

Origins of accessory small ring marker chromosomes derived from chromosome 1

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Abstract

Three patients with accessory small ring chromosomes derived from chromosome 1 are presented together with additional clinical details and cytogenetic analyses of a previously reported patient. Cytogenetic analysis was undertaken by FISH using a reverse painting probe generated from one of the patients by microdissection of the r(1) chromosome and with a BAC923C6 which maps to 1p12. Results indicated that patients with r(1) chromosomes consisting of 1q12 heterochromatin and short arm pericentric euchromatin which extends to at least the BAC923C6 were associated with a normal or mild phenotype. Patients with abnormal phenotypes possessed two types of rings. One patient had evidence for contiguous pericentric short arm euchromatin which extended from the centromere to beyond the BAC923C6. Two patients showed molecular cytogenetic results which were compatible with non-contiguous chromosome 1 euchromatin. The diversity of origin of r(1)s will hamper attempts to define phenotype/genotype relationships.

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of randomly ascertained supernumerary marker chromosomes, not exclusively small ring chromosomes. It is useful to ascertain and compare the phenotypes of patients with accessory ring chromosomes derived from the same chromosome to determine if common phenotypes and risk factors can be defined. However, since patients with rings originating from the same chromosome will be rare, it is difficult to ascertain sufficient patients for meaningful comparisons. In addition, the small ring chromosomes may contain variable regions of euchromatin originating from the pericentromeric short or long arms or contain discontinuous regions of euchromatin⁵ necessitating detailed molecular cytogenetic characterisation. Therefore establishing genotype/phenotype relationships in patients with accessory small ring chromosomes will be difficult. However, there is now evidence that the breakpoints of intrachromosomal rearrangements are not necessarily randomly distributed along the chromosome. For example, idic(15) chromosomes can be grouped into several categories with clustering of 15p breakpoints.² Therefore it is possible that a proportion of small ring chromosomes will originate from chromosome rearrangements which also show non-random breakpoints. If this is correct, then there could be a subgroup of patients with accessory ring chromosomes derived from the same chromosome with similar regions of euchromatin and therefore a characteristic phenotype.

In this report we present detailed cytogenetic characterisation of small ring chromosomes derived from chromosome 1, compare these with published cases, and discuss possible genotype/phenotype correlations.

Case reports

PATIENT A

This boy was referred at 8 years of age because of global developmental delay. His mother was hospitalised for the last 14 weeks of pregnancy because of pre-eclampsia. He was born by normal vaginal delivery at 36 weeks' gestation and weighed 2955 g (50th-90th centile). At 5 years of age he had surgery for a divergent strabismus, a feature also present in his mother and sister in childhood. There was no facial dysmorphism. Some minor dysmorphic features were present (table 1). Transient hepatomegaly of unknown cause was present and biochemical investigations were all normal. Psychological assessment at 12 years of age indicated function in the moderately intellectually disabled range. Blood lymphocytes showed the karyotype 47,XY,+r(1)/46,XY. Parental karyotypes were normal.

Table 1 Clinical comparison of phenotypically abnormal patients with r(1) accessory chromosome

Patient	A	B	C	D	Case 1
	This report	This report	This report	This report	Ref 11
Sex	M	M	M	F	M
Gestation	36	37	42	95	36
Growth					
Height (centile)	50–75	75–90	75		10–25
Lean build		+	+		
Long limbs		+			
BW (g)	2955	2450	3717	3150	2600
(Centile)	50–90	10	10–50	10–50	10–50
FTT		+			+
CNS/development					
Developmental delay/IQ	+/46–50	+/67–77	Language/ND		+/ND
Hypotonia					+
Craniofacial					
HC (centile)	2–50	25	2–50	50–98	<2
Plagiocephaly				+	+
Double scalp whorl				+	+
Protruding ears			+	+	+
Ear creases			+		
Long face			+		
Long nose			+		
Palpebral fissures					
Long		+	+		
Upward slanting		+	+		
Hypocanthus			+		
Telecanthus				+	
Epicanthus					+
Hypertelorism					+
Synophrys		+			
Strabismus	+				
High palate		+	+	+	
Trismus			+		
Smooth philtrum			+		
Protruding tongue				+	(infancy only)
Thorax/abdomen					
Pectus excavatum	+	+			
Nipples					
Widely spaced	+				
Hypoplastic	+				
Low set	+				
Hepatomegaly	+				
Chronic diarrhoea/steatorrhoea		+			
Congenital heart disease				+	small VSD
Musculoskeletal					
Kyphoscoliosis		+	+	+	
Clinodactyly V		+®	+		
Camptodactyly V		+®			
Skin					
Elbow dimples			+		
Haemangioma				+	neck, back
Other					
Chronic serous otitis media			+		

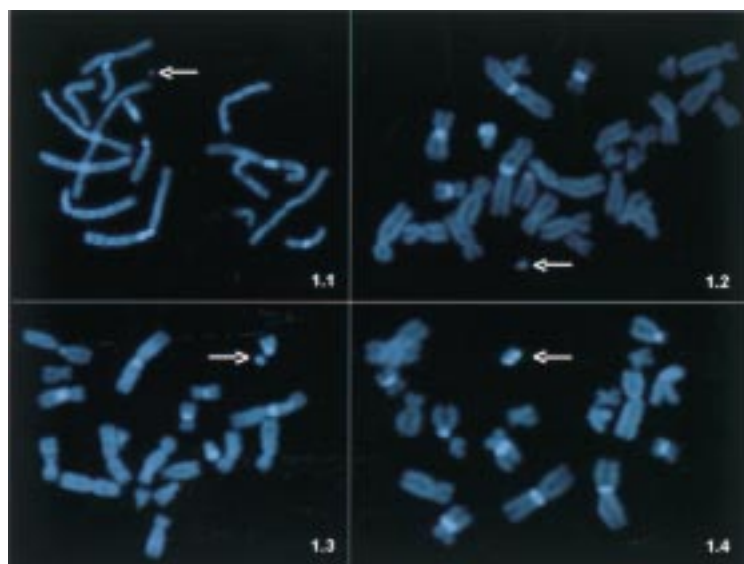


Figure 1 Distamycin A/DAPI characterisation of r(1)s. Patient karyotypes are presented with the r(1) arrowed for patients A (panel 1.1), B (panel 1.2), C (panel 1.3), and D (panel 1.4). Images are pseudo black and white.

PATIENT B

At 5 years of age this boy was referred for chromosome studies because of chronic diarrhoea, poor weight gain, and developmental delay. Investigations showed steatorrhoea and slightly abnormal pancreatic function tests. Pancreatic enzyme replacement was started. He gained weight initially but subsequently diarrhoea and weight loss recurred and were not exacerbated by cessation of the supplement. Testing at various ages showed mild intellectual disability with verbal superior to performance skills. At 15 years of age he was tall and thin with long limbs and was considerably taller than both parents and older sib. His face was mildly dysmorphic and with other clinical features as presented in table 1. Blood lymphocytes showed the karyotype 47,XY,+r(1)/46,XY. Parental karyotypes were normal.

PATIENT C

Originally reported in Callen *et al*,⁶ this boy was ascertained at the age of 2½ years because of delayed language development and dysmor-

phic features. At the age of 16, his intelligence and growth were normal. Recurrent serous otitis media had been a problem and a grommet was present in one ear. He had a very lean build with negligible subcutaneous fat, resulting in a rather gaunt facial appearance. He was dysmorphic (table 1). His inner canthal distance was 26 mm, outer canthal distance 85 mm, and interpupillary distance 57 mm. Blood lymphocytes showed the karyotype 47,XY,+r(1)/46,XY. Parental karyotypes were normal.

PATIENT D

The chromosomes of this girl were studied soon after birth because of the presence of a large, protruding tongue and a heart murmur, which was subsequently shown to result from a small ventricular septal defect. When seen at 12 weeks

and then at 2 years of age, her development was within the normal range, the ventricular septal defect had closed, and the tongue no longer protruded. Her other physical features are presented in table 1. Blood lymphocytes showed the karyotype 47,XX,+r(1)/46,XX. Parental karyotypes were normal.

Materials and methods

Standard cytogenetic and FISH procedures were used. The biotinylated probe D1Z1 was purchased from Oncor. The BAC 923C6 was fortuitously mapped to 1p12 as part of another project. This BAC (bacterial artificial chromosome) was previously reported to map to 16q24.3 in the Genome Data Base and is from the Caltech Human BAC Library A2. FISH with this probe used biotinylated DNA pre-

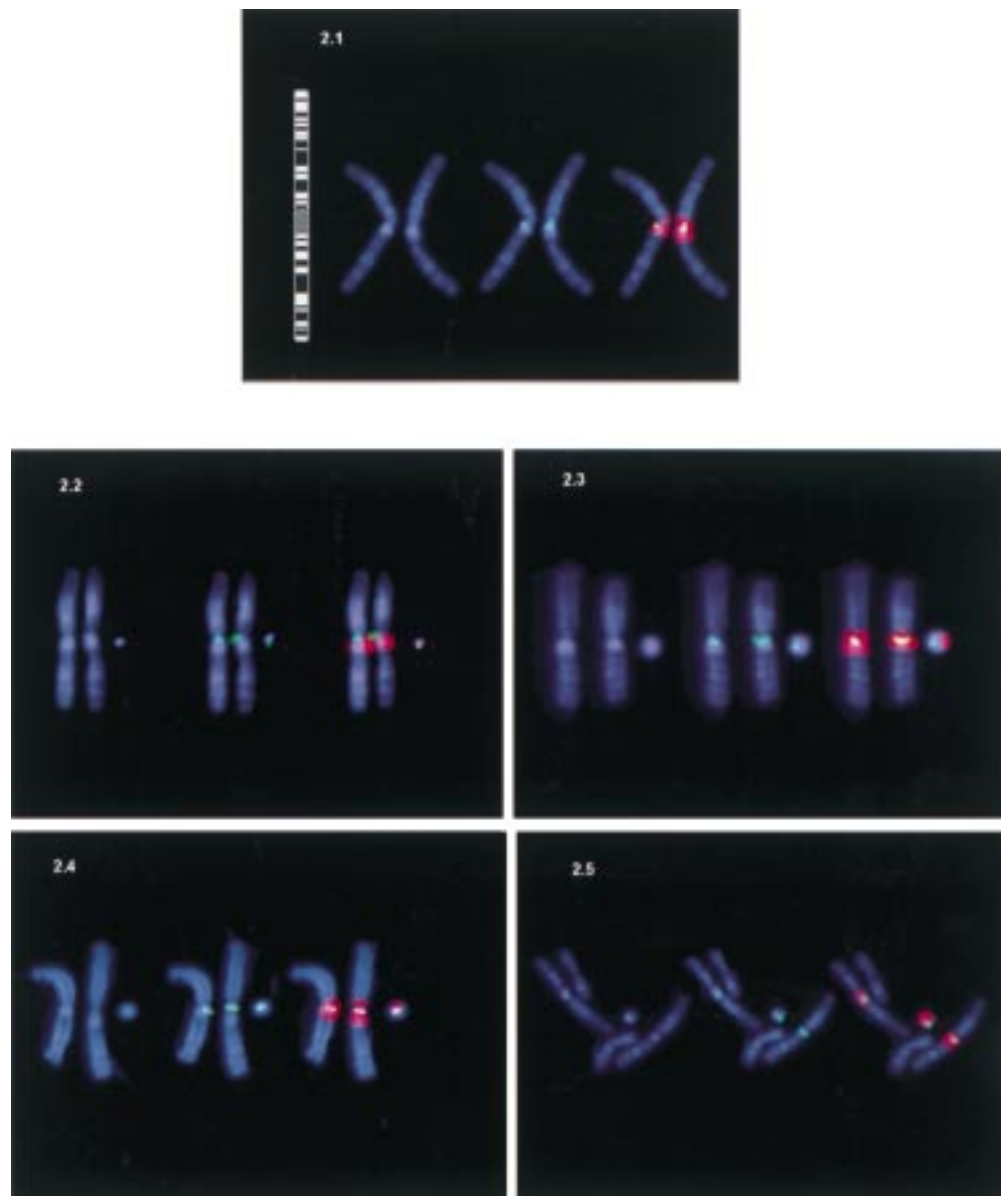


Figure 2 FISH characterisation of r(1)s. In each panel are presented three identical sets of chromosomes (panel 2.1, normal control chromosome 1 homologues; chromosome 1 homologues and ring of patient A (panel 2.2), patient B (panel 2.3), patient C (panel 2.4), and patient D (panel 2.5). The first of each set of chromosomes is counterstained blue with DAPI and the second shows the FITC signal from the centromeric probe D1Z1. The third set of chromosomes shows the red TRITC signal from reverse painting (from the microdissected r(1) of patient D) merged with the signal of the DAPI staining and the D1Z1 probe. The images were captured by a cooled CCD camera and merged using the Cytovision Ultra collection and enhancement system (Applied Imaging).

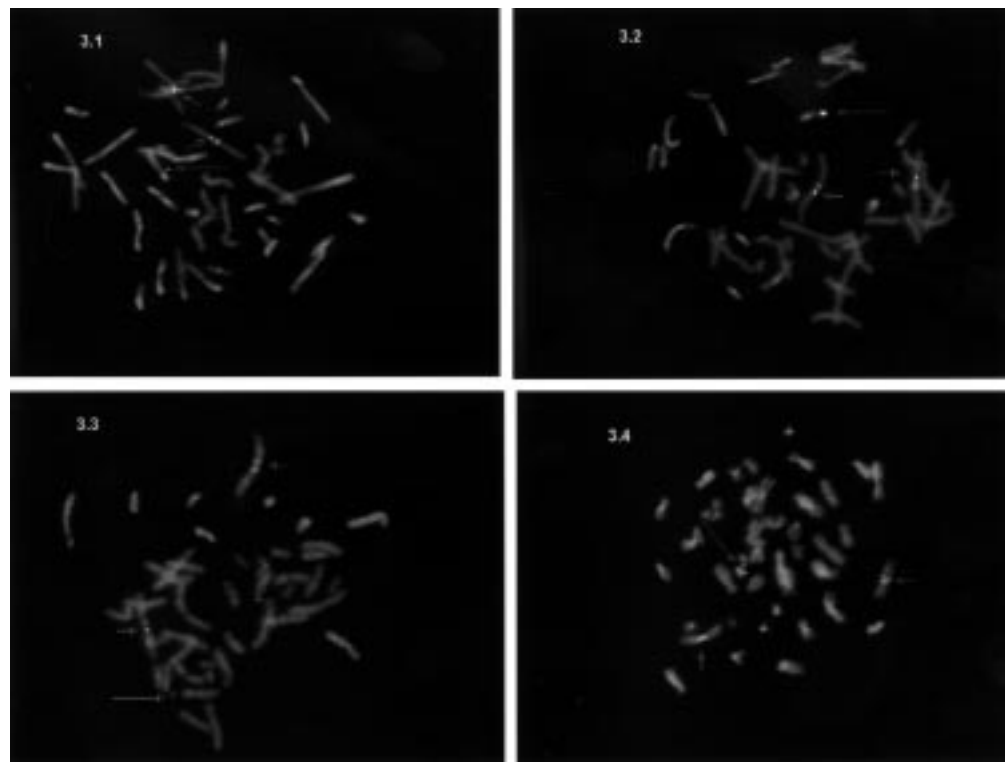


Figure 3 Metaphase spreads showing hybridisation of BAC923C6 to *r(1)*s. Indicated are normal chromosomes 1 (short arrow) and *r(1)* (long arrow). Positive hybridisation to the ring is shown in panels 3.1 (patient B) and 3.2 (patient D). Absence of hybridisation to the ring is shown in panels 3.3 (patient A) and 3.4 (patient C). Images were pseudo black and white of the original FITC signal using the Cytovision Ultra collection and enhancement system (Applied Imaging).

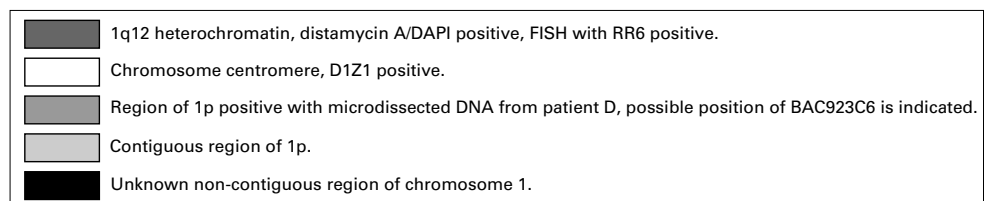
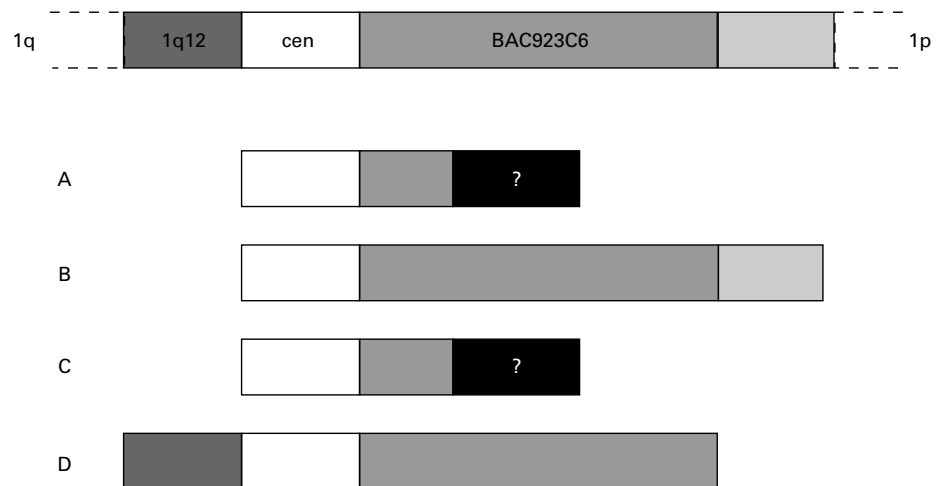


Figure 4 Proposed structure of accessory *r(1)*s as determined by molecular cytogenetics. The *r(1)*s from patients A to D are represented as linear structures. The full cytogenetic description of the karyotypes is as follows. Patient A: 47,XY,+r/46,XY.ish *r(1)*, RR6⁻, D1Z1⁺, BAC923C6⁻, rev ish 1p. Patient B: 47,XY,+r/46,XY.ish *r(1)*, RR6⁻, D1Z1⁺, BAC923C6⁺, rev ish 1p. Patient C: 47,XY,+r/46,XY.ish *r(1)*, RR6⁻, D1Z1⁺, BAC923C6⁻, rev ish 1p. Patient D: 47,XX,+r/46,XX.ish *r(1)*, RR6⁺, D1Z1⁺, BAC923C6⁺, rev ish 1p.

Table 2 Classification of small ring chromosomes 1 and relationship to phenotype

Patient reference	Sex	% r(1)	De novo	Ascertainment	Phenotype
<i>(1) 1q12 heterochromatin</i>					
Case 1, ref 10	F	25	Y	MCA	ND, del(18)(q22) also present
Case 17, refs 12 and 13	M	90	ND	Adult patient with CGL	Normal
Case 1, ref 14	M	100	Y	PND	Normal
<i>(2) 1q12 heterochromatin and euchromatin</i>					
Patient D, this report	F	15	Y	Minor dysmorphism, protruding tongue, heart murmur	Minor dysmorphism, VSD
Case 1, ref 15	F	22	Y	PND	Normal
<i>(3) Euchromatin, 1q12 heterochromatin absent</i>					
Patient A, this report	M	30	Y	Developmental delay, minor dysmorphism	Intellectual disability, minor dysmorphism
Patient B, this report	M	30	Y	Developmental delay, diarrhoea	Intellectual disability, minor dysmorphism
Patient C, this report	M	70	Y	Language delay, dysmorphic features	Minor dysmorphism, lean build
Case 1, ref 11	M	20	Y	Developmental delay, dysmorphism	Developmental delay, minor

F: female, M: male, ND: not determined.

PND: prenatal diagnosis, MCA: multiple congenital abnormalities.

pared by standard procedures. The procedure for chromosome microdissection of the ring from patient D was performed essentially as described previously.⁷ Briefly, five copies of the ring were dissected with glass microneedles controlled by a Narashige micromanipulator attached to an inverted microscope. The dissected ring fragments were transferred into a 5 µl collection drop (containing 40 mmol/l tris HCl, pH 7.5, 20 mmol/l MgCl₂, 50 mmol/l NaCl, 200 µmol/l of each dNTP, 0.1 U topoisomerase I, and 5 pmol of a universal primer (UN1) -CCGACTCGAGNNNNNNATGTG G-) and incubated at 37°C for 30 minutes. An initial eight cycles of PCR (denaturation at 94°C for one minute, annealing at 30°C for two minutes, and extension at 37°C for two minutes) were conducted by adding approximately 0.3 U of T7 DNA polymerase (Sequenase version 2.0, USB) at each cycle (Sequenase (13 units/µl) was diluted 1 to 8 in enzyme dilution buffer (USB), and 0.2 µl was added to 5 µl reaction mixture). Following this preamplification step, a conventional PCR reaction catalysed by *Taq* DNA polymerase was performed in the same tube; 50 µl PCR reaction mix was then added (10 mmol/l Tris-HCl, pH 8.4, 2 mmol/l MgCl₂, 50 mmol/l KC1, 200 µmol/l each of dNTP, 50 pmol UN1 primer, and 2 U *Taq* DNA polymerase (Perkin-Elmer/Cetus)). The reaction was heated to 95°C for three minutes followed by 35 cycles at 94°C for one minute, one minute at 56°C, two minutes at 72°C, with a five minute final extension at 72°C. An aliquot was reamplified with the same primers, labelled with digoxigenin by nick translation, and used as a probe for fluorescence in situ hybridisation studies (FISH) to metaphase chromosomes from short term lymphocyte cultures. Fluorescent images were processed using a Cytovision workstation (Applied Imaging).

Results

The ring chromosomes in each of the four patients varied in size with patient D possessing the largest and patient A the smallest. For each ring, the origin from chromosome 1 was determined by hybridisation to the probe D1Z1 which specifically hybridises to the alphoid repetitive DNA of the chromosome 1 centromere. The presence of 1q12 heterochromatin in the ring was determined by distamycin A/DAPI banding (fig 1) and confirmed by hybridisation to the probe RR6.⁶ The ring from

patient D was the only one which contained 1q12 heterochromatin.

The r(1) from patient D was microdissected and the PCR amplified products labelled with digoxigenin. Simultaneous hybridisation of the microdissected material (visualised as a red signal) and the centromere probe D1Z1 (visualised as a yellow signal) allowed the origin and structure of the markers in patients A, B, C, and D to be determined in more detail. Results from hybridisation to normal metaphases (fig 2.1) showed that the microdissected products resulted in a signal which was positive for 1q12, the centromere, and extended into the short arm distal to the centromeric hybridisation of D1Z1. In some metaphases this short arm signal of the microdissected products could not be clearly differentiated from the centromeric signal, and this was interpreted as spreading of the centromeric signal or the result of the particular physical configuration of the chromosome.

The structure of the rings in patients A, B, C, and D was then determined using simultaneous hybridisation of the microdissected products from patient D and the probe D1Z1. As expected, the ring of patient D was completely encompassed by the probes used (fig 2.5). However, in patients A, B, and C there was a region of the ring which was not labelled by either probe (fig 2.2, 2.3, and 2.4) and was therefore considered to represent additional euchromatin contained in the ring which was not contained in patient D. The representation of centromeric alphoid DNA, as shown by hybridisation with the alphoid repeat probe D1Z1 (fig 2) varied between the different rings, with patient D possessing the smallest region.

When the ring chromosomes were hybridised to BAC923C6, patients B and D were positive (fig 3.2 and 3.4) while A and C were negative (fig 3.1 and 3.3). The results of the molecular cytogenetic characterisation of these ring chromosomes are represented diagrammatically in fig 4.

Discussion

The molecular cytogenetic characterisation of these ring chromosomes showed that the ring of patient D contained 1q12 heterochromatin and a contiguous (from reverse painting using microdissected amplified DNA) region of euchromatin which hybridised to BAC923C6. The rings of patients A, B, and C did not contain 1q12 heterochromatin but all contained

euchromatin which was additional to that contained in the ring of patient D. This was shown by a lack of complete labelling of the rings with microdissected amplified DNA from patient D. Only in patient B did the ring hybridise to BAC923C6. This is interpreted as an additional contiguous region of short arm euchromatin that extends distal to BAC923C6 and is an additional region to that contained within the ring of patient D (fig 4). Painting with amplified microdissected DNA derived from the ring of patient D showed that there was additional euchromatin in patients A and C which was not contained in the ring of patient D, but this euchromatin did not hybridise to BAC923C6. An explanation for these findings is that these two rings contained non-contiguous chromosome 1 euchromatin. This has previously been shown by molecular cytogenetic studies using cloned microdissected products from a small ring chromosome derived from chromosome 4.⁵ In this case the ring consisted of three non-contiguous regions of this chromosome (4p13 or p14, centromere, and 4q31) and an origin was suggested from a conceptus with trisomy 4 with one of these chromosomes 4 consisting of a large ring chromosome formed by telomere fusion or rearrangement. Subsequently, the large ring was reduced in size by a series of breakage/fusion cycles resulting from cell division involving interlocked rings. Selective pressures during postzygotic development would ensure that cells with minimal aneuploidy would predominate. It is concluded that patients A and C have ring chromosomes 1 of a similar origin.

The phenotypes of the patients in this report, together with other reported patients with small ring chromosomes, are presented in table 2. The patient reported by Chen *et al*⁶ was excluded since the ring chromosome 1 of this patient was considerably larger, approximating the size of a G group chromosome. The structure of these rings is categorised according to presence or absence of 1q12 heterochromatin and euchromatin. Two patients with rings derived from heterochromatin alone were normal. The patient (case 1) reported by Callen *et al*⁶ also had a del(18)(q22) and the phenotype was consistent with the presence of this abnormality.

One patient with 1q12 heterochromatin and euchromatin was normal while the other had minor dysmorphism. For patient D of this report, trisomy for the pericentric short arm euchromatin extending at least to the BAC923C6 only appears to be associated with mild dysmorphism. Presumably this region is gene poor or contains genes which do not significantly compromise function when present at an increased dosage.

Detailed comparison of the phenotypes of patients with accessory r(1)s which consist of euchromatin but where 1q12 heterochromatin is absent (category 3, table 2) are presented in table 1. Based on molecular cytogenetic analysis, only patient B is likely to contain a contiguous region of pericentric short arm euchromatin. Patients A and B are considered to possess ring chromosomes derived from

non-contiguous regions of chromosome 1 and therefore are unlikely to share euchromatin between each other or with other patients. The origin of the euchromatin in the patient reported by Lanphear *et al*¹⁰ is not determined. There were no striking clinical similarities between any of the patients although patients B and C did show in a common a lean build, hand abnormalities, and a high palate.

Based on the patients in this report it will be very difficult to define a specific phenotype associated with the presence of a small ring chromosome 1. It is apparent that those accessory rings consisting of chromosome 1q12 heterochromatin or short arm euchromatin extending from the centromere at least to the BAC923C6 are associated with a normal or mild phenotype, although it should be noted that mosaicism of the ring is present in these patients. A potential ring 1 phenotype may be that present in patient B since molecular cytogenetic evidence is consistent with a contiguous region of pericentric short arm euchromatin.

Molecular characterisations of other accessory chromosomes, for example the inv dup(15)s, have shown a clustering of breakpoints. This suggests the processes leading to the generation of these chromosomes are dependent on specific DNA sequences. The molecular cytogenetic characterisation of the rings derived from chromosome 1 are not consistent with clustering of breakpoints but show the varied origin of such rings. Since both r(1)s and a ring (4) chromosome are consistent with an origin from non-contiguous regions of a chromosome, this is likely to be one of the general mechanisms for the formation of ring chromosomes. As a consequence the genetic content of similar sized markers originating from the same chromosome will vary and it will not be possible to provide definitive clinical information when counselling parents of newly ascertained cases of de novo small accessory ring chromosomes.

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