

A novel microdeletion/microduplication syndrome of 19p13.13

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Purpose: Whole genome interrogation by array-based comparative genomic hybridization has led to a rapidly increasing number of discoveries of novel microdeletion and/or microduplication syndromes. We here describe the clinical and cytogenomic correlates of a novel microdeletion/microduplication of 19p13.13. **Methods:** Among patients referred to the Cytogenetics laboratory for array-based comparative genomic hybridization analysis, we identified four with a deletion and one with a duplication within 19p13.13. Confirmatory fluorescence in situ hybridization and parental studies were performed. Detailed clinical findings and array profiles were reviewed and compared. **Results:** Patients with deletions of 19p13.13 share a unique constellation of phenotypic abnormalities. In addition to developmental disabilities, the microdeletion manifested in overgrowth, macrocephaly, and ophthalmologic and gastrointestinal findings; in contrast, the single microduplication manifested in growth delay and microcephaly. **Conclusion:** The consistent constellation of clinical findings associated with copy number variation of this region warrants the designation of microdeletion/microduplication syndrome of 19p13.13. An approximately 311–340 Kb smallest region of overlap encompassing 16 genes was identified. Candidate genes include *MAST1*, *NFIX*, and *CALR*. Identification of this syndrome has led to recommendations for diagnostic work-up and follow-up of patients with this copy number variant. Integration of detailed clinical and array data is critical for advancing both patient care and human genomic research. *Genet Med* 2010;12(8):503–511.

Key Words: array CGH, chromosome 19p13, microdeletion, microduplication, syndrome

The incorporation of microarray technology into the routine assessment of patients with unexplained developmental disabilities and/or phenotypic abnormalities has resulted in the rapid discovery of numerous recurrent microdeletions/microduplications. For example, over a period of only 2 years, previously undetected losses and/or gains for the genomic regions of 1q21.1^{1,2} and 16p11.2^{3,4} have become widely recognized as potential etiologic factors for autism and related disorders. Copy number vari-

ants (CNVs) of clinical significance that involve too small a chromosomal region to be detected by conventional cytogenetics have been identified for every chromosome pair in the human karyotype. For some CNVs, variability in expression and penetrance of clinical manifestations has complicated the establishment of clinical significance. For others, documenting clinical relevance has been facilitated by association of the CNV with more consistent and specific phenotypic findings, and with de novo inheritance. We here describe a CNV for chromosome 19p13.13 that meets the latter criteria. Despite the fact that chromosome 19 is one of the most gene-rich chromosomes (~2000 genes within 59 Mb), to date, there have been only a few published reports of individuals with deletions involving 19p.^{5–10} Critical to identifying this microdeletion/microduplication syndrome was the consensus characterization of the clinical findings by four clinical geneticists and correlation of those findings with the microarray data.

MATERIALS AND METHODS

Subjects

Five patients were referred for diagnosis to genetics clinics at the University of Minnesota Amplatz Children's Hospital and at the Children's Hospitals and Clinics of Minnesota. Array-CGH was ordered as a component of the standard work-up for patients with unexplained multiple anomalies and/or developmental disabilities. The patients' ages ranged from 5 to 26 months at the time of first examination; each was evaluated by one of four clinical geneticists. Participation in this study to permit sharing of data and publication of results was approved by the Institutional Review Boards of both the institutions. Informed consent was obtained from the parents of these patients.

Array-based comparative genomic hybridization analysis

DNA from the patients' peripheral blood was isolated, restriction digested, and labeled with fluorochrome cyanine 5 using random primers and exo-Klenow fragment DNA polymerase. DNA from pooled sex-matched controls was labeled concurrently with fluorochrome cyanine 3. The DNA of the patient and the control was combined, and an array-based comparative genomic hybridization (aCGH) was performed with a customized 44K oligonucleotide array that included targeted regions of enriched coverage on a backbone of average overall median probe spacing of 35 Kb. To estimate with greater precision the stop and start points for the patient with the smallest deletion (Patient 3), a 180K array with an average tiled spacing of 13 Kb was performed (Agilent Technologies, Santa Clara, CA). The ratio of patient to control DNA for each oligonucleotide was calculated using Feature Extraction software 9.1 or 10.5 (Agilent Technologies), and analysis was performed using DNA Analytics 4.0.85 (Agilent Technologies). Statistical algorithms used for analysis included ADM1 and ADM2 (Agilent Technologies) with threshold values set at an absolute

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Table 1. Clinical and cytogenetic features of patients with loss or gain within 19p13.12-p13.13

Clinical and cytogenetic features of patients with loss or gain within 19p13.12-p13.13					
	Patients				
	1	2	3	4	5
Cytogenetic imbalance	Del 19p13.13-p13.2	Del 19p13.13-p13.2	Del 19p13.13	Del 19p13.13-p13.2	Dup 19p13.12-p13.2
Breakpoints (minimum)	12,498,237–13,126,508	12,536,641–13,794,080	12,793,474–13,104,643	12,411,017–13,120,904	12,601,112–14,488,238
Size (min)	0.6 Mb	1.3 Mb	0.3 Mb (311 Kb)	0.7 Mb	1.9 Mb
Breakpoints (maximum)	12,476,664–13,178,511	12,521,732–13,854,243	12,779,366–13,120,858	12,335,402–13,126,464	12,582,355–14,503,887
Size (max)	0.7 Mb	1.3 Mb	0.3 Mb (341 Kb)	0.8 Mb	1.9 Mb
First examination					
Age	2 yrs	2 yrs	0.75 yrs	0.5 yrs	3 yrs
Length (%ile)	50th	>95th	90th	90th	<5th
Weight (%ile)	75th	>95th	50–75th	90th	25th
OFC (%ile)	>97th	>95th	>98th	>98th	<–2 SD
Craniofacial	Macroceph, frontal bossing	Macroceph, frontal bossing, downslanting PF	Macroceph, frontal bossing, downslanting PF	Macroceph, frontal bossing	Microceph, frontal bossing, downslanting PF
Ophthalmologic	Esotropia, nystagmus, poor fixation	Optic nerve hypoplasia, exotropia	OA, exotropia	Optic nerve hypoplasia, exotropia, nystagmus	Nystagmus
GI	Chronic diarrhea	Abd. pain, vomiting, poor feeding	None	Abd. pain, vomiting, celiac disease	Abd. pain, vomiting, FTT (G-tube)
Brain	Normal	Mild atrophy, frontal lobes	Absent rostral corpus callosum, cystic lesion	Chiari I malformation with syrinx	Normal MRI
Seizures	–	–	+	+	+
Hypotonia	+	–	+	+	Torticollis
Developmental delay	Full scale IQ 49	18-mo skills at 3 yrs; mkd speech delay	Almost nonverbal	Mod. speech delay; needs special ed.	21–29 mo skills at 4.5 yrs; mkd speech delay
Last examination					
Age	6 yrs	3 yrs	14.5 yrs	9.5 yrs	7 yrs
Height (%ile)	86th	>95th	50th	75th	<3rd
Weight (%ile)	55th	>95th	10–25th	50–75th	50th
OFC (%ile)	>97th	>95th	>98th	>98th	–2 SD

Del, deletion; dup, duplication; NM, not mentioned; NE, not evaluated; macroceph, macrocephaly; micro, microcephaly; brachy, brachycephaly; OA, optic atrophy; OFC, occipitofrontal circumference; PF, palpebral fissures; abd., abdominal; mod, moderate; mkd, marked; ed, education.

value of 0.3 on a log₂ scale and with a requirement of three consecutive oligos meeting the threshold criteria.

Fluorescence in situ hybridization analysis

Fluorescence in situ hybridization (FISH) was performed to confirm the presence of a deletion and/or duplication in the

probands and to rule out an interchromosomal rearrangement in the parents that, if found, would significantly impact recurrence risks. The BACs selected for FISH probes were RP11-654K9 (encompassing *MAST1*), RP11-963I8 (encompassing *NFIX*), RP11-14L14 (encompassing *CACNA1A*), and for Patient 5, RP11-56K21 (encompassing *PKN1*). Stabs for RP11-654K9,

Table 1. Continued

Clinical and cytogenetic features of patients with loss or gain within 19p13.12-p13.13					
Patients					
	Lysy et al ⁸	Auvin et al ⁹	Jensen et al ¹⁰	Engels et al ⁷	Stratton et al ⁶
Cytogenetic imbalance	Del 19p13.13-p13.2	Del 19p13.13	Del 19p13.12	Del 19p13.12	Dup 19p13.13-p13.2
Breakpoints (minimum)	10,256,871–13,188,698	12,615,927–13,280,259	13,838,264–16,357,778	NM	NE
Size (min)	2.9 Mb	0.6 Mb	2.5 Mb	2.1 Mb	NM
Breakpoints (maximum)	10,246,651–13,280,203	NM	NM	NM	NE
Size (max)	3.0 Mb	0.7 Mb	NM	NM	NM
First examination					
Age	Birth	Normal growth parameters at birth	Birth	Birth	Birth
Length (%ile)	<–2 SD		NM	<3rd	25–50th
Weight (%ile)	<–2 SD		<1st	NM	20th
OFC (%ile)	–2 SD		NM	<<3rd	15th
Craniofacial	Complex cranio-synostosis, frontal bulge, small nose, low nasal bridge	Macroceph, high, large forehead, flat philtrum, small mouth	Flat occiput, tall forehead, down-slanting PF, long philtrum, short nose	Micro/brachy, thin upper lip, long philtrum, small mouth	Microceph, bifrontal prominence, upslanting PF
Ophthalmologic	Orbital hypoplasia, hypertelorism, proptosis, strabismus	NM	Strabismus, optic disc cupping, ptosis, epicanthal folds	Epicanthal folds	Intermittent exotropia
GI	NM	Severe constipation	NM	NM	Colic post-natally
Brain	Mod. ventriculomegaly	Normal MRI	Mild hypopl. of corpus callosum & cerebellar vermis, prom. 4th vent	Normal	Normal head CT
Seizures	NM	+	NM	NM	NM
Hypotonia	+	+	NM	+	NM
Developmental delay	Stood at 33 mos; no language at 3.7 yrs	Sat at 2 yrs; “psychomotor delay”	Full-scale IQ 63	Crawled at 16 mos; no speech	Did not reach motor milestones, 4 mos
Last examination					
Age	3.7 yrs	2 yrs	NM	1.5 yrs	0.75 yrs
Height (%ile)	–3 SD	>+3 SD	NM	<3rd	50th
Weight (%ile)	–0.9 SD	+2 SD	NM	<3rd	5th
OFC (%ile)	–0.7 SD	+2.5 SD	NM	<<3rd	<5th

Del, deletion; dup, duplication; NM, not mentioned; NE, not evaluated; macroceph, macrocephaly; micro, microcephaly; brachy, brachycephaly; OA, optic atrophy; OFC, occipitofrontal circumference; PF, palpebral fissures; abd., abdominal; mod, moderate; mkd, marked; ed, education.

RP11-14L14, and RP11-56K21 were obtained from CHORI BAC/PAC resources, grown on Luria broth plates with chloramphenicol, and labeled by nick translation (Abbott Molecular, Abbott Park, IL). RP11-963I8, pre-labeled with Enzo-Green, was obtained from Empire Genomics (Empire

Genomics, Buffalo, NY). A probe to the 19q subtelomeric region (D19S238E; Abbott Molecular) was also used as a control. To evaluate the presence or absence of a deletion, 10 metaphase cells were scored, and to evaluate the presence or absence of a duplication, 75–100 interphase cells were scored.

RESULTS

Clinical findings

Detailed clinical history and findings for each patient are provided below and are summarized in Table 1.

Patient 1

This girl was born at term (birth weight 8 lb, 14 oz) (Fig. 1). Difficulties in the neonatal period included jaundice and inability to breastfeed requiring clipping of the frenulum. Her parents had concerns regarding her development at the age of 3 months that became more apparent as she grew older and included gross motor and speech delays over time. Ophthalmologic and visual issues included esotropia, saccadic eye movements, end-gaze nystagmus, and poor fixation. She had delayed maturation of vision that improved over time and had two surgeries to correct strabismus. Gastrointestinal problems, characterized by chronic diarrhea of unknown etiology, were present for the first 4 years.

Physical examination at 22 months showed macrocephaly (>97th percentile), length at the 50th percentile, and weight at the 75th percentile. There was marked frontal bossing, with supraorbital ridging and deep-set eyes in addition to a depressed nasal bridge, upturned nasal tip, and small mouth with a relatively high-arched palate. Her fingers and toes were long, with some deeper creases on the soles of her feet. She was hypotonic with good deep tendon reflexes. Examination at 30 months revealed height and weight >80th percentile and occipitofrontal circumference (OFC) >97th percentile, although only 0.3 cm larger than at 22 months. Follow-up at the age of 6 years showed height at the 86th percentile, weight at the 55th percentile, and OFC 1 SD above the 97th percentile. On the Wechsler preschool battery at her most recent assessment, her verbal IQ was 61 and performance IQ was 47; full-scale IQ was 49. Her fine motor and visual motor abilities have been repeatedly assessed and are comparably delayed. Radiologic examination included normal x-ray and computed tomography of her skull and normal bone age. Magnetic resonance imaging (MRI) of the brain showed mild hypoplasia of the prechiasmatic optic nerve; other-

wise, the brain was structurally normal. Echocardiogram and renal ultrasound were normal. Laboratory testing showed a normal 46, XX karyotype (550 band level resolution), normal urine metabolic screen, and normal serum amino acids, ammonia, lactate, and pyruvate.

Patient 2

This girl was born at term (birth weight 6 lb, 4 oz). At 6 months, she presented with gross and fine motor developmental delay and with an inability to focus and track visually. She was macrocephalic with an OFC of 46.5 cm (>95th percentile), and had downslanting palpebral fissures. Bayley scale of infant development at the age of 7 months was 3 months, with a gross motor level of 2–5 months and a fine motor level of 1 month. An electroencephalogram at 15 months was normal, as was her hearing. At 17 months, she presented with chronic and episodic abdominal pain with associated vomiting. Brain MRI showed mild atrophy, particularly of the frontal lobes. There was continued exotropia, and an ophthalmologic examination showed bilateral optic nerve hypoplasia. Cardiac and renal ultrasounds were normal. Cytogenetic analysis revealed a normal 46, XX karyotype. As the clinical findings were suggestive of Sotos syndrome, sequencing of *NSD1* was performed and the results were negative. Follow-up evaluations documented continued language and developmental delay, with a skill level of about 18 months at the age of 3 years, significant speech delay (speech apraxia), and persistent macrocephaly, with OFC >95th percentile.

Patient 3

This boy was born at term (birth weight 8 lb, 9 oz) and presented in infancy with large size, macrocephaly with a broad forehead, frontal bossing, downslanting palpebral fissures, hypotonia, and significant developmental delay (Fig. 1). He developed a seizure disorder at the age of 4 years, which has been controlled medically. He underwent surgery twice to correct small angle strabismus and upper lid retraction (deep set eyes with decreased blinking). From infancy to 14 years of age, his macrocephaly persisted (OFC



Fig. 1. Photographs of Patient 1 (top row, ages 22 months and 7 years, 2 months), Patient 3 (lower left), and Patient 4 (lower right).

>98th percentile), whereas his height gradually fell from the 90th to the 50th percentile and his weight from the 50–75th to the 10–25th percentile. Ophthalmologic follow-up showed optic nerve atrophy that developed between the ages of 8 years (normal optic nerves by MRI) and 11 years (postbulbar, prechiasmatic, and chiasmatic optic nerve hypoplasia).

At his most recent examination (age, 14.5 years), his height was at the 25–50th percentile, weight in the 10–25th percentile, and head circumference at the 98th percentile. Dysmorphic features included low facial tone, large ears, high palate with crowded teeth, large hands, and large flat feet. He had severe speech delay and was nonverbal except for a few selected words.

MRI of the brain showed a chronic occipital cystic lesion of unknown etiology and absence of the rostrum of the corpus callosum. Serial renal ultrasounds showed a dilation or prominence of the left renal pelvis interpreted as an extra renal pelvis that has remained stable. Cytogenetics revealed a normal 46, XY karyotype (550 band-level resolution), and molecular testing was negative for deletion or mutation of *NSD1* or *FMRI*.

Patient 4

This girl was born at term (birth weight 9 lb, 0.5 oz) and presented in infancy with hypotonia, macrocephaly, prominent eyes with large blue irises, and exotropia; optic nerve pallor was noted within the first year (Fig. 1). Other clinical findings included upturned nose, low facial tone, 6 truncal café-au-lait spots, and myoclonic jerks; she had one prolonged seizure at the age of 9 years. No epilepsy was detected on electroencephalogram. Motor and verbal delays were present, including walking at 2–3 years and dysarthric speech with no words before 2 years. Early visual inattentiveness improved over time, although she underwent strabismus surgery at 4 years. Between 7 and 9 years, she developed signs of celiac disease and is on a gluten-free diet. At her most recent examination at the age of 9.5 years, her vocabulary has markedly expanded although she requires special education services. A brain MRI at 5 months was normal but a repeat at 4 years showed a Chiari I malformation (surgically decompressed at the age of 5 years) and hypoplasia of the optic chiasm and the intracranial optic nerves. Laboratory work-up showed normal results for metabolic testing (serum amino acids, blood long-chain fatty acids, urine organic acids, muscle biopsy, and mitochondrial DNA testing), cytogenetics (750 band-level resolution), and *NFI* mutation analysis.

Patient 5

This boy was born at 36 weeks' gestation (birth weight 6 lb, 4 oz). At 2 months, he presented with feeding problems, constipation, frequent vomiting, marked irritability, and multiple infections and was noted to have a lighter complexion than his sibling. At 14 months, his weight, length, and OFC were all <5th percentile. Other clinical findings included a sloping forehead, narrow alae nasi, inverted nipples, and an unusual fat distribution over the buttocks. He had a seizure disorder, with ~4–5 seizures daily originating in a left frontotemporal focus. Because of his continued feeding difficulties and oral aversion, a Nissen fundoplication with G-tube placement was performed. Ophthalmologic examination showed horizontal nystagmus.

By 3 years, his head circumference remained significantly below the 5th percentile, with persistent failure to thrive and developmental delay (only a few words; walked at the age of 2 years). Neuropsychologic testing at 4.5 years showed an age equivalent development of 21–29 months with hyperactivity, sleep disruption (delayed sleep onset with ~3 hours' sleep per night), tantrums and obsessional and self-injurious behaviors. His receptive language was mildly impaired and his expressive

communication significantly impaired; he first spoke in sentences at the age of 6 years. There has been some improvement in the past 2–3 years. The patient's growth rate has been slow (3.2 cm/year, <1st percentile); currently, at the age of 7 years, his height is <3rd percentile, but his weight has increased to the 50th percentile as his oral aversion has improved. Family history was significant in that the patient's father has nystagmus and reported learning difficulties.

Radiologic testing included chest x-ray, abdominal ultrasound, upper gastrointestinal, flexible sigmoidoscopy, and brain MRI, all of which were normal. Laboratory tests, including electrolytes, complete blood count with differential, liver and thyroid function tests, immunoglobulins, sweat chloride, serum amino acids, and urine organic acids were all reported as normal. Cytogenetic analysis showed a normal 46, XY karyotype at 850 band-level resolution; FISH, to rule out Williams syndrome, telomere FISH, and BAC aCGH performed at an outside reference laboratory were also normal.

Array-CGH analysis

Array-CGH revealed ratio profiles consistent with regions of loss in Patients 1 to 4, and a region of gain in Patient 5. As illustrated in Figure 2, the regions of loss ranged from ~0.31 Mb (Patient 3) to 1.3 Mb (Patient 2). The region of gain in Patient 5 was ~1.9 Mb. The smallest region of overlap (SRO), delimited by the deletion found in Patient 3, spans ~311 Kb, and localizes to band 19p13.13. Based on Human Genome Build 18, the start and stop points for this SRO were 12793474 and 13104643 (minimal breakpoints) and 12779366 and 13120858 (maximal breakpoints).

Follow-up aCGH was performed on the parents of all patients (Fig. 3). Ratio profiles for the 19p13 regions were within normal limits for each of the parental specimens.

FISH analysis

The copy number losses in Patients 1–4 were confirmed by FISH as representing interstitial deletions. Consistent with the aCGH results, all showed loss of both RP11-654K9 (Fig. 4A) and RP11-963I8, whereas only Patient 2 showed loss for RP11-14L14. Patient 5, with a copy number gain, showed an interphase signal pattern consistent with a duplication. For probes RP11-654K9, RP11-963I8, RP11-14L14, and RP11-56K21, 88%, 78%, 80%, and 65% of interphase cells, respectively, showed three hybridization signals (Fig. 4B). Although the duplication could not be visualized by FISH on metaphase chromosomes, signals were present only on the #19 chromosomes, thus ruling out the possibility that the extra signal was inserted elsewhere in the genome. Parental FISH studies showed no evidence of deletion or duplication and further confirmed localization of all the BAC signals to the #19 chromosomes, ruling out the possibility of an interchromosomal insertion (Fig. 4C). Thus, the losses and gain in the probands represent de novo events.

Genes within the SRO: the SRO, delimited by the region of loss found in Patient 3, contains all or part of 16 genes, extending from *RNASEH2A* (telomeric) to *NAC1* (centromeric), including most of *RNASEH2*, all of *RTBDN*, *MAST1*, *DNASE2*, *KLF1*, *GCDH*, *SYCE2*, *FARSA*, *CALR*, *RAD23A*, *GADD45GIP1*, *DAND5*, *NFIX*, *LYL1* and *TRMT1*, and most of *NAC1* (Fig. 5) (<http://genome.ucsc.edu>; UCSC hg18 Mar 2006).^{11,12}

DISCUSSION

By aCGH, four patients with a microdeletion and one with a microduplication involving 19p13.12–19p13.2 were identified. The SRO was delimited by Patient 3 with the smallest deletion,

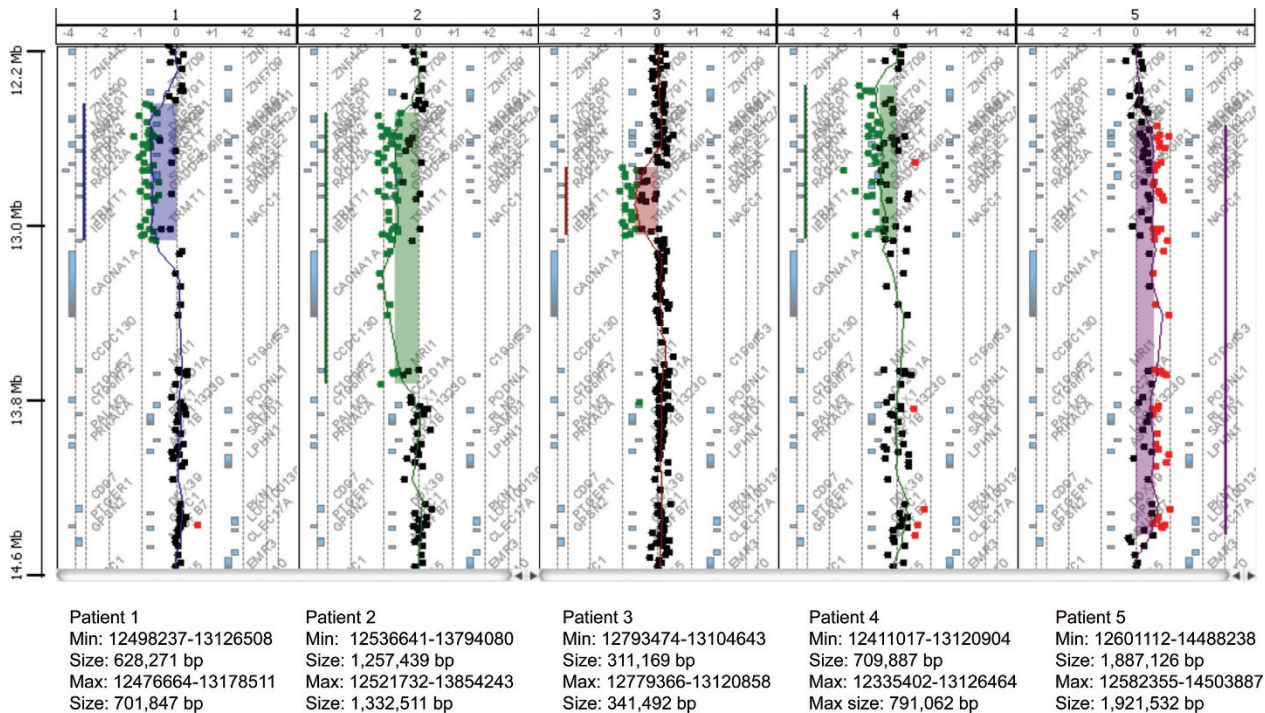


Fig. 2. Array-comparative genomic hybridization (aCGH) profiles from the four microdeletion patients (Patients 1–4) and the microduplication patient (Patient 5). The array plots for Patients 1–4 show deletions ranging from a minimum of 0.3 Mb (Patient 3) to a maximum of 1.3 Mb (Patient 2). The array plot for Patient 5 shows a 1.8–1.9 Mb duplication.

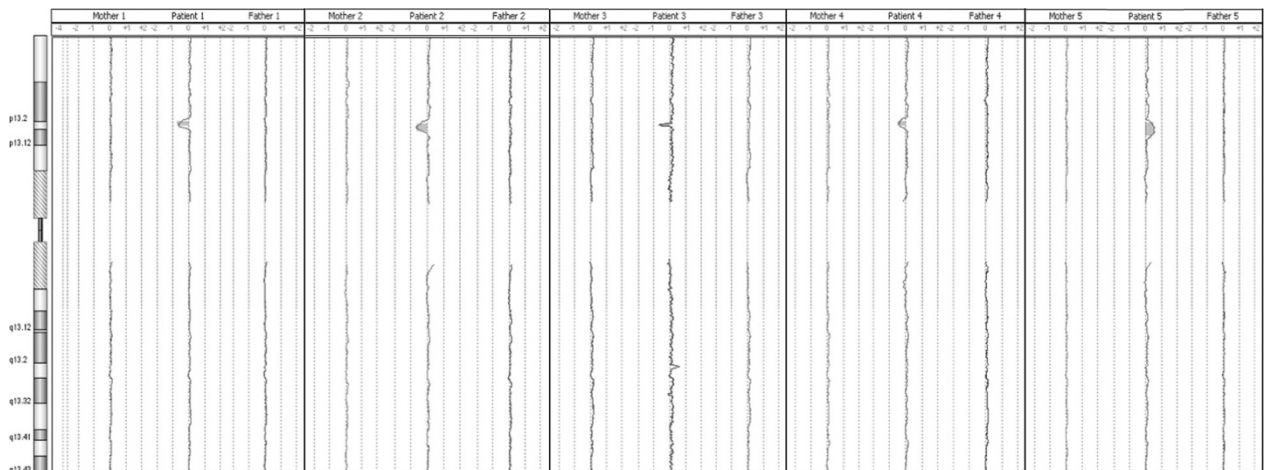


Fig. 3. Array-comparative genomic hybridization (aCGH) profiles from the parents of each patient (patient array is in the center for reference) showing no imbalance in the region of interest.

encompassing ~311 Kb (maximum 340 Kb) within 19p13.13. The minimal SRO extends from approximately bp 12793474 to bp 13104643 (Build 18).

To date, there have been only two published case reports of patients with deletions that encompass this SRO: a patient reported by Auvin et al.⁹ with an ~740 Kb deletion and one reported by Lysy et al.⁸ with a larger 3 Mb deletion. The patients of this study and the study of Auvin et al. show consistent phenotypic correlations that support the designation of this 19p13.13 clinical-cytogenomic entity as a microdeletion/

microduplication syndrome. The patient of Lysy et al.⁸ diverges significantly in phenotype; however, this is likely due to the fact that his deletion extends almost 3 Mb telomeric to those in this report and is almost 3 times larger than our largest deletion and almost 10 times larger than the critical region.

The clinical findings in the 19p13.13 deletion patients are notable for a constellation of three recurring abnormalities. The first is overgrowth. Patients 1–4 and those of Auvin et al.⁹ were macrocephalic with frontal bossing; height and weight were concordant with craniofacial size because these patients were all

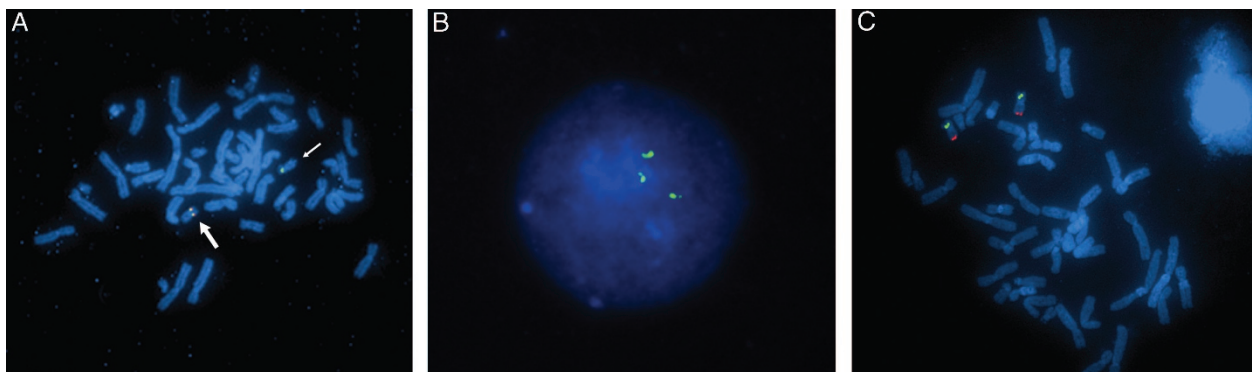


Fig. 4. A, FISH using a combination of BAC clones (RP11-654K9, red; RP11-14L14, green) on a metaphase cell from Patient 3. The normal chromosome 19 (large arrow) hybridized to both probes, resulting in a yellow fusion signal. The deleted chromosome 19 (small arrow) hybridized only to RP11-14L14, resulting in absence of the red signal. B, The interphase cell, from Patient 5, shows three signals for the RP11-56K21 probe. C, A metaphase cell from a parental specimen showing a normal signal pattern using probes RP11-96318 (green) and D19S238E (red), showing no interchromosomal rearrangement.

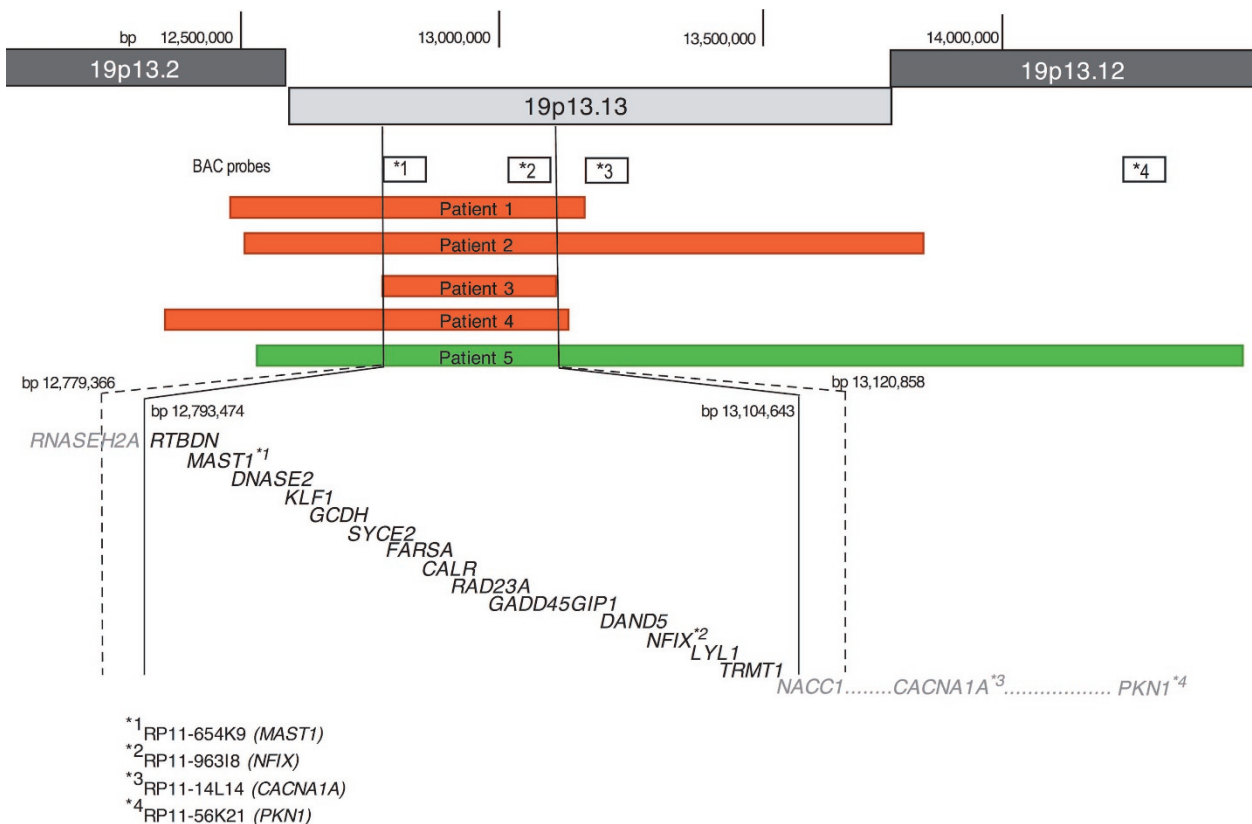


Fig. 5. Diagram showing the location of the regions of deletion (red) and duplication (green) for Patients 1–5; the solid and dashed lines designate the minimal and maximal breakpoints, respectively, of the SRO. Also noted are the BAC probes used to confirm the regions of imbalance (*1–*4), the genes to which they map, and the genes included in the SRO.

large for age. It is of particular interest that for Patients 2 and 3 and for the patient of Auvin et al.,⁹ *NSD1* testing was performed to rule out Sotos syndrome. Thus, this region of 19p13.13 may be relevant to other patients for whom Sotos syndrome is in the differential diagnosis but have negative *NSD1* testing results. The second common clinical manifestation involves ophthalmologic abnormalities (particularly strabismus) and optic nerve

atrophy or hypoplasia, the latter of which were detected by formal ophthalmologic examination and/or MRI. The third finding in this triad is gastrointestinal symptomatology, particularly abdominal pain and vomiting. Although no single finding in this triad is specific to abnormalities of 19p13.13, the constellation of findings is not one that has been described among the now numerous reports of other microdeletions/microduplications.

However, these 19p13.13 patients also demonstrated some non-specific findings; neurologic abnormalities (e.g., seizures, myoclonic jerks, hypotonia) were found in some and developmental delays and/or mental retardation were present in all. For the purposes of comparison, the few other published patients with microdeletions or microduplications within 19p13.12-19p13.2 are included in Table 1 (also see Refs. 6, 7, and 10). None of these overlaps the critical region delimited by Patient 3, and there are some marked differences in their phenotypes. In contrast to our deletion patients and that of Auvin et al.,⁹ these nonoverlapping deletion patients^{7,10} were small for age, supporting the conclusion that a gene within our defined 310 Kb SRO is responsible for the large body size. Although there is no hearing loss in our deletion patients, there is significant hearing loss for these nonoverlapping 19p deletions, suggesting that genes within these proximal and distal regions account for this aspect of their phenotype. However, some similarities do exist (e.g., strabismus^{6,10} and optic disc cupping¹⁰).

As only a single patient with a duplication was observed in our series, we are cautious in generalizing about its clinical manifestations. In the now well-established reciprocal microdeletion/microduplication syndromes involving 17p11.2 and 22q11.2, it is clear that the constellation of phenotypic findings associated with each deletion is sufficiently distinct from that associated with the corresponding duplication as to justify their designations as separate syndromes (e.g., Smith-Magenis syndrome for deletion of 17p11.2 and Potocki-Lupski syndrome for duplication of 17p11.2). Furthermore, as has been noted with imbalances involving the Williams-Beuren locus, patients with duplications may have findings opposite of those seen in patients with deletions.^{13,14} In this series, this is similarly shown by the striking difference in head circumference between those patients with a 19p13.13 deletion who were macrocephalic and the patient with a duplication who was microcephalic. This was also true for the duplication patient reported by Stratton et al.⁶ However, deletions and duplications of the same region may also share some common features, as illustrated by the finding of velopharyngeal insufficiency in both deletions and duplications of 22q11.2. Similarly, some clinical findings (e.g., ocular findings, gastrointestinal symptoms, seizures) were present among the 19p cases regardless of deletion or duplication status.¹⁵⁻¹⁷

The 16 genes mapped to the SRO are involved in diverse processes including transcription, DNA repair, and hematopoiesis. *DNASE2*¹⁸ and *KLF1*^{19,20} are involved in fetal hematopoiesis. As the presence of one functional *KLF1* allele is sufficient for human erythropoiesis, the absence of overt hematologic abnormalities in these five patients is not unexpected. Other genes within the region are involved in autosomal recessive disorders: *GCDH* and glutaric acidemia/aciduria (GA-1)²¹⁻²⁴ and *RNASEH2A* and Aicardi-Goutieres syndrome. Unless accompanied by a mutation on the non-deleted homolog, deletions of these genes would not be expected to be associated with clinical manifestations. Several of the genes in the SRO are expressed in the brain and neural tissues and represent candidate genes for clinical manifestations. *MAST1*, microtubule associated serine/threonine kinase 1, is such a candidate. It is a member of a family of microtubule-associated serine/threonine kinases²⁵ that is highly expressed in the brain. Of particular interest is the fact that *MAST1* interacts with and helps stabilize *PTEN*, mutations of which have been associated both with macrocephaly and autism.²⁶⁻²⁸ *NFLX*, a member of the NF1 transcription factor family, is another candidate gene. Mouse models have demonstrated that *Nfix* is essential for normal brain development,²⁹ with hemizygoty shown in one experiment to result in agenesis of the corpus callosum,³⁰ a finding similar to that seen in Patient 3. As discussed above, gastrointestinal issues were prominent in our

patients. A gene of interest for these findings is *CALR*, which encodes for calreticulin, a protein that acts both within the endoplasmic reticulum to bind and store calcium and within the nucleus, where it acts to regulate gene transcription by nuclear hormone receptors. Interestingly, it is also located in neurons in the human small intestine, and thus might play a role in the gastrointestinal symptoms seen in four of our five patients. In previously published reports of deletions of 19p13, including that of Auvin et al.,⁹ *CACNA1A* and *CC2D1A* have been hypothesized to play an important role in the resulting phenotype.^{9,31} Because neither *CACNA1A* nor *CC2D1A* is included in the our critical 310–340 Kb region, they are not considered candidates for the specific phenotypic constellation associated with this 19p13.13 deletion/duplication in our patients.

To further investigate the potential candidate genes *MAST1* and *CALR*, mouse models and mutation screening have been proposed. As noted previously, the start and stop points involved in the deletions and the duplication were unique to each of the patients. This fact, combined with the absence of low copy repeats or segmental duplications within or flanking the deleted/duplicated regions, argues against nonallelic homologous recombination as the mechanism giving rise to the 19p13 CNV. Most recently, microhomology at breakpoint junctions involved in repair of strand breaks has been implicated in the formation of CNV with nonrecurrent breakpoints.^{32,33} However, detailed sequencing of the breakpoints would be necessary to further investigate this possibility and to further characterize the underlying molecular mechanism.

The Database of Genomic Variants (DGV) from The Centre for Applied Genomics (TCAG) (<http://projects.tcag.ca/variation/>)³⁴ has been invaluable to cytogeneticists and others working with arrays to help identify CNVs that have been well documented in control populations and thus might represent benign variants. In the DGV, it is noted that a portion of this region (from the start point to 12919491) has been identified by Wong et al.³⁵ as deleted in 3 of 95 people, based on BAC array. Notably, none other of the larger control cohorts³⁶⁻⁴⁰ published in the literature and referenced by the DGV (representing more than 4000 control individuals) has identified a deletion or duplication for a large portion of this region. Further, as the deletion and duplication in our cases proved to be de novo, the single 2007 entry in the DGV does not outweigh the data in support of this CNV being clinically significant. Validating the breakpoints of the CNV reported in the 2007 BAC array might also provide additional insight.

In summary, the close collaboration of clinical geneticists and cytogeneticists, and the correlation of genotypic and phenotypic data, has facilitated the identification of a microdeletion/microduplication syndrome of 19p13.13. This has led to diagnostic workup recommendations for identified patients (e.g., complete ophthalmologic examination to detect strabismus and thorough evaluation, including MRI, of the optic nerves; MRI to detect structural abnormalities of the brain; sequential monitoring of occipitofrontal circumference; and elucidation of a history of gastrointestinal symptomatology including poor feeding, abdominal pain, and vomiting) as well as to the identification of genes to be targeted for research to elucidate further their function and relationship to the clinical features of this syndrome.

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