome 17-P064 (MR1) and P033 (CMT1/HNPP). However, since the P064 kit does not contain probes specifically for the RAI1 gene, we designed probes for RAI1 exons 1, 3, 4, and 6 to use in conjunction with this kit (Fig. 2, Table II for RAI1 probe sequences and sizes). These probes were directly synthesized with a 5' phosphate added to the second probe of each pair to allow ligation and purified by reversed-phase HPLC (Thermo Hybaid, Ulm, Germany). The P064 MLPA test kit was followed per manufacturer instructions except that 1.5 μl (1.33 fmol) of the RAI1 probe mix was used per P064 MLPA test with the amount of the salt solution (1.5 M KCl, 300 mM Tris-HCl pH 8.5, 1 mM EDTA) in the initial denaturation step reduced to 2.5 μl to allow for the extra 1.5 μl of the RAI1 probe.

**Fig. 2.** Detection and delineation of an ~6.3 Mb deletion in the patient. A: Chromosome 17p loci from telomere to centromere evaluated by FISH, aCGH, and MLPA are shown. Deleted loci are shown in red. PRPSAP2 was deleted by MLPA but could not be confirmed by FISH (using CTC-457L16/D17S29 probe) or aCGH. B: FISH of metaphase spreads using an RAI1-specific test probe, RP1-253P07 (green signal), and RP11-314M5 as a 17q control probe (red signal) with green and red arrows indicating their location on chromosome 17, respectively. The patient is not deleted for this probe since the test probe signal is seen on both chromosomes 17. C: Genotyper® traces comparing the peak heights of MLPA analysis of a normal control individual and the patient. Half height peaks indicating deletion for that particular probe are seen for RAI1 exon 1 and TNFRSF13B (in red). RAI1 exons 3, 4, and 6 have full length peaks and are not deleted. MLPA probes designed for RAI1 (see Table I) and the MRC-Holland kit P064 for Smith–Magenis syndrome was used (see Methods). D: aCGH data for the patient showing loss of DNA indicated by a peak that spans 17(p11.2/p13.1) (bottom). The area of deletion is diagrammatically represented by a red box on the chr. 17 ideogram (top).
mix. The binding sites of some of the probes were sequenced to rule out any polymorphism that may interfere with the data analysis. For example, the PRPSAP2 probe binding region was sequenced for validation of the experiment. The MLPA PCR product (1 μl) was run on an ABI PRISM® 3100 Genetic Analyzer, and the data were analyzed using Genotyper (version 3.7) software (Applied Biosystems, Foster City, CA). Each MLPA reaction had ~80 μm diameter spots with 150 μm center to center spacing creating an array of ~39,000 elements. The printed slides were dried overnight and UV-crosslinked (350 ml) in a Stratalinker 2400 (Stratagene, Inc., La Jolla, CA) immediately before hybridization. A complete list of the RPCI-11 BAC clones spotted on the 19K array can be found at: http://microarrays.roswellpark.org.

Reference DNA, 500 ng from each parent and 1 μg of the patient’s (test) DNA was fluorescently labeled using the BioPrime aCGH Labeling System (GE Healthcare, Piscataway, NJ). Initially, the DNA was denatured in the presence of the random primer at 99°C for 10 min in a thermal cycler and then quickly cooled to 4°C. The tubes were transferred to ice and labeled with dNTP-cyanine 3 (or dNTP-cyanine 5) and Klenow fragment before incubation at 37°C. The unincorporated nucleotides were removed using a QIAquick PCR purification column (Qiagen Inc., Valencia, CA), and the labeled probe was eluted. Prior to hybridization, the test and reference probes were combined with 100 μg human fluorometric Cot-1 (Invitrogen, Carlsbad, CA) and ethanol precipitated. Following centrifugation, the probes were resuspended in 110 μl SlideHyb Buffer #3 (Ambion, Austin, TX) containing 5 μl of 100 μg/μl Yeast tRNA (Invitrogen), heated to 95°C for 5 min and incubated for 30 min at 37°C to block repetitive elements on the probe. Hybridization to the 19K CGH arrays was performed for 16 h at 55°C using a GeneMachine hybridization station (Genomic Solutions, Ann Arbor, MI) as described previously [Cowell and Nowak, 2003]. Following post hybridization washes, the slides were spun dry and scanned immediately at 5 μm resolution using a GenePix 4000B laser scanner (Axon, Inc. Sunnyvale, CA).

Image analysis was performed using the BlueFuse package from Cambridge BlueGnome. A subgrid loess corrected log2 ratio of the background subtracted test/control was calculated for each clone to compensate for non-linear raw aCGH profiles in

<table>
<thead>
<tr>
<th>Location and size</th>
<th>Probe sequences</th>
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<tbody>
<tr>
<td>Exon 1 (118 bp)</td>
<td>5′–GGGTTCCTAAGGTTGAGGATCTGCTGAGTTCCTCAAGACTTCAGCAAGTGCCCAGTGGCGAACCAGGTGAGAGATTGTCCTAGATTGAGATCCTGGCCAC–3′</td>
</tr>
<tr>
<td>Exon 3a (103 bp)</td>
<td>5′–GGGTTCCTAAGGTTGAGGATCTGCTGAGTTCCTCAAGACTTCAGCAAGTGCCCAGTGGCGAACCAGGTGAGAGATTGTCCTAGATTGAGATCCTGGCCAC–3′</td>
</tr>
<tr>
<td>Exon 3b (108 bp)</td>
<td>5′–GGGTTCCTAAGGTTGAGGATCTGCTGAGTTCCTCAAGACTTCAGCAAGTGCCCAGTGGCGAACCAGGTGAGAGATTGTCCTAGATTGAGATCCTGGCCAC–3′</td>
</tr>
<tr>
<td>Exon 4 (113 bp)</td>
<td>5′–GGGTTCCTAACGGTTGAGGATCTGCTGAGTTCCTCAAGACTTCAGCAAGTGCCCAGTGGCGAACCAGGTGAGAGATTGTCCTAGATTGAGATCCTGGCCAC–3′</td>
</tr>
<tr>
<td>Exon 6 (123 bp)</td>
<td>5′–GGGTTCCTAAGGTTGAGGATCTGCTGAGTTCCTCAAGACTTCAGCAAGTGCCCAGTGGCGAACCAGGTGAGAGATTGTCCTAGATTGAGATCCTGGCCAC–3′</td>
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each sample. Mapping information was added to the resulting $\log_2$ test/control values. The mapping data for each BAC was found by querying the Genome browser and examined for regions of large-scale variation (LSV) in the human genome [Iafrate et al., 2004; Sharp et al., 2005; Tuzun et al., 2005]. The mode adjusted $\log_2$ test/control (ALR, short for adjusted log ratio ALR) value was calculated for each BAC clone by subtracting the mode of a non-parametric density estimate of all spots in that array from the mean of the loess corrected $\log_2$ ratios for the spots that passed the quality control steps.

### Analysis of Recombination Breakpoints

Sequence analysis to identify recombinitogenic repeat elements, including palindromic AT-rich repeats was performed using UCSC Genome Browser (www.genome.ucsc.edu). A palindrome recognition algorithm (www.bioinfo.cs.technion.ac.il) [Tsuno da et al., 1999] was used to identify palindromic AT-rich repeats sequences. BLAST analysis [Altschul et al., 1990] was used to identify other similar sequences at the breakpoint regions that could form recombination substrates. Repeats were localized to the deletion breakpoints using BLAT (www.genome.ucsc.edu).

### RESULTS

We report here on the clinical and molecular evaluation of a 10-year-old girl with SMS and moyamoya disease. Initial cytogenetic analysis of peripheral blood from this patient by karyotyping and standard GTG banding techniques revealed a chromosome 17 microdeletion, 46,XX,del(17) (p11.2p13.1). FISH studies with commercially available probes for 17p11.2 and distal 17p were negative. Due to a strong clinical suspicion of SMS and also due to the fact that currently available commercial FISH probes for SMS do not detect RAI1 deletion [Vlangos et al., 2005], an RAI1 specific probe (RP1-253P07) was used for further evaluation. FISH with the RAI1-probe showed no deletion (Fig. 1). Since RAI1 was not deleted by FISH, to completely rule out the involvement of RAI1 in this SMS patient and to delineate the proximal breakpoint of the deletion, MLPA was performed.

Array-CGH was also performed in order to delineate the distal breakpoint of the deletion and to identify other possible genomic rearrangements that might be responsible for the severe, previously unreported, clinical features in this patient.

MLPA analysis localized the centromeric extent of the deletion to exon-1 of RAI1 and the telomeric extent to between MAP2K4 and ASPA. Array-CGH analysis further delineated the distal breakpoint of the deletion to FLJ45455 on 17p13.1 and confirmed the proximal breakpoint to RAI1 on 17p11.2. The combination of FISH, MLPA, and aCGH confirmed the deletion to be ~6.3 Mb and to contain more than 25 known genes including exon 1 of RAI1. Additional genes in the area of deletion include PMP22, HS3ST3B1, COX10, HS3STX1A, ELAC2, MYOCD1, MAP2K4, ZNF18, and MYH1. Genes proximal to RAI1 were not shown to be deleted by any of these techniques, with the exception of PRPSAP2, which showed deletion with an MLPA probe (data not shown), but FISH (using CTC457L16) and aCGH did not confirm this deletion. No other genomic alterations were identified by aCGH.

The manifestations of our patient were compared to published information on SMS patients with 17p11.2 deletions as well as those with mutations (Table III). Data show that this patient has significantly short stature and growth retardation with weight, height, and head circumference consistently below the 10th centile for her age (Table I). While core SMS traits

<table>
<thead>
<tr>
<th>TABLE III. Smith–Magenis Syndrome Manifestations</th>
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<tr>
<td>Clinical findings</td>
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<td>-------------------</td>
</tr>
<tr>
<td>Craniofacial/skeletal</td>
</tr>
<tr>
<td>Brachycephaly</td>
</tr>
<tr>
<td>Midface hypoplasia</td>
</tr>
<tr>
<td>Prognathism (relative to age)</td>
</tr>
<tr>
<td>Tented upper lip</td>
</tr>
<tr>
<td>Broad, square face</td>
</tr>
<tr>
<td>Synophrys</td>
</tr>
<tr>
<td>Cleft lip/palate</td>
</tr>
<tr>
<td>Brachydactyly</td>
</tr>
<tr>
<td>Short stature</td>
</tr>
<tr>
<td>Scoliosis</td>
</tr>
<tr>
<td>Otolaryngologic</td>
</tr>
<tr>
<td>Chronic ear infections</td>
</tr>
<tr>
<td>Hearing loss</td>
</tr>
<tr>
<td>Hoarse, deep voice</td>
</tr>
<tr>
<td>Neurological/behavioral</td>
</tr>
<tr>
<td>Variable mental retardation</td>
</tr>
<tr>
<td>Speech delay</td>
</tr>
<tr>
<td>Motor delay</td>
</tr>
<tr>
<td>Hypotonia</td>
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<tr>
<td>Seizures by history</td>
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<tr>
<td>Sleep disturbance</td>
</tr>
<tr>
<td>Self-hugging/hand-wringer</td>
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<tr>
<td>Attention-seeking</td>
</tr>
<tr>
<td>Self-injurious behaviors</td>
</tr>
<tr>
<td>Onychotillomania</td>
</tr>
<tr>
<td>Polyembolokoilomania</td>
</tr>
<tr>
<td>Head-banging/face-slapping</td>
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<tr>
<td>Ocular</td>
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<tr>
<td>Myopia</td>
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<tr>
<td>Strabismus</td>
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<tr>
<td>Other manifestations</td>
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<tr>
<td>Cardiovascular abnormalities</td>
</tr>
<tr>
<td>Renal/urinary tract abnormality</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
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<tr>
<td>Dental anomalies</td>
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</table>

*aModified from Girirajan et al. (2006).*
such as mental retardation, sleep disturbance, self-injurious, and stereotypical behavior are suggestive of an RAI1-mutation phenotype, the presence of short stature, significant speech and motor delay, and hypotonia are more consistent with 17p11.2 deletion [Giriirajan et al., 2006]. Patients will also likely show signs of hereditary neuropathy with liability to pressure palsies (HNPP) due to deletion of the PMP22 gene in 17p12. The unusual finding of moyamoya disease in this patient could be independent of the chromosomal deletion or may be due to hemizygosity of other genes in the deletion region.

In an attempt to explain the mechanism for the large deletion in this patient, the genomic structure of the breakpoint region was analyzed for predisposing architecture. Palindromic AT rich repeats near the proximal and distal breakpoints: (AT)_{18} in intron 1 of RAI1 and (AT)_{15}, (AT)_{30}, (AT)_{25}, (AT)_{28}, and (AT)_{23} located in FLJ45455 distal to MAP2K4 were identified. These AT rich repeats have been implicated as possible recombination substrates leading to uncommon deletions [Shaw et al., 2004b].

**DISCUSSION**

We present here the molecular analysis of a large chromosome 17 deletion in a patient with SMS and moyamoya disease using a combination of FISH, MLPA, and aCGH. While initial karyotyping and GTG banding showed a microdeletion of 17p11.2 consistent with SMS, FISH studies did not support this diagnosis. In order to map the extent of the deletion and to confirm a diagnosis of SMS, FISH, with an RAI1-specific probe, RP1-253P07 was performed, but the metaphase chromosomes of this patient showed no deletion with this probe. However, probes distal to RAI1 were deleted by FISH. Deletion analysis at the proximal end was continued for two reasons: first, RAI1 was not completely ruled out in this case due to the presence of core SMS manifestations, including mental retardation, sleep disturbance, and behaviors that are solely due to RAI1 [Giriirajan et al., 2006] and second, to map the proximal extent of the deletion at higher resolution using MLPA. Thus, the higher resolution of the MLPA experiments demonstrated the proximal deletion breakpoint to reside within the 5' end of the RAI1 gene, between exons 1 and 3. Using an array of BAC clones, we were able to localize the distal breakpoint to FLJ45455 at 17p13.1, and to confirm the proximal end of the deletion within the RAI1 gene. We conclude that haploinsufficiency of RAI1 due to loss of exon-1 and regulatory regions at the 5' end of the gene are responsible for the core SMS signs in the patient.

Central nervous system manifestations have been previously reported in SMS: Smith et al. [1986] reported an infant with SMS with foreshortened frontal lobe with frontal delection of neurons and small choroid plexus hemangioma in the lateral ventricle; Greenberg et al. [1996] reported structural anomalies in the brain in the form of ventriculomegaly in 50% of the cases; Boddaert et al. [2004] reported lenticulo-insular brain anomalies with bilateral decrease of grey matter associated with hypoperfusion of these regions; and Natacci et al. [2000] identified a case of SMS carrying an atypical deletion with clinical findings of Joubert syndrome (hypoplasia of cerebellar vermis). The patient has frontal and parietal lobe ischemia and white matter changes in the periventricular region. These neuropathological findings are secondary to the vasculopathy (occlusion of the circle of Willis) rather than a primary structural defect. Further studies of SMS patients with cerebrovascular manifestations might provide insight into the underlying pathogenesis of CNS anomalies in SMS.

Moyamoya disease, resulting from spontaneous occlusion of the terminal portion of the internal carotid artery (circle of Willis) at the base of the brain has an estimated incidence of ≥3 per 100,000 in Asian populations [Ikeda et al., 1999]. About 9% of cases with moyamoya disease are familial with either polygenic or autosomal dominant inheritance with female predominance and likely genetic anticipation [Ikeda et al., 1999; Mineharu et al., 2006; Nanba et al., 2006]. By linkage analysis, disease loci have been mapped to 3q24.2-p26 [Ikeda et al., 1999], 17q25 [Yamauchi et al., 2000], and 8q23 [Sakurai et al., 2004] in Japanese patients with moyamoya; however, no causative gene has been identified. It is the most critical cause of childhood stroke in the Japanese population [Yamauchi et al., 2000]. Moyamoya in our patient is a sporadic event and its co-occurrence with SMS is responsible for the severe phenotype. Also, co-occurrence of moyamoya disease with various other disorders such as neurofibromatosis [Yamauchi et al., 2000], Alagille syndrome [Rachmel et al., 1989], Noonan syndrome [Ganesan and Kirkham, 1997], Turner syndrome [Kawai et al., 1993], Prader Willi syndrome [Kusuhara et al., 1996], Costello syndrome [Shihara et al., 2005], Turner syndrome [Spengos et al., 2006], systemic lupus erythematosi [El Ramahi and Al Rayes, 2000], Behcet disease [Joo et al., 2006], holoprosencephaly [Chen et al., 2006], Graves disease [Kushima et al., 1991], Fanconi anemia [Pavlakis et al., 1995], cardio-facio-cutaneous syndrome [Ishiguro et al., 2002], sickle-cell disease [Seeler et al., 1978], Wilms tumor [Watanabe et al., 1985], Shy-Drager syndrome [Imaoka et al., 1990], hypomelanosis of Ito, encephalotrigeminal angiomatosis, and incontinentia pigmenti [Echenne et al., 1995] have been reported. It is also of interest that our patient also has ventricular septal defect, since...
association of moyamoya disease has been reported in children with congenital heart disease [Lutterman et al., 1998]. Thus, children with SMS carrying a large deletion extending distally beyond 17p11.2 who present with congenital heart disease should be evaluated for moyamoya disease, especially in cases where the deletion extends to 17p13. Hemizygosity of a candidate gene within the deletion region (17p11.2-p13.1) or mutations at another undetermined locus occurring independently of this deletion could be responsible for the moyamoya disease in our case. It is important to note that by aCGH, we have ruled out any other genomic copy number changes in our patient that could be responsible for the moyamoya disease. Further, a review of genes in the deleted region did not detect any obvious candidates responsible for vasculogenesis. Thus, evaluation of more patients with moyamoya disease and chromosome 17p rearrangements may help identify a susceptibility locus in this region.

We have also identified palindromes of AT rich repeat elements in the vicinity of the deletion breakpoints that could potentially form recombination substrates for unequal crossing over. Palindromic AT-rich repeats present in normal chromosomes 11, 17, and 22 have the potential to form intrastrand base-pairing resulting in the formation of hairpins or cruciform structures; the center of the palindrome that forms the tip of hairpin is susceptible to double strand breaks leading to illegitimate recombination [Edelmann et al., 2001; Kurahashi and Emanuel, 2001]. Similar mechanisms have been identified in Saccharomyces cerevisiae where palindromes are highly unstable resulting in cleavage of DNA and loss of hairpin structure, resulting in stabilization of the locus [Gordenin et al., 1993]. Thus, these AT-rich repeats at the breakpoint intervals might have contributed to the deletion in this patient.

Our study using aCGH, MLPA, and FISH shows a complementary approach toward defining the breakpoints of the deletion carried by the patient. MLPA has been used for the detection of gene deletions replacing techniques such as FISH, Southern hybridization, and pulsed field gel electrophoresis (PFGE) [Douglas et al., 2005; Vorstman et al., 2006]. Similarly, aCGH has been useful in the delineation of large genomic deletions and duplications, which otherwise is cumbersome by FISH, and useful also in the high throughput evaluation of a large cohort of patients with mental retardation [Vissers et al., 2003]. Even though use of these techniques individually in the evaluation of chromosomal rearrangements has been widely reported [Veltman et al., 2002; Shaw et al., 2004a,b; Lalic et al., 2005; Langerak et al., 2005; Monfort et al., 2006], rapid diagnosis with higher sensitivity has utilized a combination of these approaches [Hermsen et al., 2005; Kirchhoff et al., 2005; de Vries et al., 2005; Lam et al., 2006].

Thus, we have delineated a ~6.3 Mb deletion at a higher resolution using FISH, MLPA, and aCGH than would have been possible using any one method alone. This allowed for accurate diagnosis of SMS and involvement of the RAII gene as causative for the core SMS characteristics seen in our patient. We recommend use of aCGH for initial detection of genomic rearrangements in patients with undiagnosed mental retardation or known multiple congenital anomaly/mental retardation syndromes with severe/unusual phenotypes, followed by a combination of FISH and MLPA to determine the extent of the abnormality at higher resolution.

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