LETTER TO JMG

Clinical and molecular cytogenetic characterisation of a newly recognised microdeletion syndrome involving 2p15-16.1

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J Med Genet 2007;**44**:269–276. doi: 10.1136/jmg.2006.045013

Background: During whole genome microarray-based comparative genomic hybridisation (array CGH) screening of subjects with idiopathic intellectual disability, we identified two unrelated individuals with a similar de novo interstitial microdeletion at 2p15-2p16.1. Both individuals share a similar clinical phenotype including moderate to severe intellectual disability, autism/autistic features, microcephaly, structural brain anomalies including cortical dysplasia/pachygyria, renal anomalies (multicystic kidney, hydronephrosis), digital camptodactyly, visual impairment, strabismus, neuromotor deficits, communication and attention impairments, and a distinctive pattern of craniofacial features. Dysmorphic craniofacial features include progressive microcephaly, flat occiput, widened inner canthal distance, small palpebral fissures, ptosis, long and straight eyelashes, broad and high nasal root extending to a widened, prominent nasal tip with elongated, smooth philtrum, rounding of the upper vermillion border and everted lower lips.

Methods: Clinical assessments, and cytogenetic, array CGH and fluorescence in situ hybridisation (FISH) analyses were performed.

Results: The microdeletions discovered in each individual measured 4.5 Mb and 5.7 Mb, spanning the chromosome 2p region from 57.2 to 61.7 Mb and from 56 to 61.7 Mb, respectively. Each deleted clone in this range demonstrated a dosage reduction from two to one copy in each proband except for clone RP11-79K21, which was present in three copies in each proband and in four copies in their respective parents (two per each chromosome 2 homologue).

Discussion: The common constellation of features found in the two affected subjects indicates that they have a newly recognised microdeletion syndrome involving haploinsufficiency of one or more genes deleted within at least a 4.5-Mb segment of the 2p15-16.1 region.

Traditionally the first step in establishing an association between submicroscopic chromosomal changes and a distinct set of phenotypic features is the observation of visible recurrent chromosomal rearrangements upon routine karyotyping in individuals who share a similar phenotype, frequently including idiopathic intellectual disability (IQ<70).[1 2] The advent of new technologies, such as testing for the presence of submicroscopic abnormalities of chromosomal ends[3–10] and screening of the whole genome for subtle chromosomal changes in individuals with idiopathic intellectual disability using array comparative genomic hybridisation (array CGH),[11–20] has dramatically increased the number of novel microdeletions and microduplications associated with a wide range of clinical phenotypes, leading in some cases to the identification of the respective culprit genes.[19] In a recent review of whole genome screening in individuals with undefined causes of intellectual disability using array CGH, Menten *et al*[20] summarised results in 432 individuals and reported that 20% of affected individuals had subchromosomal imbalances that were mostly randomly distributed over all chromosomes. Non-random involvement of chromosomal regions included interstitial subtelomeric deletions of chromosome 1p36, and genomic alterations of two additional loci at 1q21.1 and 5q35.1, for which both duplications and deletions were observed. It was noted that these sites may mark an increased risk for recurrent rearrangements due to non-homologous recombination between suspected flanking low copy repeats. However, in the absence of detailed clinical descriptions and correlation of findings relating to such recurrent gene dosage anomalies, it is impossible to determine their pathological significance and phenotypic consequence. More recently array CGH allowed the identification and detailed clinical description of a recurrent microdeletion of 9q22.3 detected in two individuals with idiopathic intellectual disability.[21]

We report here the clinical and molecular cytogenetic findings discerned in two individuals for whom we have discovered a similar microdeletion at 2p15-2p16.1 identified in the course of systematic whole genome screening of 88 individuals with idiopathic intellectual disability and 70 individuals with an autism spectrum disorder (ASD) and intellectual disability using the 1-Mb resolution array. Based on

Key points

- We have described a newly recognised, recurrent microdeletion of the 2p15-16.1 region, detected in two individuals with idiopathic intellectual disability and autism spectrum disorder/autistic features and an otherwise remarkably similar clinical phenotype.
- The microdeletion region includes one of the two copies of the previously unrecognised repeat sequence on chromosome 2p.
- The two cases we describe are, to the best of our knowledge, the only cases for which the same 4.5-Mb deletion of chromosome 2p15-16.1 (from 57.2 to 61.7 Mb) has been identified.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ADOS-G, Autism Diagnostic Observation Schedule - Generic; array CGH, array comparative genomic hybridisation; ASD, autism spectrum disorder; CIHR, Canadian Institutes for Health Research; FISH, fluorescence in situ hybridisation; HEIDI, Healthcare Equity for Intellectually Disabled Individuals; IPD, intrapupillary distance; IUGR, intrauterine growth retardation; MRI, magnetic resonance imaging; OCD, outer canthal distance; X-MR, X linked mental retardation

their largely concordant phenotypic similarities, described herein, it is very likely that these two individuals constitute a pattern of clinical findings representative of a new microdeletion syndrome involving 2p15-16.1. In providing detailed clinical characterisation and comparison of the phenotypes found in common between the two probands manifesting nearly identical microdeletions at 2p15-2p16.1, we aim to facilitate the more global identification of additional subjects with idiopathic intellectual disability and/or ASD/autistic features bearing the same microdeletion. Moreover, we hope that this will lead to improved recognition and management of prospective developmental, behavioural and medical health care needs associated with 2p15-2p16.1 deletion syndrome.

METHODS

Clinical assessments

The two probands, subject 1 and subject 2, volunteered to participate in a Canadian Institutes for Health Research (CIHR) funded study to identify novel microdeletions and microduplications in individuals with idiopathic intellectual disability and/or ASD in collaboration with the Healthcare Equity for Intellectually Disabled Individuals (HEIDI) research program and the Autism Spectrum Disorders – Canadian-American Research Consortium, aimed at improving the diagnosis and health management of persons with these disorders across their lifespan. We have established a screening methodology for identifying genetic causes of idiopathic intellectual disability/ASD due to submicroscopic microdeletions and microduplications using the 1-Mb resolution array CGH. Ethics approval for clinical research involving human subjects was obtained through the Clinical Research Ethics Board of the University of British Columbia, Vancouver, Canada.

The clinical genetic, morphometric, medical and cognitive evaluations for all subjects were prioritised according to the criteria of de Vries *et al*[22] in order to select individuals for array CGH analysis who had a higher likelihood of manifesting a segmental subchromosomal copy number variation potentially harbouring an underlying intellectual disability and/or ASD susceptibility gene. All individuals undergoing study had had negative high-resolution karyotype testing at 500 band level resolution and negative Fragile X testing. As part of these studies a broad spectrum of clinical phenotyping measures, including a comprehensive and standardised clinical genetic evaluation (family, pregnancy and medical history, physical examination, craniofacial anthropometry, standardised somatic morphological characterisation and 2D photogrammetry) as well as specific psychometric measures offering insight into past and current developmental level, speech and language skills, adaptive behaviour, social subtypes and behavioural problems, were applied for both probands.

Cytogenetic analysis

Routine cytogenetic G-band analysis was performed for both probands, and for the parents of subject 2, as they had previously experienced two unexplained spontaneous pregnancy losses.

Array CGH analysis

Array CGH analysis was performed as described by Tyson *et al*[14] and Koochek *et al*.[23] Briefly, commercially available 1-Mb resolution BAC arrays (Spectral Genomics, Houston, TX) were hybridised to genomic DNA from the probands mixed with a pool of control DNAs (Promega, Madison, WI). Dye swap experiments were performed for both probands to minimise dye related artefacts. Data analysis was performed using Spectralware 2 software (Spectral Genomics). Identification of clones with a significant gain or loss was based on previously established cut-off values of 1.2 and 0.8 as described by us[14] and others using the same array platform.[15 24]

Fluorescence in situ hybridisation analyses

Fluorescence in situ hybridisation (FISH) analyses were performed using the BAC DNA clones (Spectral Genomics) that showed deletion by array CGH as described by Tyson *et al*[14] and Rajcan-Separovic *et al*.[25] Slides were viewed on a Zeiss Axioplan 2 fluorescence microscope and images captured using MacProbe software (Applied Imaging, Santa Clara, CA). For each FISH probe 5–10 metaphase cells were analysed. Information regarding clone positions and band designations was obtained from the NCBI, May 2004 version and UCSC database, build 36.1.

RESULTS

Clinical findings in the affected probands

Subject 1 (fig 1, table 1) is an 8-year-old non-verbal female born to non-consanguineous, 23- and 26-year-old Caucasian parents, with a paternal medical history of X-linked ichthyosis due to steroid sulphatase deficiency. Polyhydramnios and left hydronephrosis, leading to multicystic dysplasia due to suspected uteropelvic junction obstruction, was observed prenatally together with intrauterine growth retardation (IUGR), although all growth parameters at birth were within normal limits. Subsequently, transient postnatal growth retardation and failure to thrive ensued together with progressive microcephaly. Other features included moderately severe global developmental delay, mild visual impairment due to optic nerve hypoplasia and perisylvian migration disorder of the brain (marked by pseudobulbar palsy with dysarthria, dysphagia, facial diplegia, drooling, intellectual disability and bilateral perisylvian cortical dysplasia confirmed by cranial magnetic resonance imaging (MRI)). Dysmorphic features included brachycephaly, bitemporal narrowing, flattened occiput, prominent nasal bridge and tip, widened inner canthal distance (ICD; +1.5 SD; −1 SD for both outer canthal (OCD) and intrapupillary (IPD) distances), telecanthus, strabismus, ptosis (fig1A–C), smooth philtrum, everted lower lip, increased internipple distance, camptodactyly of digits 3–5 bilaterally (fig 1D), metatarsus abductus and a non-functional left multicystic kidney. Neurodevelopmental features included normal hearing and EEG testing, spasticity of the lower extremities, severely delayed receptive and expressive language skills, attention deficit hyperactivity disorder (ADHD), and a diagnosis of ASD based on standardised DSM-IV criteria confirmed by objective testing using the Autism Diagnostic Observation Schedule - Generic (ADOS-G; Module 1) (Western Psychological Services, Los Angeles, CA, 2002).[26 27] Routine cytogenetic studies at amniocentesis and postnatally (450–500 band level) were normal, as were FISH studies for Angelman and Miller-Dieker syndromes.

Subject 2 (fig 2, table 1) is a 6-year-old non-verbal male of mixed Chinese-Caucasian descent born to healthy, non-consanguineous 29- and 32-year-old parents with a prior history of two spontaneous pregnancy losses, for which parental karyotype testing was normal. The pregnancy was uncomplicated and all fetal ultrasound-detected growth parameters were within normal limits. Microcephaly was observed at birth. Subsequently, progressive growth retardation, persistent microcephaly, moderate intellectual disability and global developmental delay were documented, including mild visual impairment due to hyperopia and optic nerve hypoplasia. Dysmorphic features included brachycephaly, bitemporal narrowing, flattened occiput, prominent metopic suture, prominent nasal bridge and tip, widened ICD (+1.5 SD; OCD and IPD at –1 SD), telecanthus, strabismus, ptosis (fig 2A–D), camptodactyly of the 5th digit bilaterally, metatarsus abductus and left

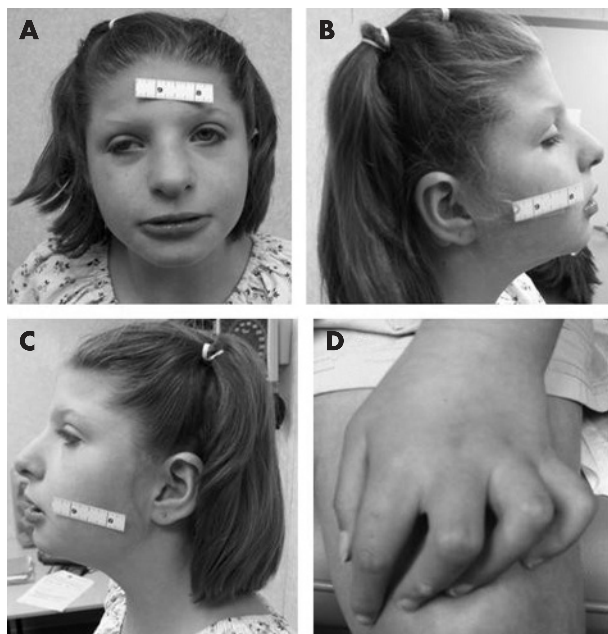

Figure 1 Frontal (A), right (B) and left (C) lateral craniofacial views of subject 1 and (D) camptodactyly of digits 3–5 of the left hand. Parental/guardian informed consent was obtained for publication of this figure.

hydronephrosis suggestive of obstruction or reflux. The genitalia were hypoplastic and testosterone testing (after hCG stimulation) suggested an aetiology due to hypothalamic hypogonadism. Neurodevelopmental features included bilateral sensorineural hearing loss, EEG findings of disorganised background activity with mid/left frontal/occipital spike waves, spasticity of the lower extremities, severely delayed receptive and expressive language skills and autistic features (not meeting DSM-IV or ADOS-G criteria for an ASD) with significant attentional deficits. Cranial MRI revealed an enlarged 4th ventricle, mild hypoplasia of the inferior cerebellar vermis, and small anterior pituitary and pons. The cortex appeared generally thickened and the subcortical tissue was hyperintense, suggestive of either dysmyelination or cortical dysplasia. Routine cytogenetic testing postnatally (500 band level) was normal.

Array CGH analysis

Subject 1 had a de novo deletion of five clones from 2p15-16.1 (RP11-81L7, RP11-90D1, RP11-81L13, RP11-79K21 and RP11-355B11; fig 3A). She also demonstrated a paternally-inherited deletion of two clones in the Xp22.31 region (RP11-294K6 and RP11-143E20, not shown). The minimum sizes of the 2p and Xp deletions were 4.5 Mb and 1.2 Mb, respectively.

Subject 2 had the same de novo five clone microdeletion as determined in subject 1, as well as a loss of two adjacent, telomeric clones from 2p16.1 (RP11-494H5 and RP11-482H16;

Table 1 Comparison of clinical features for subject 1 and subject 2

Clinical features	Subject 1	Subject 2
Fetal features	IUGR, polyhydramnios, left hydronephrosis and multicystic kidney due to UPJ obstruction	None
Postnatal growth retardation	No	Yes
Feeding problems	Yes, poor suck/swallow, GI reflux, FTT	Yes
Height (percentile)	75–90%	<3%
Weight (percentile)	75–90%	5–10%
OFC (percentile)	<<2%	<<2%
Cranium size and shape	Microcephaly, brachycephaly, bitemporal narrowing, flat occiput, facial asymmetry	Microcephaly, brachycephaly, bitemporal narrowing, flat occiput, prominent metopic suture
Oral cavity	High palate; oral-motor dysfunction, excessive drooling, bruxism	High palate; oral-motor dysfunction
Forehead	Short (in keeping with microcephaly)	Short (in keeping with microcephaly)
Eyes	Ptosis, widened inner canthal distance, telecanthus, short palpebral fissures, long, straight eyelashes	Ptosis, widened inner canthal distance, telecanthus, short palpebral fissures, long, straight eyelashes, long and thin eyebrows
Vision	Strabismus and optic nerve hypoplasia	Hyperopia, optic nerve hypoplasia
Ears	Large (relative to microcephaly), normally formed	Large (relative to microcephaly), thinning of scapha helices, large lobes
Hearing	Normal	Bilateral sensorineural loss at 2000–6000 Hz
Nose	Broad and high nasal root and prominent tip	Broad and high nasal root and prominent tip
Mouth	Smooth upper vermillion border, smooth and widened philtrum, everted lower lip	Smooth upper vermillion border, smooth and widened philtrum, everted lower lip
Thorax/vertebral	↑ Internipple distance	↑ Internipple distance, supernumerary nipple
Cardiopulmonary	Frequent upper respiratory infections	Laryngomalacia, obstructive sleep apnea
GI/genitourinary	Non-functioning left multicystic kidney, hydronephrosis	Micropenis, small testes, left hydronephrosis
Endocrine	Normal	Hypothalamic hypogonadism
Hands	Camptodactyly of digits 3–5 bilaterally	Camptodactyly of the 5th digit bilaterally
Feet	Metatarsus abductus	Metatarsus abductus, bilateral 2nd/3rd toe syndactyly and medial deviation of the 4th toe
Musculoskeletal	Bilateral tight Achilles tendons and calcaneovalgus	Bilateral tight Achilles tendons and calcaneovalgus
Neurological	Spasticity of lower extremities, unsteady gait/balance , pseudobulbar palsy with dysarthria, dysphagia, facial diplegia, drooling. Normal EEG	Spasticity of lower extremities, unsteady gait/balance. EEG findings of disorganised background activity with mid/left frontal/occipital spikes.
Neurodevelopment	Moderate intellectual disability Moderate adaptive delay	Moderate intellectual disability Moderate adaptive delay
Language skills	Severely delayed receptive and expressive skills	Severely delayed receptive and expressive skills
Behaviour	ASD, ADHD	Autistic features, attention deficits
Neuroimaging	Bilateral perisylvian cortical dysplasia	Dysmyelination, cortical dysplasia, small anterior pituitary and pons

ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; EEG, electroencephalogram; FTT, failure to thrive; IUGR, intrauterine growth retardation; OFC, occipitofrontal circumference; UPJ, uteropelvic junction.

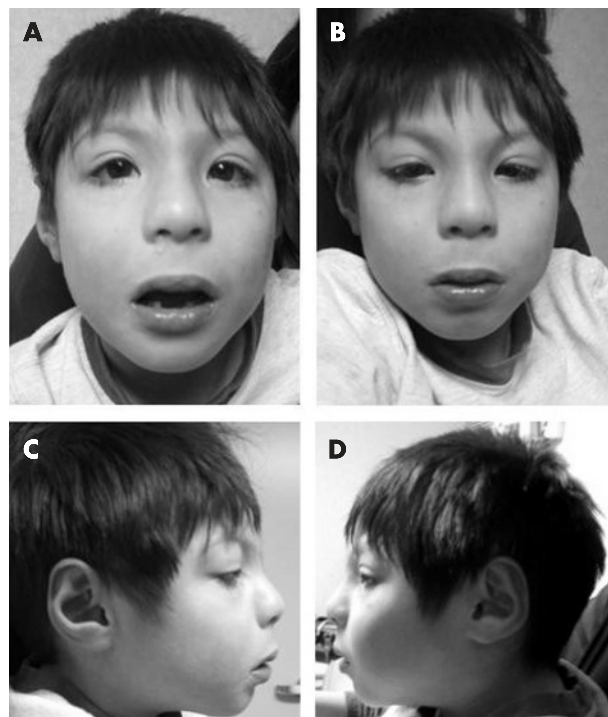

Figure 2 Frontal (A, B), right (C) and left (D) lateral craniofacial views of subject 2. Parental/guardian informed consent was obtained for publication of this figure.

fig 3B). The 2p15-16.1 microdeletion in subject 2 was 5.7 Mb in size. Figure 3C illustrates the clones from 2p15-16.1 deleted in subjects 1 and 2 and the bordering balanced clones on the array.

FISH confirmation of the deletions

FISH analysis was performed on samples from subjects 1 and 2 and their parents using directly labelled BAC DNA probes for all the clones showing a deletion on the array. The 2p15-16.1 deletion was found to be de novo in both probands, while the Xp22.31 deletion in subject 1 was inherited from her nullisomic father. Figure 4 demonstrates the typical de novo deletion of two clones from the 2p15-16.1 region as determined by FISH in both probands (clones at the centromeric and telomeric margins of the 4.5-Mb deletion from 2p15-16.1 common to both subjects 1 and 2 are shown: RP11-355B11 and RP11-81L7). One of the clones illustrating a deletion on the array in both probands (RP11-79K21) showed an unexpected pattern of four copies per genome (two copies for each chromosome 2 homologue) in each of the parents of subjects 1 and 2, as well as in all three unrelated control subjects tested for this clone, whereas only three of four copies were seen in the affected probands (fig 5). Clone RP11-79K21 was therefore considered to exist as an intrachromosomal duplication in unaffected individuals, while in the affected probands one of the four clone complements was missing. The two copies of RP11-79K21 on the normal homologue were separated on the 2p arm, with one copy mapping to 2p15-16 and the other copy mapping closer to the 2p telomere. Macroscopically, as determined by FISH (fig 5), they appeared to be separated by at least 20–30 Mb of DNA, as they mapped to the distal and proximal third of the 2p short arm (the total length of 2p is approximately 90 Mb). Based on the intensity of the FISH signals, the two copies of RP11-79K21 appeared different in size, with the proximal 2p15-16 copy showing a much stronger signal. It is likely that the difference in FISH signal intensity for the two hybridisation sites reflects a different number of repeats of DNA sequences from clone RP11-79K21 within each site. The stronger proximal FISH signal was missing in the affected probands (fig 5). To the best of our knowledge, clone RP11-79K21 has not yet been reported as a common polymorphism (http://projects.tcag.ca/variation/). According to the NCBI and USCS databases (http://www.ncbi.nlm.nih.gov/genome/guide/human/ and http://genome.ucsc.edu/cgi-bin/hgGateway) this clone has been fully sequenced and its location on 2p16.1 has been confirmed. Except for the Spectral Genomics 1-Mb array, this specific clone has not been incorporated in commonly used array platforms (for example, the Welcome Trust Sanger Institute 1-MB array and the Tiling path array set) according to the Decipher catalogue of array platforms and clone content (http://www.sanger.ac.uk/PostGenomics/decipher/). This may explain why the repetitive nature of clone RP11-79K21 has not yet been recognised and investigated in detail.

FISH analysis of the Xp deletion in subject 1 using clones RP11-294K6 and RP11-143E20 showed that the former clone was deleted in subject 1 and her father, who was therefore nullisomic for this DNA sequence. Clone RP11-143E20 appeared to be only partially deleted in subject 1 as two FISH signals of different intensity were seen on her X chromosome homologues (with one signal much fainter than the other). Her father's X chromosome also had a very faint FISH signal with RP11-143E20. Both the RP11-294K6 and RP11-143E20 probes showed a normal two signal pattern on the two chromosome X homologues in the mother of subject 1 (not shown).

DISCUSSION

We report the novel clinical and molecular cytogenetic findings of two unrelated individuals with idiopathic intellectual disability found to share a strikingly similar pattern of neurosensory (mild visual impairment), neurodevelopmental (moderate developmental and adaptive delay), behavioural (ASD/autistic features) and somatic congenital anomalies associated with an interstitial microdeletion of the 2p15-16.1 region, cryptic upon G-banding, and identified by 1-Mb array CGH. The commonality of findings for these two individuals represents the first description of a newly recognised 2p15-16.1 microdeletion syndrome. Despite the slight heterogeneity in the size of the deletion in subjects 1 and 2, measuring 4.5 and 5.7 Mb, respectively, with a common deletion of five clones beginning from the centromeric end of 2p15 (RP11-355B11, RP11- 79K21, RP11-81L13, RP11-90D1 and RP11-81L7), several common phenotypic features have emerged. These include moderate to severe intellectual disability, ASD or autistic features, progressive microcephaly with cortical dysplasia/pachygyria evident on cranial MRI, optic nerve hypoplasia, ptosis, shortened palpebral fissures, widened inner canthal distance, broadened nasal root and tip, everted lower lips, digital camptodactyly, renal anomalies and spasticity of the lower extremities (see table 1 for comparison).

The common 4.5-Mb deletion interval of 2p15-2p16.1 seen in subjects 1 and 2 (from 57.2 to 61.7) includes several known genes with a variety of functions such as vaccinia related kinase 2 (C-REL, OMIM 164910), Fanconi anaemia, complementation group L (FANCL, OMIM 608111), B-cell CLL/lymphoma 11A isoform 3 (BCLA11, OMIM 606557), poly(A) polymerase gamma (PAPOLG), exportin 1 (XPO1, OMIM 602559) and peroxisome biogenesis factor 13 (PEX13, OMIM 601789). A number of genes with hypothetical proteins have also been assigned to this region (for example, AF312211, FLJ32312, AK131267 and KIAA1841). The additional 1.2-Mb deletion region in subject 2 does not contain any known genes. One of the genes deleted in both subjects 1 and 2 is PEX13, known to

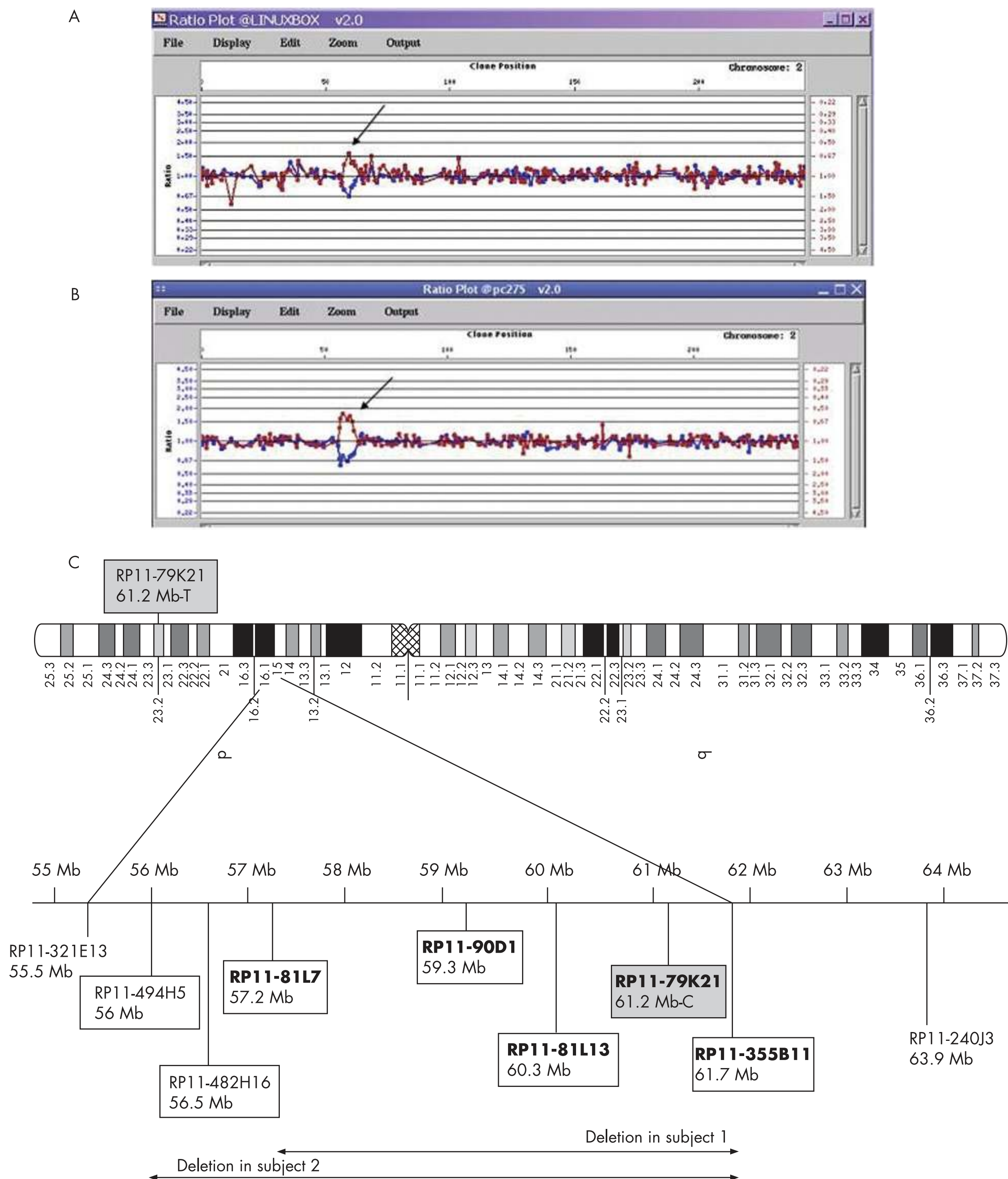

Figure 3 (A,B) Array profile for subjects 1 (A) and 2 (B) showing loss of five and seven clones, respectively, from 2p15-16.1. (C) Clones from 2p15-16.1 showing a deletion in subjects 1 and 2 (boxed) and the bordering telomeric and centromeric balanced clones on the array. Clone names in bold represent clones deleted in both subjects, clones boxed but not in bold are deleted in subject 2 only. Clone RP11-79K21 is shaded as it shows two copies per homologue in normal individuals (T-telomeric copy, approximately 1/3 of the 2p length from the telomere and C-centromeric copy, approximately at 2/3 of the 2p length from the telomere). In normal individuals both the centromeric and the telomeric copy are present on each homologue. In subjects 1 and 2 the centromeric copy of this clone is deleted and included in the 2p15-16.1 deletion.

be associated with Zellweger syndrome[28] (OMIM 601789), an autosomal recessive and lethal peroxisome biogenesis disorder. PEX13 is one of the large number of PEX genes which, when mutated, contributes to a range of phenotypes from the less severe neonatal adrenoleukodystrophy to the lethal Zellweger syndrome forms. Overlapping features of the latter disorders encompass retinopathy, impaired hearing, seizures, stippled epiphyses, enlarged liver and psychomotor delay across all

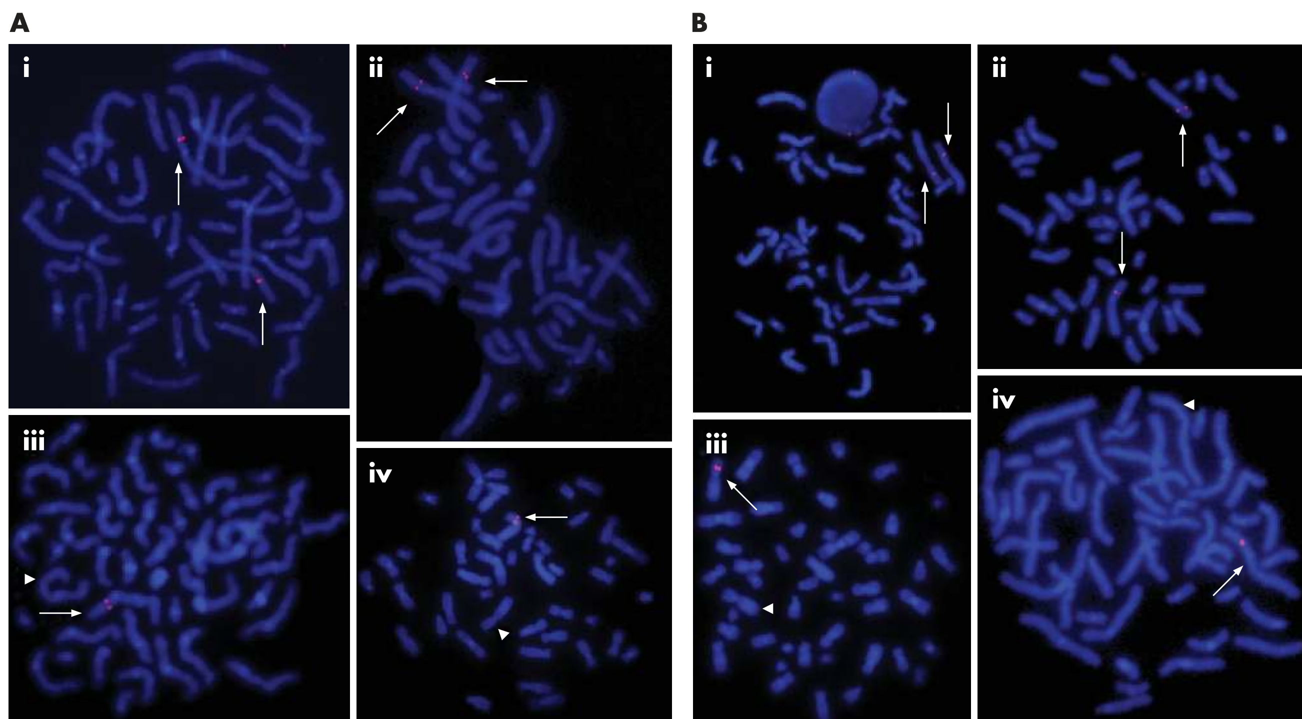

Figure 4 (A) FISH analysis using the RP11-355B11 BAC clone (at 2p15) marking the most centromeric margin of the 2p15-16.1 deletion: (i) subject 2's mother, (ii) subject's 2 father, (iii) subject 2, and (iv) subject 1. Arrow indicates the normal chromosome 2 and the arrowhead indicates the deleted chromosome 2. (B) FISH analysis of RP11-81L7 (at 2p16.1) marking the most telomeric clone from the common 2p15-16.1 deletion region (4.5 Mb). (i) Subject 1's mother, (ii) subject 1's father; (iii) subject 1, and (iv) subject 2. Arrow indicates the normal chromosome 2 and the arrowhead indicates the deleted chromosome 2.

phenotypic forms.[29] The probands we describe here did not have laboratory evidence of a disorder of peroxisomal biogenesis (normal long chain fatty acids and phytanic acid) nor a clinical constellation of features compatible with either Zellweger or neonatal adrenoleukodystrophy. Nonetheless, it cannot be excluded that their phenotype could be partly attributed to the loss of function of only one copy of the PEX13 gene, in addition to the contribution of any number of other contiguous known and unknown genes in the involved 2p15-16.1 segmental deletion. In addition to PEX13, the homozygous loss of function of the Fanconi anaemia, complementation group L gene (FANCL) is known to be associated with congenital anomalies in the autosomal recessive Fanconi anaemia phenotype consisting of anaemia, pigmentary changes in the skin, and malformations of the heart, kidney and limbs (aplasia of the radius, thumb deformity). Deletions of genes other than PEX13 and FANCL from the 2p15-16.1 region have not yet been associated with distinct and recognisable constitutional disorders. However, proto-oncogene REL-C has shown an increase in copy number in B-cell lymphoma, which was demonstrable as an amplification of the clone RP11-373L24, containing the REL-C gene and the proximal RP11-79K21 repeat.[30 31] A second tumour related gene from the 2p15-16.1 region, BCL11A proto-oncogene, has also been found to be co-amplified with REL-C, over-expressed or deregulated due to chromosomal translocations.[32] These findings suggest that the 2p15-16.1 region is very unstable and prone to both constitutional deletions and acquired amplifications leading to quite diverse and non-overlapping phenotypic sequelae.

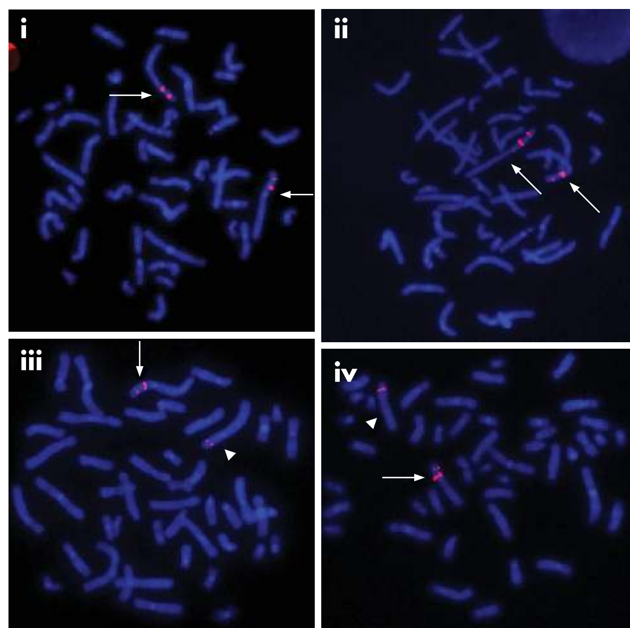

Figure 5 FISH analysis using probe RP11-79K21 demonstrated an unexpected intrachromosomal duplication, thus a normal four-copy number complement was seen in (i) subject 1's mother and (ii) subject 1's father, and three copies were seen in (iii) subject 1 and (iv) subject 2. The normal chromosome 2 is indicated with an arrow and the deleted chromosome 2 is indicated with an arrowhead.

Recently, Chandler *et al*[33] reported one unrelated and three related children with a novel neurodevelopmental disorder characterised by congenital microcephaly, marked mental handicap and intractable seizures leading to death before age 3 years. Brain imaging revealed some cerebral abnormalities similar to those of subjects 1 and 2: a simplified gyral pattern, an irregular cortex with deficiency of supratentorial white matter with cystic changes involving the temporal and occipital

horns, a lack of myelination, severe hypoplasia of the corpus callosum and brain stem with flattening of the pons. One patient had microgenitalia and another had congenital hypothyroidism. An autosomal recessive syndrome was suspected on the basis of consanguinity and absence of parental involvement, with homozygosity mapping identifying a candidate region spanning at least 44 genes and 16 Mb on chromosome 2p16, for which no definitive candidate genes were identified. The candidate region from Chandler *et al*[33] spanned from 49.5 to 67 Mb compared to the noted deletion at 57–62 Mb in our subjects 1 and 2. It is quite plausible that findings by Chandler *et al*[33] represent the most severe manifestation of nullisomy for a candidate gene that could in part contribute to the features seen with contiguous haploinsufficiency of genes at 2p15-16.1.

Although one cannot entirely rule out potential intergenic, epigenetic or parent of origin effects of genes deleted within the Xp22.31 region upon the 2p15-16.1 microdeletion phenotype in subject 1, we believe that the similarity in phenotype to subject 2, confirmation of paternal inheritance of the Xp22.31 deletion from a developmentally normal father and finding of random X chromosome inactivation for an X-linked recessively transmitted disorder in subject 1 attests to a phenotype primarily being due to haploinsufficiency of genes within the 2p15-16.1 region. Moreover, the frequency of deletions of the STS gene is relatively high in the population (1:2000–1:6000 males)[34] with the phenotype primarily limited to males, and systemically to the dermatologic findings of X-linked ichthyosis due to steroid sulphatase deficiency. However, the STS gene can also be deleted in some males with intellectual disability. An X linked mental retardation (X-MR) gene has long been suspected to exist in the Xp22.31 region[35]; however, any phenotypic influence would be expected to be less in a carrier female with normal, random X chromosome inactivation.

In summary, we have described a newly recognised, recurrent microdeletion of the 2p15-16.1 region, detected in two individuals with idiopathic intellectual disability and ASD/autistic features and an otherwise remarkably similar clinical phenotype. The microdeletion region includes one of the two copies of a previously unrecognised repeat sequence on chromosome 2p. Whole genome array analysis has so far been performed on close to 500 individuals with idiopathic intellectual disability[21] and the two cases we describe are, to the best of our knowledge, the only cases for which the same 4.5-Mb deletion of a chromosome 2p15-16.1 (from 57.2 to 61.7 Mb) has been identified. Large scale screening of individuals with intellectual disability and/or ASD/autistic features for the same microdeletion, who broadly fit the described phenotype, will be the next step in establishing its population prevalence, consequence and guidelines for anticipatory health care management across the lifespan.

ELECTRONIC DATABASE INFORMATION

The following URLs have been mentioned in this article: Autism Spectrum Disorders Canadian-American Research Consortium (ASD-CARC): http://www.autismresearch.ca and http://www.asdcarc.com; Database of Genomic Variants: http://projects.tcag.ca/variation/; Decipher database: http://www.sanger.ac.uk/PostGenomics/decipher/; NCBI database: http://www.ncbi.nlm.nih.gov/genome/guide/human/; and USCS database: http://genome.ucsc.edu/cgi-bin/hgGateway

ACKNOWLEDGEMENTS

We extend our sincere appreciation to subjects 1 and 2 and their family members for their enthusiastic support of this study.

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This work was supported by grants from the Canadian Institutes for Health Research (MOP-74502, ERS principal investigator; RT-64217, MESL principal investigator) and Vancouver Foundation (BCM05-0016, ERS principal investigator), a CIHR Interdisciplinary Health Research Team grant (RT-43820) to the Autism Spectrum Disorders Canadian-American Research Consortium (ASD-CARC: http://www.autismresearch.ca) (JJAH, principal investigator) and a grant from the Ontario Mental Health Foundation (JJAH). Y Qiao is a trainee with the CIHR/NAAR STIHR Inter-Institute Autism Spectrum Disorders Training Program (principal investigator: JJAH). E Rajcan-Separovic is supported by a CIHR Institute of Genetics Clinician Investigator Award (2005–07). MES Lewis sincerely appreciates the support provided by a Michael Smith Foundation for Health Research Career Investigator (Scholar) Award (2005–10).

Competing interests: None.

E Rajcan-Separovic, C Harvard, Y Qiao, ECR Mickelson, JJA Holden and MES Lewis are members of The Autism Spectrum Disorders – Canadian American Research Consortium (ASD-CARC; www.autismresearch.ca, www.asdcarc.com).

Further information: Patient details are published with the consent of their families.

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Received 21 June 2006
Revised 20 July 2006
Accepted 3 August 2006
Published Online First 8 September 2006

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