Duplications in addition to terminal deletions are present in a proportion of ring chromosomes: clues to the mechanisms of formation

E Rossi,^{1*} M Riegel,^{2*} J Messa,¹ S Gimelli,¹ P Maraschio,¹ R Ciccone,¹ M Stroppi,³ P Riva,³ C S Perrotta,⁴ T Mattina,⁴ L Memo,⁵ A Baumer,² V Kucinskas,⁶ C Castellan,⁷ A Schinzel,² O Zuffardi^{1,8}

¹ Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy; ² Institut fuer Medizinische Genetik der Universitaet Zuerich, Schwerzenbach, Switzerland; ³ Dipartimento di Biologia e Genetica, Università di Milano, Milano, Italy; ⁴ Divisione di Genetica Medica, Università di Catania, Catania, Italy; ⁵ UO Patologia Neonatale, Ospedale Cà Foncello, Treviso, Italy; ⁶ Human Genetics Center, Santariskiu Hospital, University of Vilnius, Vilnius, Lithuania; ⁷ Genetische Beratungsstelle, Bozen, Italy; ⁸ Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Correspondence to: Dr O Zuffardi, Genetica Medica, Università di Pavia, Pavia, Italy; zuffardi@unipv.it

*The first two authors contributed equally to the work

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ABSTRACT

Background and methods: Ring chromosomes are often associated with abnormal phenotypes because of loss of genomic material at one or both ends. In some cases no deletion has been detected and the abnormal phenotype has been attributed to mitotic ring instability. We investigated 33 different ring chromosomes in patients with phenotypic abnormalities by array based comparative genomic hybridisation (CGH) and fluorescence in situ hybridisation (FISH).

Results: In seven cases we found not only the expected terminal deletion but also a contiguous duplication. FISH analysis in some of these cases demonstrated that the duplication was inverted. Thus these ring chromosomes derived through a classical inv dup del rearrangement consisting of a deletion and an inverted duplication.

Discussion: Inv dup del rearrangements have been reported for several chromosomes, but hardly ever in ring chromosomes. Our findings highlight a new mechanism for the formation of some ring chromosomes and show that inv dup del rearrangements may be stabilised not only through telomere healing and telomere capture but also through circularisation. This type of mechanism must be kept in mind when evaluating possible genotype–phenotype correlations in ring chromosomes since in these cases: (1) the deletion may be larger or smaller than first estimated based on the size of the ring, with a different impact on the phenotype; and (2) the associated duplication will in general cause further phenotypic anomalies and might confuse the genotype–phenotype correlation. Moreover, these findings explain some phenotypic peculiarities which previously were attributed to a wide phenotypic variation or hidden mosaicism related to the instability of the ring.

“ring syndrome”¹¹ that in cases with intact ring chromosomes is characterised, independently of the chromosome involved, by severe growth failure, minor dysmorphic features, and mild to moderate mental retardation, without major malformations. In a review of 207 cases, Kosztolányi² estimated that one fifth of subjects with autosomal rings are affected by the “ring syndrome” phenotype. Indeed, more recent papers have demonstrated that intact ring chromosomes may cause areas of hypopigmentation along the lines of Blaschko as the only sign of ring-induced mosaicism,³ or specific features such as a characteristic type of epilepsy and electroencephalographic pattern as reported for several ring (20) chromosomes,⁴ thus weakening the hypothesis of the “ring syndrome”. Moreover, fluorescent in situ hybridisation (FISH) analysis at first, and more recently whole genome array screenings, have demonstrated that in most of the cases a cryptic deletion is at the basis of the phenotypic abnormalities in apparently intact rings.^{5–7} Recently the case of an r(14) has been reported with the combination of an inverted duplication with a terminal deletion characterised using high resolution molecular karyotyping and FISH.⁸ The patient presented overlapping clinical features described in terminal deletion, duplication and ring chromosome 14 cases. By examining 33 probands with ring chromosomes through array based comparative genomic hybridisation (CGH) we detected the same situation in seven of them, suggesting both a new mechanism of ring formation and a warning for clinical geneticists to consider this possibility while performing genotype–phenotype correlations.

MATERIALS AND METHODS

Patients

The initial chromosome examination which detected a ring chromosome was performed in 17 cases in different cytogenetic laboratories in Italy and in 16 cases in Zurich, Switzerland. In one case (case 33, from Zurich), the ring chromosome was transmitted from a mildly affected mother with low mosaicism to a non-mosaic daughter, while in all the other cases the ring formation had occurred de novo. Three cases, 8 (case 14 in Ballarati *et al*⁹), 9 (case 10 in Ballarati *et al*⁹ and Guala *et al*¹⁰) and 25 (Baumer *et al*¹¹) had already been published.

Ring chromosomes are usually associated with abnormal phenotypes due to the loss of material at both or at least one chromosome end. Thus, in principle, the abnormal phenotypes are essentially due to haploinsufficiency of those dosage sensitive genes contained in the deleted segment(s). The finding of ring chromosomes without apparent loss of genetic material in subjects with abnormal phenotypes led investigators to hypothesise that the ring formation with the related difficulties in the sister chromatid separation at cell division induced the generation of secondary aneuploid cells. Some aneuploidies, being lethal at the cellular level, would in turn give rise to increased cell death rate. Altogether this situation should lead to the

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Array CGH

In 28 cases array based CGH was performed using the Agilent Human Genome CGH Microarray Kit 44B (Agilent Technologies, Santa Clara, California, USA), and in six cases the 244A Kit. In one case (case 21) we used both platforms. These platforms are high resolution oligonucleotide based microarrays that allow genome-wide survey and molecular profiling of genomic aberrations with a resolution of about 100 kb and 20 kb, respectively. Labelling and hybridisation were performed following the protocols provided by Agilent. Briefly, 500 ng of purified DNA of a patient and of a control (Promega Corporation, Madison, Wisconsin, USA) were double-digested with *RsaI* and *AluI* for 2 h at 37°C. After 20 min at 65°C, DNA of each digested sample was labelled, by the Agilent random primers labelling kit, for 2 h using Cy5-dUTP for the patient DNA and Cy3-dUTP for the control DNA. Labelled products were column purified and prepared according to the Agilent protocol. After probe denaturation and pre-annealing with 50 µg of Cot-1 DNA (Invitrogen, Carlsbad, California, USA), hybridisation was performed at 65°C with rotation for 40 h. After two washing steps the arrays were analysed with the Agilent scanner and the Feature Extraction software (v8.0; v9.1.3). Graphical overview was obtained using the CGH analytics software (v3.1; v3.4). All the breakpoint sequences were analysed with self chain and segmental duplication repeats tools from UCSC (<http://genome.ucsc.edu/>).

FISH

To confirm array CGH results and demonstrate the inversion of the duplication in patients 7 and 13, FISH experiments were performed. All bacterial artificial chromosome (BAC) probes used were selected according to the UCSC Human Genome Browser and were obtained from the human library RPCI-11. DNA extraction and FISH experiments were carried out as previously described.¹² The inversion of the duplication was determined in two cases by dual colour FISH experiments according to standard protocols.

Genotyping

Genotyping of polymorphic loci was performed by amplification with primers labelled with fluorescent probes (ABI 5-Fam and Hex) followed by analysis on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster, California, USA). The UCSC Genome Browser maps and sequences were used as references. Amplification was performed with Taq Gold (Applied Biosystems) using standard protocols. In case 13, primers were designed in the duplicated region. The sequences of the informative markers are the following:

- ▶ 15DUP3 left: CTCAGCGAGAGACCATCCTC, right: AAGAGAGCAGGAGGGAAGC;
- ▶ 15DUP4 left: CCAAAAAGTGCATGGTGTG, right: AGGGAATGTGACCTGTGGAG;
- ▶ 15DUP11 left: CAGAATTCGGGACAGGAAAA, right: CCAATCGCCTGCTCTCTTAG;
- ▶ 15DUP13 left: TGGAGAACACCTGGAAGAGG, right: CGGCCGTTCTCATGTATCTT;
- ▶ 15DUP15 left: TAAGCAAGCACCAAGCACAC, right: TTGTTGCTTCTGTGGGAAAA

RESULTS

We identified seven dup del or inv dup del rearrangements among 33 different ring chromosomes (fig 1). The denomination of “inv dup del” was reserved to those two cases (cases 7

and 13) in which, thanks to the availability of lymphoblastoid cell lines, we demonstrated by dual colour FISH that the duplication was inverted. In case 7 the BACs used were RP11-164b1 (biotin labelled) and RP11-122a8 (digoxigenin labelled) (fig 2A); in case 13 the inversion was identified by BAC RP11-1072p10 (biotin labelled) and RP11-522b15 (digoxigenin labelled) (fig 2B). The orientation of the duplication could not be investigated in the five cases with a “dup del” rearrangement (cases 5, 10, 21, 26, 33). The chromosomes involved were: chromosome 13 (cases 5, 7 and 10), chromosome 15 (case 13), chromosome 18 (cases 21 and 26) and chromosome 22 (case 33).

Data on array-CGH results and parental origin of the 33 cases are given in table 1.

The clinical features of the seven patients with inv dup del or dup del ring chromosomes and the array CGH findings with the resulting genomic imbalance are listed in table 2.

In the two inv dup del cases (cases 7 and 13) microsatellite analysis in patients and parents with markers mapping to the deleted regions (D13S173, D13S1315: case 7; D15S642, D15S107: case 13) indicated a paternal origin of the rearrangement in both cases (fig 3). Moreover, microsatellite markers of the duplicated region showed that the rearrangement was intrachromosomal since only two alleles, the paternal one showing a double peak area, had been detected for all the informative markers (D13S1283, D13S790, D13S1323; 15DUP3, 15DUP4, 15DUP11, 15DUP13, 15DUP15) (fig 3A,B).

In case 33, with a dup del(22q), the rearrangement was probably maternal in origin. We did not perform microsatellite analysis since parental DNA was not available. However, the mother who had some phenotypic abnormalities showed a low mosaicism (1 out of 50) for the same ring in lymphocytes.

In case 21 with an inv dup del(18p), the array CGH results with the Agilent Kit 44B (100 kb resolution) identified in 18p a single deleted oligonucleotide (170,229–170,285 kb). A second analysis with the Agilent Kit 244A, with a resolution of about 20 kb, confirmed a real terminal deletion of 207.5 kb (fig 1F). In this case, as in case 26, a second deletion was detected at 18q.

DISCUSSION

Ring formation in the seven dup del/inv dup del rearrangements

The classic mode of formation of ring chromosomes is breakage in both arms of a chromosome, loss of distal fragments followed by fusion of the proximal broken ends. In this case the patient has a partial monosomy for the distal short arm and distal long arm. In other cases, especially patients with no or very mild abnormal phenotype, loss of the telomeres with retention of subtelomeric sequences has been reported. It has been assumed that even this small genetic loss may contribute to an abnormal phenotype (patient 2 in Sigurdardottir *et al*¹³). The other mode of formation is telomere-to-telomere fusion, with telomeric and subtelomeric sequences being retained, and in this case no genetic material would be lost. It has been hypothesised that the phenotypic abnormalities in this type of ring chromosome are due essentially to what is called “dynamic mosaicism” with pre- and postnatal growth retardation and microcephaly as consistent features.^{2–14} Several examples show the presence of specific phenotypes otherwise typically associated with dominant gene mutations in patients carrying ring chromosomes not deleted for the responsible genes.^{15–16} Thus, hemizygoty of the responsible gene(s) in a significant proportion of somatic cells due to the ring instability may explain the phenotype.

Obviously, cryptic deletions of critical sequences distal to the commercial subtelomeric clone in one of the two arms had also

Figure 1 Array based comparative genomic hybridisation (CGH) profiles of six out of seven inv dup del rearrangements; A: case 5; B: case 7; C: case 13; D: case 33; E: case 26; F: case 21. In case 21 the array CGH profile refers to experiment with the CGH Microarray Kit 244A; in all the other cases with the CGH Microarray Kit 44B. The green arrows in E and F indicate the small deletions.

Figure 2 (A) Case 7, dual colour fluorescence in situ hybridisation (FISH) with bacterial artificial chromosome (BAC) RP11-164b1, green fluorescence, and RP11-122a8, red fluorescence. (B) Case 13, dual colour FISH with BAC RP11-1072p10, green fluorescence, and RP11-522b15, red fluorescence. In both cases FISH analysis on patients' metaphases demonstrates that the duplication is inverted in the ring chromosome.

Table 1 Array based comparative genomic hybridisation (CGH) results, genomic imbalance and parental origin of the 33 cases

Case	Ring	Array results	Genomic imbalance*	Parental origin
Case 1 (AM)	r4	del 4p15.1pter	del 4p: 28.5 Mb	nt
Case 2 (SS)	r5	del 5q35.3qter	del 5q: 0.6 Mb	nt
Case 3 (FT)	r13	del 13q31.3qter	del 13q: 26.5 Mb	Paternal
Case 4 (SF)	r13	del 13q32.1qter	del: 18.2 Mb	Maternal
Case 5 (GJ)	r13	dup del 13q	del 13q: 5.8 Mb	Paternal
			dup 13q: 5.9 Mb	
Case 6 (PS)	r13	del 13q33.3qter	del: 8.2 Mb	nt
Case 7 (AG)	r13	inv dup del 13q	del 13q: 10 Mb	Paternal
			dup 13q: 20 Mb	
Case 8 (MR)	r13	del 13q34qter	del 13q: 4.5 Mb	nt
Case 9 (MA)	r13	del 13q31.1qter	del 13q: 33.7 Mb	Paternal
Case 10 (AV)	r13	dup del 13q	del 13q: 10 Mb	nt
			dup 13q: 22.8 Mb	
Case 11 (BR)	r13	del 13q33.2qter	del 13q: 9.97 Mb	nt
Case 12 (DJ)	r15	del 15q26.3qter	del 15q: 2.3 Mb	Paternal
Case 13 (FD)	r15	inv dup del 15q	del 15q: 5 Mb	Paternal
			dup 15q: 2 Mb	
Case 14 (TS)	r15	del 15q26.3qter	del 15q: 4.8 Mb	Paternal
Case 15 (BT)	r15	del 15q26.3qter	del 15q: 4.6 Mb	Paternal
Case 16 (PM)	r15	del 15q26.3qter	del 15q: 4.9 Mb	Paternal
Case 17 (MM)	r15	del 15q26.3qter	del 15q: 3.8 Mb	Maternal
Case 18 (MG)	r15	del 15q26.3qter	del 15q: 2.2 Mb	Maternal
Case 19 (CC)	r15	del 15q26.3qter	del 15q: 2.1 Mb	nt
Case 20 (AM)	r15	del 15q26.3qter	del 15q: 1.6 Mb	Paternal
Case 21 (LM)	r18	dup del 18p del 18q22.1qter	del 18p: 207.5 kb dup 18p: 5.4 Mb	nt
			del 18q: 12.8 Mb	
Case 22 (SE)	r18	del 18pterp11.12	del 18p: 11.7 Mb	Paternal
		del 18q22.3qter	del 18q: 6.7 Mb	
Case 23 (FN)	r18	dup 18p	the whole 18p dup: 15.4 Mb	Paternal
		del 18q23qter	del 18q: 882 kb	
Case 24 (TE)	r18	del 18p11.21qter	del 18p: 13.7 Mb	nt
		del 18q22.3qter	del 18q: 5.5 Mb	
Case 25 (GF)	r18	del 18p11.32pter	del 18p: 1 Mb	Maternal
		del 18q22.3qter	del 18q: 5.4 Mb	
Case 26 (TM)	r18	dup del 18p; del 18q22.3qter	del 18p: 500 kb dup 18p: 14.8 Mb	nt
			del 18q: 7.8 Mb	
Case 27 (GM)	r20	No imbalances		
Case 28 (CM)	r20	No imbalances		
Case 29 (NA)	r20	No imbalances		
Case 30 (GA)	r21	dup 21q21.3q22.2	dup 21q: 11.2 Mb	nt
		trp 21q22.2q22.3	trp 21q: 5.8 Mb	
Case 31 (KJ)	r22	del 22q13.31qter	del 22q: 3.1 Mb	Maternal
Case 32 (MD)	r22	del 22q13.32qter	del 22q: 2.6 Mb	Paternal
Case 33 (AM)	r22	dup del 22q	del 22q: 946 kb dup 22q: 785 kb	Maternal

*Map positions are based on genome the Assembly May 2004; nt, not tested.

been suspected to be responsible for some phenotypic abnormalities.¹⁷

Our study demonstrates that some ring chromosomes have been formed through a more complex rearrangement leading to concurrent deletion and contiguous duplication. Although the inversion of the duplicated region has been demonstrated only in two cases (cases 7 and 13) in whom lymphoblastoid cell lines were available, we assume that the duplication was inverted also in all the other ring cases with a duplication contiguous to a terminal deletion. Thus, the rings sharing the same type of rearrangement were 7 out of 33. This suggests a different mechanism at the basis of such ring chromosomes with the formation of an intermediate inv dup del chromosome that circularises to stabilise itself. Obviously this mechanism has important implications for the phenotype which is not only due

to the simple deletion as generally assumed for ring chromosomes but also to the associated duplication.

A single case of a ring chromosome 14 containing a terminal deletion and an additional inverted duplication proximal to the deletion has been recently reported.⁸ There are a few reports in the literature, suggesting similar rearrangements. One case is a ring chromosome 9 with an inverted duplication of the distal segment of 9p.¹⁸ However, in this case the inverted duplication was only suggested by high resolution banding, and there was no apparent concomitant distal 9p deletion. Another case is an r(21) with partial trisomy for much of 21q and partial monosomy for distal 21q (patient 10 in McGinniss *et al*¹⁹) found in a patient with mild Down stigmata. The molecular and cytogenetic data of this patient were consistent with the model of r(21) formation in which asymmetric breakage and

Table 2 Array CGH results with the resulting genomic imbalance, the parental origin and the clinical features of the seven patients with dup del or inv dup del rearrangements

Case	Array results	Genomic imbalance*	Clinical features
Case 5 (GJ)	dup del 13q	del 13q: 5.8 Mb (from 108.248 Mb to telomere); dup 13q: 5.9 Mb (from 101.920 to 107.896 Mb)	Age at examination: birth Oligohydramnios, intrauterine growth retardation (36 weeks–1400 g/39 cm), cystic kidneys, hypotelorism, narrow forehead, dysplastic and low set ears, short neck, 2 umbilical vessels, bilateral cryptorchidism, arthrogryotic restriction of movements in almost all limb joints, deviation towards the middle of fingers 2 and 5; hypoplastic lungs, died 9 h after delivery.
Case 7 (AG)	inv dup 13q	del 13q: 10 Mb (from 104.243 Mb to telomere); dup 13q: 20 Mb (from 82.097 to 103.639 Mb)	Age at examination: birth, 2 years At birth muscular hypotonia and anal atresia; growth parameters always below the 3rd centile; profound psychomotor delay and mild dysmorphic features. Imaging studies revealed patent ductus arteriosus, pale optic discs, mild cerebral atrophy and rostral hypoplasia.
Case 10 (AV)	dup del 13q	del 13q: 10 Mb (from 104.008 to 114.123 Mb); dup 13q: 22.8 Mb (from 81.242 to 104.047 Mb)	Age at examination: 12 years Facial dysmorphisms included: low forehead, downslanting palpebral fissures, misshapen ears; microcephaly, deafness, spastic cerebral palsy, severe motor and mental retardation
Case 13 (FD)	inv dup 15q	del 15q: 5 Mb (from 95.258 Mb to telomere); dup 15q: 2 Mb (from 93.502 to 95.128 Mb)	Age at examination: birth, 3, 6, 9 years Referred at 3 years because of growth retardation and dysmorphic features; slightly retarded psychomotor development. Growth parameters at birth, at 3, 6 and 9 years well below the 3rd centile. Total IQ was 67. Asymmetric café au lait spots, hypochromic spots and naevi on limbs and chest, microcephaly, triangular face, abnormal hand ossification with an additional phalanx on the third finger
Case 21 (LM)	dup del 18p; del18q22.1qter	del 18p: 0.207 Mb (from telomere to 0.207 Mb); dup 18p: 5.4 Mb (from 0.215 to 5.814 Mb); del 18q: 12.8 Mb (from 63.221 Mb to telomere)	Age at examination: 35 years Asymmetric and broad face, prominent nasal bridge, attached lobuli of auricles, normal external ear canal, narrow and tapering fingers, proximally implanted and retroflexible thumbs, talipes equinovarus on both sides, growth and mental retardation
Case 26 (TM)	dup del 18p; del18q22.3qter	del 18p: 0.500 Mb (from telomere to 0.467 Mb); dup 18p: 14.8 Mb (from 0.571 to 17.137 Mb); del 18q: 7.8 Mb (from 68 Mb to telomere)	Age at examination: birth, 5 years At birth: cleft lip and palate, mild muscular hypotonia, heart defect (pulmonary stenosis, ventricular septal defect (VSD), surgery at 1 year). Delayed psychomotor development; all growth parameters at 5 years: << 3rd centile. Abnormal EEG pattern. Facial dysmorphisms
Case 33 (AM)	dup del 22q	del 22q: 946 kb (from 48.745 Mb to telomere); dup 22q: 785 kb (from 47.857 to 48.641 Mb)	Age at examination: 11 years Narrow forehead, full orbits, deep-set eyes with downslanting palpebral fissures, bulbous nose, flat philtrum, full lips, macrostomia, small and prominent ears, truncal obesity, limited excursions in elbows, tapering fingers, transverse palmar crease on the right, moderate to severe mental retardation

*Genomic distances are based on the Assembly May 2004.

Figure 3 Microsatellites analysis of the duplicated region in case 7 (A: 15DUP15) and in case 13 (B: D13S1283). In both cases paternal alleles in the patients show a double peak area.

reunion of the long arms of an intermediate isochromosome or robertsonian translocation chromosome generated a large r(21). An unusual r(21) in mosaic state, in a child who had some manifestations of Down syndrome, has been reported by Palmer.²⁰ Molecular studies demonstrated that the ring carried a duplicated region and a concomitant distal deletion and suggested the following mechanism: an initial robertsonian translocation occurred between one paternal and one maternal chromosome 21 in a trisomic cell, followed by distal breakage and deletion in both long arms of the translocation, leading to the formation of a ring chromosome. Three older reports described four mosaic cases each involving two cell lines, one with an isochromosome or robertsonian translocation chromosome and the other with a ring chromosome.^{21–23} In these cases the authors suggested a post-zygotic secondary event in the translocation chromosome to explain the ring formation.

Our findings indicate a different mechanism of formation of the deleted and duplicated ring chromosome. In fact, the duplicated region never involved the proximal portion of 13q, 15q, 18p, 22q as expected in an isochromosome or a robertsonian chromosome but rather involved more distal regions contiguous to the deletion region. The most well known mechanism for formation of inv dup del type rearrangements is non-allelic homologous recombination between homologous segmental duplications located at the rearrangement's breakpoints,^{24–26} leading to an intermediate dicentric chromosome. In our cases, the cytogenetic breakpoints do not fall in regions known to contain segmental duplications; therefore, the formation of the dicentric chromosomes cannot be explained by non-allelic homologous recombination (NAHR). The resolution of the array platforms used is too low to reveal if specific DNA motifs leading to instability such

as palindromic AT-rich repeats²⁷ or other non-B DNA structures²⁸ are present at the rearrangements' breakpoints. Moreover, results from microsatellite marker examinations of the duplicated region in one r(13) (case 7) and in one r(15) (case 13) suggest that the rearrangement is intrachromosomal, indicating that at least in these cases non-homologous end joining can be responsible for the formation of the dicentric, as demonstrated initially in maize^{29,30} and more recently in humans—for example, in some cases of inv dup del(1p).³¹

An asymmetric breakage of the dicentric would lead to two different abnormal chromosomes, one inv dup del chromosome and a simply deleted one, that could be stabilised by different mechanisms. Telomere healing and telomere capture are the most well known mechanisms to stabilise broken chromosomes and there are many descriptions in the literature of how these work.^{32–35} In our cases the stabilisation of the inv dup del chromosome is achieved through the formation of the ring, representing a new mechanism through which such inv dup del chromosome can stabilise itself. According to our hypothesis the mechanism leading to dup del rings is a multi-step process: a prezygotic event leading to the formation of the dicentric, a postzygotic breakage leading to the inv dup del chromosome, and the circularisation of the inv dup del in some cells. The finding that these rings are present in most of the cells and no cell lines showing either the inv dup del or the simply deleted chromosome have been found, can be explained assuming an early selection versus the most viable cell line(s). In this case an important selection factor may be the restriction of telomerase expression to the first embryonic stages³⁶ impairing the broken chromosomes to be stabilised by telomere healing. An example showing that post-zygotic events may produce a mosaicism present in the fetus but not in the adult is provided by the classical inv dup del(8p). We³⁷ and others³⁸ demonstrated that it can be found in mosaic with a del(8p) during the fetal life, although in the great majority of postnatal cases the inv dup del(8p) is present in all cells.

Ring formation in the other cases

In the other 26 cases, array CGH identified the following rearrangements.

In 18 cases a distal deletion in one arm was present. For the 16 cases involving acrocentric chromosomes (chromosomes 13, 15 and 22) a second deletion at the short arm is likely and cannot be excluded since these regions are not represented in the array platforms. No recurrent breakpoints had been found in rings involving the same chromosome and none of the breakpoints was characterised by the presence of segmental duplications. In cases 1 and 2, concerning an r(4) and an r(5), a single deletion at the level of the short and long arm respectively had been detected. Although a second very small deletion at the opposite arm cannot be excluded while using higher resolution array platforms, it is possible that ring chromosome formation in these cases acted as an alternative way to stabilise a broken chromosome next to telomere healing and capture. In three cases of r(18) (cases 22, 24 and 25) array CGH identified two distal deletions in both arms. A similar situation in two ring chromosomes 18 characterised by FISH and microsatellite analysis has been described (cases 4 and 5 in Stankiewicz *et al*).⁵ In these cases the ring chromosome could have been formed through a classical mechanism involving two breakages in both arms, loss of the distal fragments followed by fusion of the proximal broken ends. In case 23, with an r(18), the whole 18p arm was duplicated and a small distal deletion was present at the opposite end. The phenotype was mild, according to

duplication of the whole 18p. Concerning the rearrangement, we cannot exclude a small distal deletion in 18p with a similar mechanism as demonstrated in other inv dup del rearrangements; alternatively, a pericentric inversion of chromosome 18 preceding the ring formation or an 18p direct/inverted duplication could be possible. A further blood sample from the patient and further analysis are necessary to delineate the real situation.

In three patients with epilepsy and a ring chromosome 20 in mosaic state (30–40%) (cases 27–29), we could not identify any genomic loss even using the platform at about 20 kb resolution. Many other similar cases have been reported,^{4,14,39} and the ring in these cases might be derived from telomere–telomere fusion, as already demonstrated by quantitative FISH analysis in one mosaic r(20) patient.⁴

In case 30, with a ring chromosome 21 in a patient with Down syndrome, array CGH identified an interstitial duplication followed by a distal triplication; a distal 21q deletion could not be shown.

Genotype–phenotype correlations

The findings that 21% of the ring chromosomes we studied show not only the expected deletion(s) but also a contiguous duplication have important genotype–phenotype implications. It is essential to emphasise that, at the beginning, the identification of two out of seven inv dup del rearrangements in ring chromosomes was absolutely fortuitous. Cases 7 and 13 belong to two series of patients, with deletions of chromosome 13 and chromosome 15, respectively, that were analysed by array CGH and FISH for genotype–phenotype correlation studies. These unexpected findings prompted the investigations of further cases.

It is obvious that in a ring chromosome, phenotypic correlations cannot be done any more assuming a simple deletion before having excluded additional duplicated segments. So far, it had been assumed that a ring of the length of the normal homologue has lost little material, and thus the adverse consequences for the phenotype are minor, and vice versa. This would obviously not be true if a relatively large ring has a deletion plus a duplication which compensate each other with respect to size alteration. We cannot extrapolate from our dup del ring cases which specific traits were due to the deletion and which ones to the duplication: some of the patients were very young at clinical examination and several of them have been examined by different clinicians. However, at least in case 5, with the r(13) having a deletion and a duplication of approximately 6 Mb each, oligohydramnios and cystic kidney may be attributed to trisomy 13^{40,41} and not to monosomy for the distal 13q33.3-qter region.⁹ In case 13 with the r(15) having a deletion of 5 Mb and a duplication of 2 Mb, abnormal hand ossification with an additional phalanx on the third finger was observed. Similar findings have been reported in an old case with partial trisomy 15q⁴² but never in cases with a simple 15q deletions.^{7,41,43} Thus, if up to now the explanation for the spectrum and severity of symptoms in patients with apparently identical ring chromosomes was attributed to mitotic instability of the ring, causing the formation, in a mosaic state, of secondary rings with either larger deletion or additional duplication or both, from now on the possibility that the ring is not only deleted but also duplicated should also be taken into consideration.

In the other ring cases where the expected deletion had been confirmed by array CGH the phenotype was more or less severe according to the extension of the deleted region. For example, case 9 with a 13q deletion of 33.7 Mb died at the age of 3 years

because of severe congenital anomalies,¹⁰ whereas case 8 with a 13q deletion of 4.5 Mb has moderate mental retardation but no malformations (cases 10 and 14 in Ballarati *et al*⁹).

In case 30 segmental triplication of chromosome 21 could be demonstrated. Previous assumptions of mosaic tetrasomy of 21 turned out to be erroneous: these cases with mosaicism for a metacentric extra chromosome turned out to be examples of mosaic tetrasomy 12p—the Pallister-Killian syndrome.⁴¹ The 11.2 Mb duplication in our patient covers the 21q21.3-q22.2 region considered to be most important for Down syndrome phenotype, hence explaining her Down syndrome phenotype. We have recently observed another case with the phenotype of mild and atypical Down syndrome in whom array CGH results demonstrated a complex rearrangement of chromosome 21 with deleted and duplicated and also one triplicated segment (AS, unpublished observation). The reason why such cases were not detected before is probably that, unless there is an extra chromosome, these cases would not be investigated for potential segmental triplication with molecular methods; array-CGH, however, shows the triplication without special focused examinations.

Conclusions

We consider that re-examination using array CGH will solve any discrepancy in patients with a clinical phenotype not congruent with the size, the banding pattern of the ring and the FISH results. Array CGH can show additional aneuploid segments, either deletions or duplications or both. Therefore we suggest that all ring patients are (re-)examined using array CGH.

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