Ring chromosome 13: lack of distinct syndromes based on different breakpoints on 13q

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Abstract

A stillborn male child with anencephaly and multiple malformations was found to have the karyotype 46,XY,r(13)(p11q21.1). The breakpoint at 13q21.1, determined by high resolution banding, is the most proximal breakpoint ever reported in patients with ring chromosome 13. In situ hybridisation with the probe L1.26 confirmed the derivation from chromosome 13 and DNA polymorphism analysis showed maternal origin of the ring chromosome.

Our results, together with a review of previous reports of cases with ring chromosome 13 with identified breakpoints, could neither support the theory of distinct clinical syndromes based on different breakpoints on 13q nor correlate the severity of symptoms with instability of the ring.

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A ring chromosome formation of an acrocentric chromosome is often associated with increased severity of clinical symptoms compared to deletion of the same segment but without ring formation.¹ The most consistent clinical symptoms have been reported in ring chromosome 13 patients.¹ Since the first report of a ring chromosome 13,² many attempts have been made to correlate the clinical phenotype to cytogenetic findings.^{1,3,4}

Niebuhr and Ottosen⁴ identified three groups based on the clinical findings among patients with ring chromosome 13 and interstitial deletions in 13q. Breakpoints at 13q33 or

13q34 are found in the majority of reported cases of ring chromosome 13. A smaller group shows breakpoints at 13q31 or 13q32; more proximal breakpoints in the long arm have only been reported twice^{5,6} and one was derived from a maternal Robertsonian translocation chromosome (t(13q13q)).⁶

We present a case of ring chromosome 13 with very severe symptoms, namely anencephaly and multiple malformations. Anencephaly has only been reported in one previous case by Schmidt *et al.*⁵ The previously reported 22 cases of ring chromosome 13 with identified breakpoints are reviewed.^{1,3-19} The clinical findings were compared to the breakpoints and the stability of the ring, in an effort to test the theory of several clinical subgroups of patients with ring chromosome 13.^{1,20}

Material and methods

CASE REPORT

A male infant was stillborn at 29 weeks' gestation. Anencephaly was suspected after ultrasound investigation and labour was induced. He was the first child of a 25 year old mother and a 30 year old father. Both parents were healthy and non-consanguineous. The pregnancy was normal until the actual referral based on the symptoms of intrauterine growth retardation. The child was in breech position in labour which was otherwise uneventful. Birth weight was 650 g (less than 10th centile for 29 weeks of gestation) and birth length was 31 cm.

Observation of the infant at birth showed (fig 1) anencephaly, cranial bone defect at the vault, broad nasal bridge, hypertelorism, low

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Figure 1 The stillborn male infant with a ring chromosome 13. Note the proximally placed thumb on the right hand.

set ears, high arched palate, short philtrum, micrognathia, hypoplastic thumb on the left hand, imperforate anus, and varus position of the left foot with abnormal overlapping second toe.

Pathological examination showed a small baby with a 7 cm long cranium bone defect at the vault. Typical anencephaly with sparse cortical tissue and no sign of cerebellar tissue was found. Hypoplasia of the gallbladder was detected at necropsy and by histological examination. The suprarenal glands seemed hypoplastic but had normal histological architecture. Both kidneys were normal but a stenotic area was found in the left ureter near the bladder. No genital malformations were found. The imperforate anal canal was covered with skin but no atresia was observed.

CYTOGENETIC ANALYSIS

Metaphase chromosome preparations from the proband were made from cultured skin fibroblasts (H 131-90) and stained with quinacrine mustard (QFQ banding) using standard methods. A total of 200 metaphases was analysed. Chromosome analyses of the parents were performed on lymphocyte cultures and both were found to have normal karyotypes. To determine the breakpoints involved in formation of the ring chromosome, high resolution banding analysis was performed using prometaphase chromosomes with RBA, GTG, and QFQ banding and AgNOR silver staining.

MOLECULAR ANALYSIS

Genomic DNA was isolated from cultured fibroblasts from the proband and from parental leucocytes. The DNA polymorphisms studied were the dinucleotide repeats of locus *D13S71*²¹ and the *FLT1* gene assigned to chromosome 13q12.²² The polymorphisms were detected after PCR amplification of genomic DNA.²³ The primers used and the PCR programmes were as previously described.^{21,22} End labelling of primers, polyacrylamide gel electrophoresis of the amplification products, and autoradiography have been described elsewhere.^{24,25}

IN SITU HYBRIDISATION

The preparation of human metaphase spreads and the in situ hybridisation protocol were

performed essentially as described by Pinkel *et al*²⁶ with only a few modifications. The probe L1.26 (from American Type Culture Collection), specific for α satellite DNA sequences from the centromeres of chromosomes 13 and 21, was used. The probe was labelled by nick translation with Biotin-11-dUTP (Sigma) according to standard methods.²⁷ Hybridised probe was detected with fluorescein conjugated avidin and amplified using biotinylated anti-avidin antibody together with a new layer of fluorescein conjugated avidin. Chromosomes and interphase nuclei were counterstained by propidium iodide (Sigma). Slides were mounted in antifade solution and examined with a Leitz Diaplan microscope connected to a confocal laser scanning computer. Photographs were taken on an Agfachrome 50 RS colour slide film or Kodak technical pan TP651 black and white film directly from the screen.

Results

Cytogenetic analysis showed 46 chromosomes including a small ring chromosome 13 in 92% of the cells (fig 2, table 1). In 8% of the cells, only 45 chromosomes were seen and no ring chromosome (table 1). A normal cell line was not found in 200 cells examined. The ring chromosome was negative by silver staining indicating that no satellites were present. High resolution banding showed the breakpoints to be at p11 and q21.1, and the karyotype was thus 46,XY,r(13)(p11q21.1) (fig 2).

The results of the DNA polymorphism analysis are given in table 2. Locus *FLT1* showed two alleles in the proband, while *D13S71* showed only one allele with no maternal allele contribution (fig 3), indicating maternal origin of the ring and showing that the *D13S71* locus is distal to the breakpoint of the ring chromosome (13q21.1).

By using in situ hybridisation with a centromere probe specific for chromosomes 13 and 21 (L1.26), 68 metaphases showed four signals (table 1). In 32 metaphases only three signals were detected and in two metaphases five signals were found, indicating a dicentric ring chromosome (table 1, fig 4).

Discussion

We have reviewed 22 previous cases of ring chromosome 13 with identified breakpoints

Figure 2 Chromosomes 13 in prometaphase stained with RBA, QFQ, AgNOR, and GTG banding techniques.

Figure 3 Autoradiogram showing the alleles of the *D13S71* (CA)_n polymorphism in the ring chromosome 13 family.

Table 1 Distribution of cell lines in a case with ring chromosome 13.

	QFQ banding	in situ hybridisation
46,XY,r(13) single ring	184	68
45,XY,-13	16	32
68,XY,r(13) double ring	0	2
Total cell count	200	102

QFQ banding and later in situ hybridisation were performed on the same fibroblast culture, which reflects the in vitro instability of the ring.

Table 2 DNA polymorphism analysis in a ring chromosome 13 family.

Locus	D13S71 (CA) _n	FLT1 (GT) _n
Father	13	12
r(13)	1	12
Mother	24	22

The numbers represent the different alleles at a specific locus.

(tables 3 and 4). All cases had microcephaly/anencephaly, severe mental retardation, and multiple malformations. Other clinical findings, found in more than 50% of cases, include: growth retardation (21/23), facial dysmorphism (for example, epicanthus, broad nasal bridge, and hypertelorism) (22/23), abnormal ears (14/23), high arched palate (14/23), and foot or toe abnormalities (12/23). Clinical traits, found in more than 25% of the cases but less than 50%, are low hair line or alopecia, micrognathia, short philtrum, short or webbed neck, functional renal defect, and genital, anal, skeletal, and thumb anomalies. Less frequent abnormalities associated with ring chromosome 13 were hypoplasia of different organs, cardiac anomalies, epilepsy and pigment anomalies.

Of 23 cases of ring chromosome 13 with identified breakpoints the mean gestational age at birth was 37.8 weeks and the mean birth weight was 2027 g, which is below the 10th centile for 38 weeks. The sex distribution was equal, 11 were males and 12 were females. The parental origin of the ring chromosome has only been determined in seven out of 23 cases (tables 3 and 4).

According to the classification of Niebuhr and Ottosen⁴ and Niebuhr,²⁰ based on specific

clinical features among patients with ring chromosome 13 or deletion of the long arm of chromosome 13, it was suggested that breakpoints at 13q33 and 13q34 are frequently found in patients with severe mental retardation, microcephaly with true hypertelorism, frontal bossing erasing the nasal bridge, protruding upper incisors, and large ears with deep sulci. In the second group, they recorded breakpoints at 13q31 and 13q32. These patients exhibited almost all the features of the first group in addition to aplasia or hypoplasia of the thumbs, foot or toe abnormalities, severe genital malformations, anal atresia, and eye malformations. The third group, with a breakpoint at 13q21, was characterised by a large number of patients with retinoblastoma. Niebuhr and Ottosen⁴ found no cases with a ring chromosome 13 in the latter group, but only patients with interstitial deletion in the 13q21 region. These probably had deletion of 13q14, because more recent reviews²⁸ have shown that a candidate gene for retinoblastoma lies within the 13q14 region.

We have reviewed 22 previous cases of ring chromosome 13 with identified breakpoints on 13q and ordered them into three groups according to the site of the breakpoint. The clinical features are shown in tables 3 and 4 in order of increasing size of the deleted segment. According to tables 3 and 4, facial dysmorphism is more often found in cases with larger deletions, that is, in groups 2 and 3 rather than in group 1, and so are malformations of the genital and anal region and of the extremities, but not exclusively so. Aplasia/hypoplasia or proximally placed thumbs are found with equal frequency in the three groups. Even within the three groups, where the breakpoints are quite similar, considerable phenotypic variability is found. However, the most striking difference between the groups is that in group 3 two out of three cases have anencephaly. There was no information about microcephaly in the third case,⁶ but it was a very small fetus which was stillborn at term. Thus, a distinct clinical syndrome for each group based on different breakpoints on 13q seems unlikely. An attempt to map different clinical traits to specific segments on 13q using ring chromosome 13 patients will therefore be inaccurate.

It has been a subject of discussion whether the stability of the ring could have any effect on the severity of symptoms.^{7,10,13} A stable ring has only been reported twice,^{16,17} but the total cell count in those reports was only 36 and 50, respectively. Thus, at least 21 cases out of 23 seem to have a considerable degree of instability showing as loss of the ring or the presence of a single, double, or triple ring. Ascertainment was made from different tissue cultures, for example, blood, skin, and amniotic fluid (tables 3 and 4), so a comparison of the influence of mitotic instability on the clinical severity should be made with caution. Nevertheless, the present review shows that instability among ring chromosomes 13 does not influence the severity of symptoms. This conclusion is also in agreement with the findings of McCorquodale *et al.*¹³

Figure 4 The result of in situ hybridisation on metaphase spread using probe L1.26 specific for chromosomes 13 and 21. Two signals in close proximity indicate a double ring chromosome 13.

Table 3 Ring chromosomes 13 with breakpoints at q34/q33.

	Reference														
	7	3	8	9	10	11 Case 2	12 Case 2	13	14	15	16	17 Case 1	11 Case 1	1 Case 3	All 14 cases (mean value)
Gestational age (wk)	NI	40	41	38	40	<37	NI	37	40	NI	36	40	NI	44	39.3
Birth weight (g)	2050	2700	2350	2160	NI	1600	2350	2155	1950	NI	2100	2660	NI	4350	2402
Birth length (cm)	48	NI	47	NI	NI	40	48	46	48	NI	NI	47	NI	NI	46.3
Sex	M	M	M	F	F	F	M	F	M	F	F	M	F	F	6/8
Age mother/father	31/31	NI	23/26	41/48	21/23	NI	20/23	17/NI	21/NI	25/23	33/36	NI	41/46	17/19	26.4/30.6
Anencephaly															0% (0/14)
Microcephaly	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100% (14/14)
Mental retardation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100% (14/14)
Growth retardation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	86% (12/14)
Hypotonia															7% (1/14)
Facial dysmorphism	+	+	+	+	+		+	+	+	+	+	+	+	+	93% (13/14)
Protruding forehead								+			+				21% (3/14)
Hair (low line, alopecia)		L		Al	Al					L	L				33% (5/14)
Broad nasal bridge	+			+	+		+	+	+		+	+		+	64% (9/14)
Epicanthus	+	+	+	+	+		+	+	+		+	+	+	+	64% (9/14)
Hypertelorism		+					+	+			+	+		+	43% (6/14)
Micrognathia	+									+	+				29% (4/14)
Short philtrum									+					+	14% (2/14)
High arched palate	+			+	+					+		+	Cl+		43% (6/14)
Abnormal ears	+	+			+	+				+	+	+			50% (7/14)
Low set ears											+			+	21% (3/14)
Short neck/webbing		+/-			+/-					-/+	+/-			-/-	29% (4/14)
Other abnormalities	Ho	Co	Ca/Pi	Pi	Ho				Ep				Ca		
Renal defect (functional)	+														7% (1/14)
Genital malformation					+		+			+					21% (3/14)
Imperforate anus										+Aa					7% (1/14)
Skeletal anomalies					+	+			+				+		29% (4/14)
Foot anomalies					+					+			+	+	29% (4/14)
Toe anomalies					+								+	+	14% (2/14)
Hand anomalies						+				+	+		+	+	43% (6/14)
Hypoplasia 1st finger										Pp	Pp		Pp		21% (3/14)
<i>Cytogenetic studies</i>															
Cultures (S/B/M/A)	S/B	B	NI	S/B	B	B	B	S/B	S/B	S/B	B	B	B	B/M	
Origin of ring (mother/father)	NI	NI	NI	NI	NI	NI	M	M	M+	F	NI	NI	NI	NI	
Total cell count	600	NI	NI	100	228	351	NI	500	294	500	36	50	309	158	
45,-r(13) (%)	6	US	0	5	5	8	US	0.2%	17	US	0	0	7	2	
46,r(13) (%)	84		95	92	80	82		96	81	70	100	100	85	89	
46,r(13) double ring (%)	7		5	3	12	5		4	2		0	0	4	5	
Breakpoints	p11q34	q34	p13q34	p11q34	p11q34	p12q34*	p11q34	p13q34	p12q33	p11q33	p12q33	q33*	p11q33*	p11q33	

Aa = anteriorly placed anus; Al = alopecia areata; cultures: skin/blood/bone marrow/amniotic fluid; Ca = cardiac anomaly; Cl = cleft lip; Co = coloboma; Ep = epilepsy; Ho = hypoplasia of different organs; NI = no information; Pi = pigment anomaly; Pp = proximally placed; US = unstable; *prometaphase technique; †maternal translocated chromosome.

Table 4 Ring chromosomes 13 with breakpoints at q32/q31 (left 6 cases) and q2/q1 (right 3 cases).

	References						References					
	18	12 Case 1	1 Case 1	1 Case 2	4	19	All 6 cases (mean value)	5	Present	6	All 3 cases (mean value)	All 23 cases (mean value)
Gestational age (wk)	NI	NI	37	34	39	NI	36.7	33	29	40	34	37.8
Birth weight (g)	2425	1850	2220	1680	1800	1900	1979	900	650	690	747	2027
Birth length (cm)	NI	44	NI	41	42	46	43.3	32	31	NI	31.5	40.2
Sex	M	M	F	F	M	M	4/2	F	M	F	1/2	11/12
Age mother/father	25/24	33/30	27/29	24/23	24/24	27/31	26.7/26.8	41/NI	25/30	17/NI	27.7	26.7/29.1
Anencephaly								+	+		67% (2/3)	9% (2/23)
Microcephaly	+	+	+	+	+	+	100% (6/6)			NI	33% (1/3)	91% (21/23)
Mental retardation	+	+	+	+	+	+	100% (6/6)	SB	SB	SB		100% (20/23)
Growth retardation	+	+	+	+	+	+	100% (6/6)	+	+	+	100% (3/3)	91% (21/23)
Hypotonia							33% (2/6)				0% (0/3)	13% (3/23)
Facial dysmorphism	+	+	+	+	+	+	100% (6/6)	+	+	+	100% (3/3)	96% (22/23)
Protruding forehead							17% (1/6)				0% (0/3)	17% (4/23)
Hair (low line, alopecia)		Al	L				33% (2/6)				0% (0/3)	30% (7/23)
Broad nasal bridge	+	+	+	+	+	+	100% (6/6)	+	+	+	100% (3/3)	78% (18/23)
Epicanthus	+	+	+	+	+	+	33% (2/6)		+	+	67% (2/3)	57% (13/23)
Hypertelorism		+		+	+	+	83% (5/6)	+	+	+	100% (3/3)	61% (14/23)
Micrognathia		+		+	+	+	50% (3/6)		+	+	33% (1/3)	35% (8/23)
Short philtrum	+		+	+	+	+	83% (5/6)	+	+	+	100% (3/3)	43% (10/23)
High arched palate	+		+	+	+	+	83% (5/6)	+	+	+	100% (3/3)	61% (14/23)
Abnormal ears	+	+	+	+	+	+	100% (5/6)	+	+	+	67% (2/3)	61% (14/23)
Low set ears		+	+	+	+	+	83% (5/6)	+	+	+	67% (2/3)	43% (10/23)
Short neck/webbing			-/+	-/-		+/+	33% (2/6)				33% (1/3)	30% (7/23)
Other abnormalities	Mo	Pi	Co/Ho	Ca/Ho		Co/Ho		Ho		Ca/Ho		
Renal defect (functional)			+	+			33% (2/6)	+		+	67% (2/3)	22% (5/20)
Genital malformation	+		+	+	+	+	100% (6/6)	+		+	67% (2/3)	48% (11/23)
Imperforate anus			Aa	+Aa			50% (3/6)	+	+	Aa	100% (3/3)	30% (7/23)
Skeletal anomalies	+			+	+	+	67% (4/6)	+			33% (1/3)	39% (9/23)
Foot anomalies			+	+	+	+	50% (3/6)	+	+		67% (2/3)	39% (9/23)
Toe anomalies			+	+	+	+	50% (3/6)	+	+	+	100% (3/3)	35% (8/23)
Hand anomalies		+	+	+	+	+	83% (5/6)	+	+	+	67% (2/3)	57% (13/23)
Hypoplasia 1st finger					A	+Pp	33% (2/6)	+	+	+	67% (2/3)	30% (7/23)
<i>Cytogenetic studies</i>												
Cultures (S/B/M/A)	S/B	S/B	B	B	S/B	B		A	S	S+		
Origin of ring (mother/father)	NI	F	NI	NI	NI	NI		NI	M	M		5/2
Total cell count	144	NI	58	126	294	NI		30	200	NI		
46,-r(13) (%)	3	US	3	5	12			7	8	9		
46,r(13) (%)	94		88	79	79			93	92			
46,r(13) double ring (%)	3		3	2	2							
Breakpoints	q32	p13q32	p11q32	p11q32	p12q31	p12q31		q2?	p11q21.1*	q11q14*		

A = aplasia; Aa = anteriorly placed anus; Al = alopecia areata; cultures: skin/blood/bone marrow/amniotic fluid; Ca = cardiac anomaly; Co = coloboma; Ha = hypoplasia of the adrenals; Ho = hypoplasia of different organs; Mo = micropthalmia; NI = no information; P = pigment anomaly; Pp = proximally placed; SB = stillborn; US = unstable; *prometaphase technique; †parental translocation chromosome.

Reviewing the previously reported cases of ring chromosome 13, we could find no support for the existence of different syndromes based on different breakpoints on 13q or instability of the ring. However, this report shows that larger deletions of 13q are more likely to cause a severe clinical outcome than smaller deletions and that deletion of the segment 13q2→qter in ring chromosome 13 can reflect the most serious symptom ever reported among patients with ring chromosomes, namely anencephaly.

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