Molecular and cytogenetic characterisation of an unusual case of partial trisomy/partial monosomy 13 mosaicism: 46,XX,r(13)(p11q14)/46,XX,der(13)t(13;13)(q10;q14)

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Abstract

A female infant with multiple malformations and mental retardation was noted to have a rare de novo chromosome abnormality involving mosaicism with two cell lines, one with a ring chromosome 13, and the other with partial trisomy 13 owing to a complex rearrangement. Cytogenetic examination excluded the presence of a t(13q;13q) cell line and showed a cell line with a marker chromosome containing two chromosome 13 long arms joined together after deletion of a part (q11→q14) of one of them. In addition, the absence of a cell line with two normal chromosomes 13 or a cell line with a t(13q;13q) implies that the ring (13) and the marker (13) arose from a single event at the first cleavage division.

The two cell lines were present in different proportions in both peripheral blood lymphocytes and skin fibroblasts. The results of microsatellite characterisation clearly indicate the paternal origin and the absence of recombination, supporting the postzygotic origin of both the ring and the marker chromosome.

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Chromosomal mosaicism is the result of a postzygotic event and involves several distinct cell lines. If karyotypic changes leading to a stable form of mosaicism occur early after conception, clinically significant developmental consequences may ensue. It is therefore important to document specific forms of karyotypic mosaicism together with their clinical findings, particularly for rare forms of mosaicism, by applying molecular methods enabling characterisation of complex rearrangements which would be difficult to analyse using conventional cytogenetic techniques.[1–3]

We characterised an unusual form of mosaicism with two cell lines, one with a ring chromosome 13 and the other with a partial trisomy 13 owing to a complex rearrangement, by means of such different approaches as in situ hybridisation and microsatellite polymorphism typing.

Patient and methods

CASE REPORT

The patient is the first child of healthy, non-consanguineous parents. She was born at 38 weeks of gestation by normal delivery to a 27 year old mother. The pregnancy was uncomplicated, with no exposure to known teratogens. At birth she weighed 1760 g (<10th centile), had a head circumference of 23 cm (<10th centile), and was 43 cm long (<10th centile).

Observation of the infant at birth showed microcephaly, a large, receding forehead, symmetrical eyes with epicanthic folds and short, upward slanting palpebral fissures, low set, dysplastic ears, depressed nasal bridge, and micrognathia. She was admitted to hospital for persistent hypotonia and feeding difficulties and consequent failure to thrive. Routine laboratory findings were within normal ranges, with the exception of mildly raised serum ALT and AST and total bilirubin increased to 5.3 mg/dl. Serum protein electrophoresis showed increased α_2 globulin (22.7%, normal 6.8-12%) and reduced γ globulin (7.5%, normal 13-23%). Serological tests for rubella, herpes simplex virus, toxoplasmosis, cytomegalovirus, HBV, and HCV infections were negative.

Abdominal ultrasonography was normal. Cardiac examination showed a pansystolic cardiac murmur; two dimensional echocardiography and colour Doppler identified a muscular ventricular septal defect in association with pulmonary valve stenosis with good haemodynamic compensation. Chest *x* ray showed rib anomalies with posterior fusion of the sixth and seventh ribs bilaterally and fusion of the ninth and tenth left ribs.

Perinatal complications included respiratory difficulties and retarded growth. By 6 months, delayed development was evident with severe psychomotor and mental retardation.

The patient came to our attention for genetic consultation at 1½ years of age; previous assessments had been unable to identify an underlying cause for her multiple congenital anomalies, retarded growth, and significant developmental delay in all areas. The patient was severely mentally retarded. Head control and sitting were absent. She had no language and only shouted or cried. Visuospatial and constructive skills were impossible to assess, given her cognitive development. Hearing screening showed severe sensorineural impairment. Hypertonia was present with typical limb

deformities, equinovarus deformity of the feet, limited hip adduction, and flexion contractures with partial overlapping of the fingers.

Physical examination (fig 1) showed mild microcephaly with occipital flattening, a narrow forehead, a nuchal median capillary haemangioma, a flat nose with slightly bulbous nasal tip, malformed ears, a short neck with extra skin at the nape and a low posterior hairline, and pectus excavatum with significant chest deformity. Ocular abnormalities were apparent including mild microphthalmia, hypertelorism, and divergent strabismus. Serial blood cell counts, polymorphonuclear leucocyte morphology, and fetal haemoglobin level were normal. The family history was unremarkable. Cytogenetic, fluorescence in situ hybridisation, and molecular studies were performed.

CYTOGENETIC STUDIES

Chromosome analysis was performed according to standard methods on cultured cells from the patient's peripheral blood and skin biopsies, and from the peripheral blood of each parent. Lymphoblastoid cell lines were set up from lymphocytes of the patient and both parents. High resolution prometaphase chromosomes were examined by trypsin G banding,[4] reverse G banding,[5] and C banding.[6]

FISH ANALYSIS

FISH was performed on metaphase chromosomes from the patient's tissues using three DNA probes: α satellite DNA pZ21A provided by Dr M Rocchi (Institute of Genetics, Bari, Italy), wcp13 (Technogenetics), and LSI^{TM}13/RB-1 (Vysis). Probe pZ21A was labelled by nick translation using biotin-11-dUTP (Ortho) according to the supplier's instructions. FISH was carried out as previously described.[7]

MOLECULAR ANALYSIS

DNA was extracted from the patient's peripheral blood lymphocytes and skin fibroblasts and from blood samples from each parent using standard methodology. Molecular analysis was performed using PCR amplification of microsatellite polymorphisms spanning the whole of chromosome 13.

The following loci were examined: D13S232, D13S115, D13S221, D13S218, D13S171, D13S325, D13S118, D13S124, D13S162, D13S317, D13S170, D13S71, D13S225, D13S158, and D13S173 (table 1). Information on the primers is available from the Genome Data Base. PCR amplification conditions were as previously described.[3 8] Aliquots of 5 µl of amplified DNA were mixed with 7 µl of 95% formamide, 20 mmol/l EDTA, and 0.05% each of bromophenol blue and xylene cyanol loading dye, and run on 6% polyacrylamide (19:1, acrylamide:bisacrylamide) gel (8 mol/l urea and 0.5 × tris borate-EDTA). The DNA fragments were visualised by silver staining.[9]

Results

CHROMOSOME INVESTIGATIONS

The results of the routine cytogenetic investigations are reported in table 2 and illustrated in fig 2. High resolution karyotyping of 200 peripheral blood lymphocytes and 200 skin fibroblasts showed two different populations. On banding, one population was 46,XX,r(13)(::p11→q14::) and the other 46,XX,der(13); the marker was an abnormal submetacentric chromosome replacing the chromosome 13. C banding showed one centromeric region both on the ring and on the rearranged chromosome. The R banding pattern of this abnormal chromosome was consistent with its being made up of two chromosome 13 long arms joined together after deletion of a part (q11→q14) of one long arm. We describe this chromosome as follows: der(13)(13qter→cen::13q14→qter).

No other cell lines were identified in 200 cells analysed from lymphocyte and fibroblast cultures, including no karyotypically normal cells. The parental G banded chromosomes were normal.

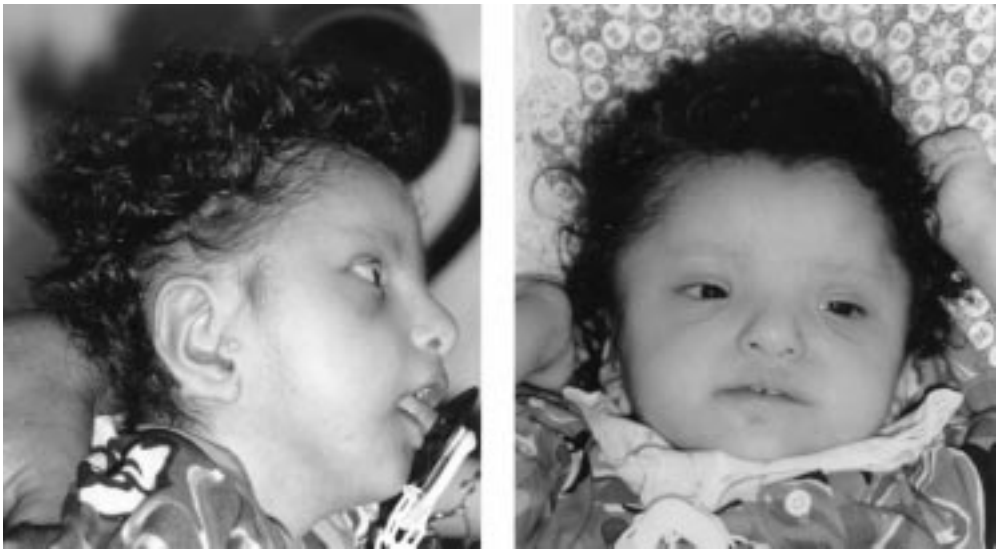

Figure 1 The patient aged 18 months.

Table 1 Microsatellite results

Locus	*Localisation*	*Father*	*Proband lymphocytes**	*Proband fibroblasts**	*Mother*
D13S232	13q11	3 3	3 1	3 1	1 2
D13S115	13q11-q12.1	2 1	2 2	2 2	2 2
D13S221	13q12.1	2 1	2 3	2 3	3 2
D13S218	13q12.2-q14.1	1 1	1 1	1 1	1 1
D13S171	13q12.3-q13	1 1	1 1	1 1	1 1
D13S325	13q14.1-q14.2	1 1	11 2	1* 2	2 1
D13S118	13q14.3	2 1	11 2	1* 2	2 1
D13S124	13q21	2 2	22 1	2* 1	1 2
D13S162	13q21.2-q31	3 3	33 2	3* 2	2 1
D13S317	13q22	1 1	11 2	1* 2	2 2
D13S170	13q31	3 3	33 1	3* 1	1 2
D13S71	13q32	2 2	22 1	2* 1	1 1
D13S225	13q32	4 2	44 1	4* 1	1 3
D13S158	13q32-q33	3 4	33 1	3* 1	1 2
D13S173	13q32-q34	3 3	33 2	3* 2	2 1

*Paternal allele (left) and maternal allele (right). The asterisk indicates the presence of a faint band.

Table 2 Percentage distribution of the two different cell lines with respect to tissue type

Tissue	*46,XX,r(13)(::p11→q14::) ish r(13)(pZ21A+,wcp13+,Rb-1-)*	*46,XX,der(13)(13qter→cen::13q14→qter) ish der(13)(pZ21A+,wcp13+,Rb-1x2)*
Blood lymphocytes	5%	95%
Skin fibroblasts	97%	3%

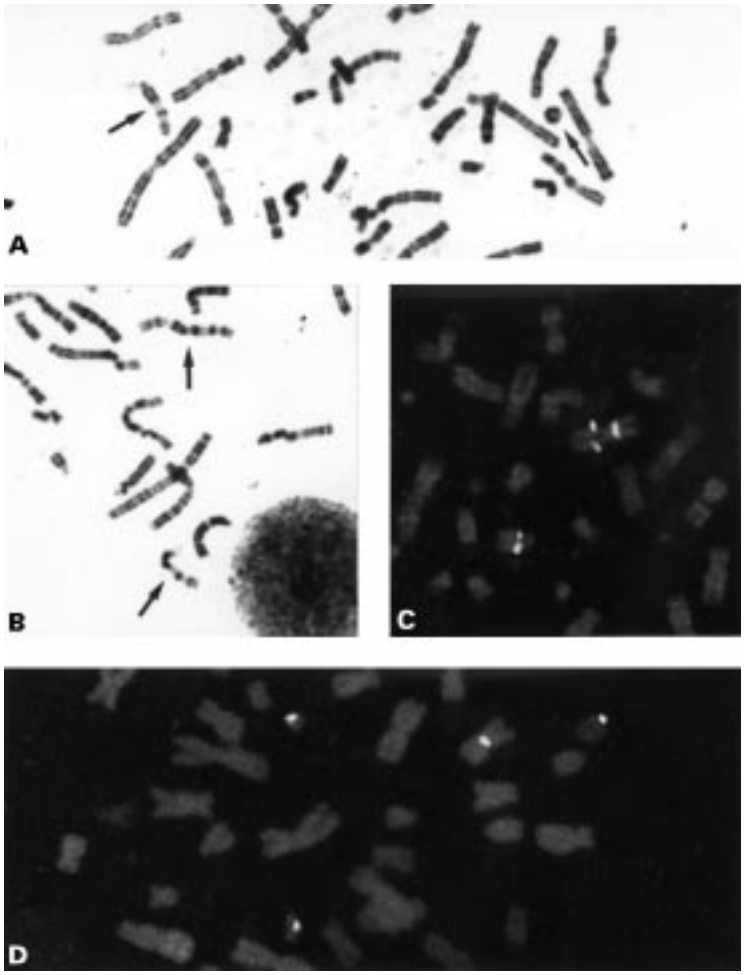

Figure 2 Lymphocyte R banded metaphases from the patient's two cell lines, one containing a normal and a ring chromosome 13 (A), and the other a normal and a derivative chromosome 13 (B). (C) FISH with LSI™13/Rb-1, mapping to band 13q14. In the rearranged chromosome 13, two sets of hybridisation signals were present. (D) Hybridisation signals from a satellite DNA pZ21A probe were present on the two chromosomes 21, the normal chromosome 13, and the der(13), indicating the presence of a single centromeric region on the rearranged 13.

FISH

FISH analysis was performed on metaphase chromosomes using pZ21A, an α satellite probe specific for the centromeric region of chromosomes 13 and 21. In all the metaphases, only one signal was detected on the ring 13 and on the rearranged chromosome (fig 2D). Hybridisation with wcp13, a probe which hybridises to most regions along the entire length of the 13 q arm and the centromere region, showed complete painting of the normal chromosome 13, the ring, and the rearranged chromosome.

On the basis of the banding pattern of the short arm, we also performed FISH with LSI™13/RB-1, a probe which maps to band 13q14. No signals were present on the ring chromosome, while the rearranged chromosome showed two sets of hybridisation signals (fig 2C), one on the long arm and the other on the short arm, indicating that the RB-1 sequence is retained on the chromosome 13 region which forms the short arm of the rearranged chromosome.

MOLECULAR ANALYSIS

In the patient's skin fibroblast DNA sample, the paternal and maternal allele of all the markers located between the 13q11 and 13q14 region showed the same intensity, whereas those mapping between the 13q14 and the 13q34 region presented a difference in intensity, the paternal ones being very faint (fig 3).

These results, consistent with the fibroblast cytogenetic findings, indicate that the ring contains only the proximal region of 13q and is of paternal origin. As for the markers located between the 13q14 region and the telomere, more intense maternal bands imply the loss of the distal region of the chromosome 13 of paternal origin in the majority of cells (97%) (fig 3). The faint paternal bands represent the contribution of the remaining 3% of cells containing the rearranged chromosome which carries two doses of the markers (one on each arm).

In the blood lymphocyte DNA sample, the situation complements the one described above (fig 3). In fact, the intensity of parental contributions is identical for the markers located proximally, as both the 46,XX,r(13)(::p11→q14::).ish r(13)(pZ21A+,wcp13+,Rb-1-) and the 46,XX,der(13)(13qter→cen::13q14→qter).ish der(13)(pZ21A+,wcp13+,Rb-1x2) cell lines are disomic for two doses of each of these markers. All the markers located distally to the q14 region show more intense paternal bands, as nearly 95% of lymphocytes bear the rearranged chromosome 13 of paternal origin, which carries two copies of the loci typed.

Discussion

The results of the present study reaffirm the importance of analysing different tissues and combining cytogenetic with molecular techniques to investigate any apparent discrepancy between karyotype and phenotype. In our patient, the discordance between the lymphocyte derived translocation trisomy 13

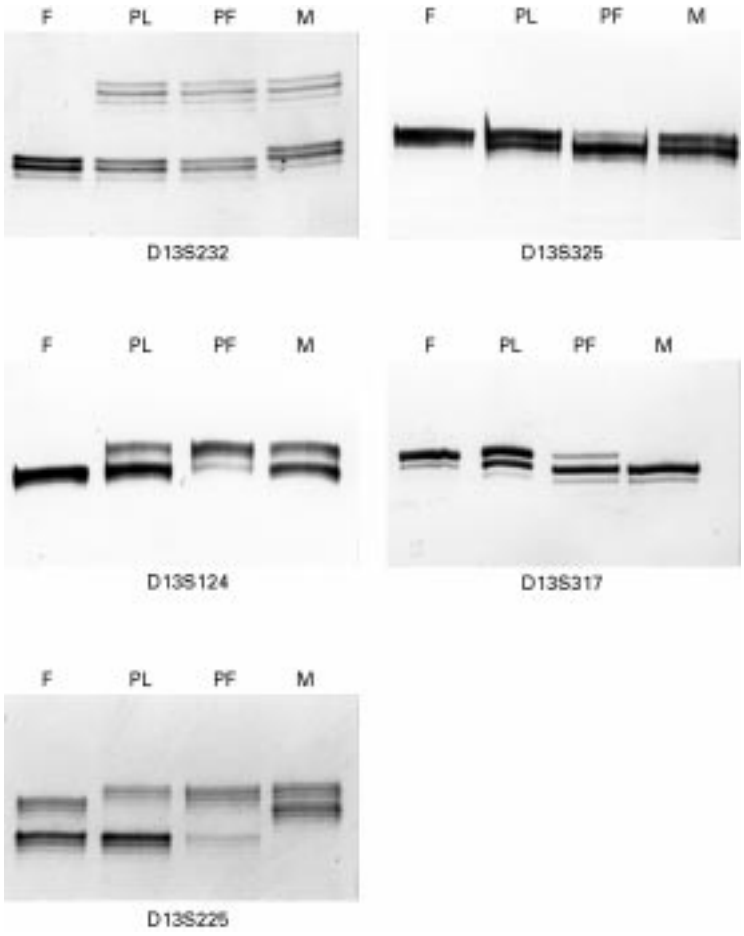

Figure 3 Representative microsatellite polymorphism results from father (F), patient blood lymphocytes (PL) and skin fibroblasts (PF), and mother (M). For marker D13S232, located in 13q11, the paternal and maternal allele shows the same intensity in the patient's lymphocytes and skin fibroblasts. For markers D13S325, D13S124, D13S317, and D13S225, mapping between 13q14 and 13qter, the paternal allele seems very faint in the skin fibroblasts, and more intense than the maternal one in the blood lymphocytes. No recombination was evident.

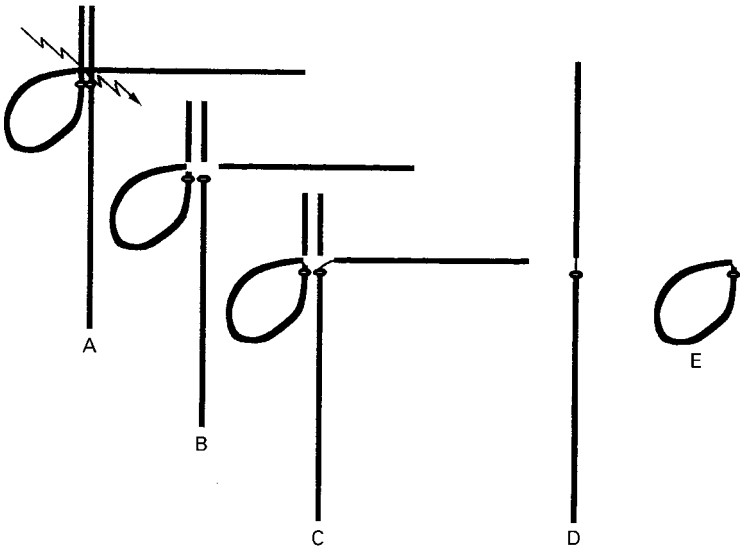

Figure 4 Possible mechanism underlying the formation of the two abnormal chromosomes (D and E). A break occurring in the overlapping region between the long arm of one chromatid and the short arms of both chromatids of a normal chromosome 13 at the two chromatid stage (A) generates five fragments (B). Erroneous rejoining (C) gives rise to the marker 13 chromosome (D) and ring 13 chromosome (E), whereas the short arms of both 13 chromatids are lost.

karyotype and the atypical non-Patau phenotype is clearly attributable to the tissue specific mosaicism. The examination of two different tissues showed the presence of two cell lines: one, containing the ring, is monosomic for the distal portion of chromosome 13, the other, containing the marker chromosome, is trisomic for the same portion.

It is not known what the proportion of these two cell lines may be in vivo in the various tissues, and in any case the complementary presence of monosomy and trisomy for the same distal portion of chromosome 13 in the same tissue made phenotype-karyotype correlation impossible. However, the typical severe malformations, that is, cleft lip, polydactyly, holoprosencephaly, and scalp defects, associated with trisomy 13, or the more common features of distal 13 trisomy,[10 11] that is, bushy eyebrows, long curved eyelashes, prominent nasal bridge, long philtrum, highly arched palate, and hexadactyly, are absent in our patient. Nor are the severe malformations recently described by Brown *et al*[12 13] in deletion of the 13q32 region present. As for the ring chromosome, an exhaustive review[14] of previously reported cases with identified breakpoints showed the lack of distinct clinical syndromes based on different breakpoints and the absence of any correlation between the severity of the symptoms and ring instability.

Patients cytogenetically similar to the one described here have been reported by Oka *et al*,[15] Duarte *et al*,[16] Jalal *et al*,[17] and Duckett *et al*.[18] A comparison of the clinical features of all these patients is shown in table 3. Although variations in the gene content of the rearranged chromosomes and the distribution of the two cell lines in the different tissues may account for clinical differences among the five cases, it is difficult to explain why all these subjects are less severely affected than might have been expected on the basis of their karyotype.[17 18]

There could be ascertainment bias, all the most severely malformed cases having died in utero. Alternatively, we could speculate that, at the tissue level, the complementary presence of trisomic and monosomic cells for the same chromosome 13 region could have altered the deleterious effects that either of the two chromosome anomalies alone usually induces.

Recently, with the application of molecular methods, the majority of homologous rearrangements of chromosome 13 had been shown to be isochromosomes, with an approximately equal ratio of maternally to paternally derived cases (4:3).[3 8 19] In addition, according to Shaffer *et al*,[8] most 13;13 isochromosomes seem to have a postzygotic origin, although the lack of detectable recombination events does not completely exclude a meiotic origin. The authors of the four mosaic cases reported in table 3 postulated that their cases could represent the result of two different events, a meiotic mutation in one of the parents with consequent t(13q;13q) formation, and a secondary mitotic event with breakage in both long arms of one chromatid of the translocated chromosome and rejoining to produce a ring.[17 18] As in none of these cases had molecular investigations

Table 3 Comparison of clinical features in four cases of t(13q;13q)/r(13) mosaicism, our patient, r(13)q2/q1, and partial 13q14→13qter trisomy† (modified by Duckett et al[18])*

Clinical features	*Oka et al*[15]	*Duarte et al*[16]	*Jalal et al*[17]	*Duckett et al*[18]	*Our case*	*r(13) q2/q1*	*Trisomy 13 q14→qter*
Peripheral blood karyotype							
% trisomy 13	75	74	34	53	95		
% ring 13	25	26	66	47	5		
Growth retardation	+	+	+	+	+	3/3	7/11
Psychomotor retardation	+	+	ND	ND	+	ND	11/11
Position or articular defects	−	−	−	−	+	ND	
Hypotonia	−	−	−	−	+	ND	3/11
Haemangioma	−	−	−	−	+	0/3	4/11
Microcephaly	+	+	+	−	+	ND	3/11
Micrognathia	−	−	+	−	+	1/3	4/11
Cleft/high arched palate	+	+	+	−	−	3/3	9/11
Short webbed neck	−	−	+	−	+	1/3	2/11
Broad nasal bridge	+	+	+	+	+	3/3	1/11
Low set/dysplastic ears	+	+	+	−	+	2/3	9/11
Short philtrum	−	−	−	−	−	3/3	0/11
Hypertelorism	+	+	+	+	+	3/3	0/11
Epicanthus	+	+	+	+	+	2/3	3/11
Short upward slanting palpebral fissures	−	−	+	−	+	1/3	4/11
Absent/hypoplastic thumbs	−	+	+	−	−	2/3	0/11
Hypoplastic fingers/toes	+	+	+	+	−	3/3	0/11
Clinodactyly/syndactyly	−	−	+	−	−	1/3	1/11
Hexadactyly	−	−	−	−	−	0/3	6/11
Congenital heart defects	+	−	−	−	+	1/3	1/11
Renal anomalies	−	−	+	−	−	2/3	1/11
Anterior/imperforate anus	−	+	+	+	−	3/3	0/11
Neural tube defects	−	−	+	−	−	2/3	0/11
Vertebral or costal abnormalities	−	−	−	+	+	1/3	6/11
Genital malformations	−	−	−	−	−	2/3	7/11
Increased PMN projections							
HbF persistence	−	−	−	−	−	0/3	1/11
Other	Duplicated oesophagus	Microphthalmia			Sensorineural deafness	Hypoplastic organs	

*Brandt *et al*.[14]
†Tharapel *et al*.[11]

been performed, there is no evidence to support or oppose this hypothesis.

In our case a meiotic origin seems highly improbable for the following reasons: (1) cytogenetic examination excluded the presence of a t(13q;13q) cell line and showed a cell line with a marker chromosome containing two chromosome 13 long arms joined together after deletion of a part (q11→q14) of one of them; (2) the absence of a cell line with two normal chromosomes 13 or a cell line with a t(13q;13q) implies that the ring (13) and the marker (13) arose from a single event at the first cleavage division; (3) the results of microsatellite characterisation clearly indicate the paternal origin and the absence of recombination (table 1), supporting the postzygotic origin of both the ring and the marker chromosomes.

As shown in fig 4, we hypothesise a single postzygotic breakage event generating five different fragments (B). The erroneous rejoining (C) of the proximal part of the long arm of one chromatid with its short arm would then form the ring (E), while the union of the distal long arm of the same chromatid with the proximal broken end of the short arm of the remaining chromatid would give rise to the marker 13 chromosome (D). The pter region of both chromatids would be lost.

Similar mechanisms have been suggested for chromosomal mosaicism cases involving different chromosomes.[20–22] However, our case is the first to lead to the hypothesis, on the basis of FISH and molecular analyses, that a single somatic mutagenic event could account for these complex de novo rearrangements, about which conventional cytogenetic techniques are unable to provide precise information.

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