

## HUMAN RING CHROMOSOMES – NEW INSIGHTS FOR THEIR CLINICAL SIGNIFICANCE

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### ABSTRACT

Twenty-nine as yet unreported ring chromosomes were characterized in detail by cytogenetic and molecular techniques. For FISH (fluorescence *in situ* hybridization) previously published high resolution approaches such as multicolor banding (MCB), subcentromere-specific multi-color-FISH (cenM-FISH) and two to three-color-FISH applying locus-specific probes were used. Overall, ring chromosome derived from chromosomes 4 (one case), 10 (one case), 13 (five cases), 14, (three cases), 18 (two cases), 21 (eight cases), 22 (three cases), X (five cases) and Y (one case) were studied. Eight cases were detected prenatally, eight due developmental delay and dysmorphic signs, and nine in connection

with infertility and/or Turner syndrome. In general, this report together with data from the literature, supports the idea that ring chromosome patients fall into two groups: group one with (severe) clinical signs and symptoms due to the ring chromosome and group two with no obvious clinical problems apart from infertility.

**Keywords:** Ring chromosomes; Fluorescence *in situ* hybridization (FISH); Genotype-phenotype correlations.

### INTRODUCTION

It is common sense that ring chromosomes result from two terminal breaks on both chromosome arms followed by fusion of the broken ends, leading to the loss of genetic material. Alternatively, they can be formed by telomere-telomere fusion without deletion [1] or the so-called McClintock mechanism [2]. Also more complex mechanisms of ring chromosome formation have been proposed [3,4]. Ring chromosomes are also observed as small supernumerary marker chromosomes (sSMC) [5,6], however, their formation seems to be completely different from that of ring chromosomes in a numerically normal karyotype [7,8].

Phenotypes associated with ring chromosomes can be highly variable, since in addition to the primary deletion associated with ring formation, secondary loss or gain of material may occur due to

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ring chromosome instability. It has also been reported that the phenotype of ring chromosome patients can overlap that of the deletion of both ends of the respective chromosome syndromes without ring formation. Moreover, there have also been numerous reports on ring chromosomes without clinical consequences, apart from possible infertility, if no relevant genetic material was lost due to ring chromosome formation [1]. Here we report ring chromosomes observed in 29 patients with (severe) clinical problems, and/or solely infertility, evaluated by cytogenetic and molecular techniques.

**MATERIALS AND METHODS**

Twenty-nine cases with ring chromosomes were studied for different clinical reasons (see Table 1). Eight cases were detected prenatally (amniocytes

studied), eight due developmental delay and dysmorphic signs, and nine in connection with infertility and/or Turner syndrome; in four cases the reason for the study was not transmitted to the laboratory at Jena, Germany (peripheral blood studied). Chromosomes were prepared according to standard procedures. The cases were studied using standard banding cytogenetics and by means of FISH (fluorescence *in situ* hybridization). Previously published high resolution approaches such as multicolor banding (MCB) [9,10], subcentromere-specific multicolor-FISH (cenM-FISH) [6] and two to three-color-FISH applying locus-specific and/or commercially available centromere specific probes (Abbott/Vysis, Wiesbaden, Germany and/or Kreatech, Amsterdam, The Netherlands) were used. Locus-specific probes and also commercial probes, such as subtelomeric ones (Abbott/Vysis) or bacterial artificial chromosome

**Table 1.** Details on the 29 studied ring chromosome cases according to their chromosomal origin, karyotype, age at diagnosis and clinical signs. [DD: developmental delay; DS: dysmorphic signs; IUGR: intrauterine growth retardation; NA: not available; TOP: termination of pregnancy; y = year(s)]

Case #	Chromosome of Origin	Karyotype	Age at Diagnosis	Clinical Signs
R-1	4	46,XX,r(4)(p16.2q34.1)[14]/ 46,XX,r(4)(:p11->q32.1::q32.1->p11::)[4]/ 46,XX,r(4)(p11q35.1)[3]/ 45,XX,-4[3]/ 46,XX,r(4)(:p16.2->q35.1::q12->q35.1::)[2]/ 46,XX,r(4)(:p16.2->q27::q11->q32.1::)[2]/ 47,XX,r(4)(:p16.2->q35.1::q12->q35.1::),+r(4)(p11q35.1)[1]/ 47,XX,-4,+r(4)(:p16.2->q27::q11->q32.1::)x2[1]/ 47,XX,-4,+r(4)(:p11->q32.1::q32.1->p11::)x2[1]/ 47,XX,-4,r(4)(:p16.2->q34.1::)x2[1]/ 46,XX,r(4)(:p16.2->q34.1::p16.2->q34.1::)[1]/ 46,XX,r(4)(p16.2q35.1)[1]/ 45,XX,-4,-14,+der(4)t(4;14)(q32.1;q11.2)[1]	5 years	DD DS
R-2	10	47,XY,del(10)(q10q25.3),+r(10)(q10q25.3)[23]/ 46,XY,del(10)(q10q25.3)[6]/ 47,XY,del(10)(q10q25.3),+r(10)(:q10->q25.3::q10->q25.3::)[1]	5 years	DD DS
R-3	13	46,XY,r(13)(p11.2q33.3~34)[9]/ 46,XY,r(13)(:p11.2q33.3~34::p11.2q33.3~34::)[1]	prenatal	IUGR, DS TOP
R-4	13	46,XX,r(13)(p11.1q33.3)[13]/ 46,XX,r(13,13)(:p11.1->q33.3::p11.1->q33.3::)[1]/ 45,XX,-13[1]	6 years	DD, DS microcephaly dwarfism
R-5	13	46,XX,r(13)(p11q32.3)[93%]/ 46,XX,r(13)(:p11->q32.3::p11->q32.2::)[7%]	adult	DD DS
R-6	13	45,XX,-13[50]/ 46,XX,r(13)(p11.1q33~34)[50]	prenatal	IUGR TOP
R-7	13	46,XY,r(13)(p1?2q34)	NA	NA
R-8	14	46,XX,r(14)(:p12->q32.2::q32.2->q23::)[20]/ 46,XX,r(14)(p12q32.2)[4]/ 46,XX,del(14)(q21)[1]/ 46,XX[1]	newborn	DD DS

Continue

Table 1. Continued

Case #	Chromosome of Origin	Karyotype	Age at Diagnosis	Clinical Signs
R-9	14	46,XY,r(14)(p13q32.2)[82]/ 45,XY,-14[18]	1 year	DD DS
R-10	14	46,XX,r(14)(p1?3q24.3)	prenatal	DS TOP
R-11	18	46,XX,r(18)(p11.1q12.3~21.1)[7]/ der(18)(:p11.1->q12.3~21.1:)[12]	prenatal	NA
R-12	18	46,XX,r(18)(p11.21q23)[7]/ 45,XX,-18[3]	prenatal	hydrocephalus TOP
R-13	21	45,XX,-21[50%]/ 46,XX,r(21)(p12q22.3)[30%]/ 46,XX,del(21)(q22.3)[20%]	11 years	DD DS
R-14	21	46,XX,r(21)(p1?3q22.1)[64]/ 45,XX,-21[26]/ 46,XX[10]	1 year	DD DS
R-15	21	46,XY,r(21)(:p11->q22::p11q22::)[10]/ 46,XY,r(21)(:p11->q22::)[7]/ 45,XY,-21[7]/ 46,XY,der(21)(:p11->q22::p11->q22:)[2]/ 46,XY,der(21)(:p11->q22:)[1]/ 46,XY,r(21)(:p11->q22::p11q2?1:)[1]/ 46,XY,der(21)(:q22->p11::p11->q22:)[1]/ 47,XY,r(21)(:p11->q22::,+r(21,21)(:p11->q22::p11q22::)[1]	prenatal	DS TOP
R-16	21	46,XY,r(21)(p12q22.3)[23]/ 46,XY,r(21;21)(:p12->q22.3::p12->q22.3:)[4]/ 45,XY,-21[2]/ 46,XY,r(21)(p12q21)[1]	prenatal	NA
R-17	21	46,XY,r(21)(p1?2q22.3)[21]/ 46,XY,del(21)(:p1?2->q22.3:)[13]/ 46,XY,r(21)(:p1?2->q22.3::q22.3->p1?2::p1?2->q22.3::q22.3->p1?2:)[1]	32 years	infertility
R-18	21	46,XY,r(21)(p11.1q22.??)[9]/ 46,XY,r(21;21)(:p11.1->q22.??::p11.1->q22.??:)[1]	NA	NA
R-19	21	46,XN,der(21)(:q11.2->p11.1~11.2::p11.1~11.2->q22.3:)[8]/ 46,XN,del(21)(:p11.1~11.2->q22.3:)[7]/ 46,XN,r(21)(:p11.1~11.2->q22.3:)[4]/ 45,XN,-21[1]	prenatal	NA
R-20	21	46,XX,r(21)(:p11.2->q22.3:)[33]/ 46,XX,r(21)(:p11.2->q22.3::p11.2->q22.3:)[5]/ 47,XX,r(21)(:p11.2->q22.3:),+del(21)(p11.2:)[1]/ 46,XX[1]	17 years	premature ovarian insufficiency
R-21	22	46,XN,r(22)(p1?2q1?3)	26 y	infertility
R-22	22	46,XY,r(22)(p11q13.3)	NA	NA
R-23	22	46,XX,r(22)(:p12->q11.1::p11.1->q11.1::q11.1->p12:)[3]/ 46,XX,der(22)(:q11.1->p12::p12->q11.1:)[2]/ 46,XX,r(22)(:p12>q11.1:)[2]/ 46,XX,r(22)(:p11.1->q11.1::p11.1->q11.1:)[1]	NA	NA
R-24	X	45,X[56]/ 46,X,r(X)(p11.1q24)[44]	16 y	Turner syndrome
R-25	X	45,X[19]/ 46,X,r(X)(p11.2q13.?) [11]	40 y	infertility
R-26	X	45,X[46]/ 46,X,r(X)(p11.??2q13.3)[4]/ 47,X,r(X)(p11.??2q13.3)x2[1]	30 y	Turner syndrome
R-27	X	45,X[17]/ 46,X,r(X)(p11.23q28)[7]	28 y	infertility
R-28	X	45,X[70%]/ 46,X,r(X)(p22.1~22.2->q21.1)[27%]/ 46,X,r(X)(:p22.1~22.2->q21.1::q21.1->p22.1~22.2:)[2]/ 47,X,-X,+r(X)(:p22.1~22.2->q21.1::q21.1->p22.1~22.2:)[1]	26 y	infertility
R-29	Y	45,X[?%]/46,X,r(Y)(:p11.3->q11.2?3::q11.2?3->p11.3:)[?%]	adult	infertility

(BAC) probes, were applied. Labeling and application of the probes was done according to the manufacturer's instructions or as reported [11].

**RESULTS AND DISCUSSION**

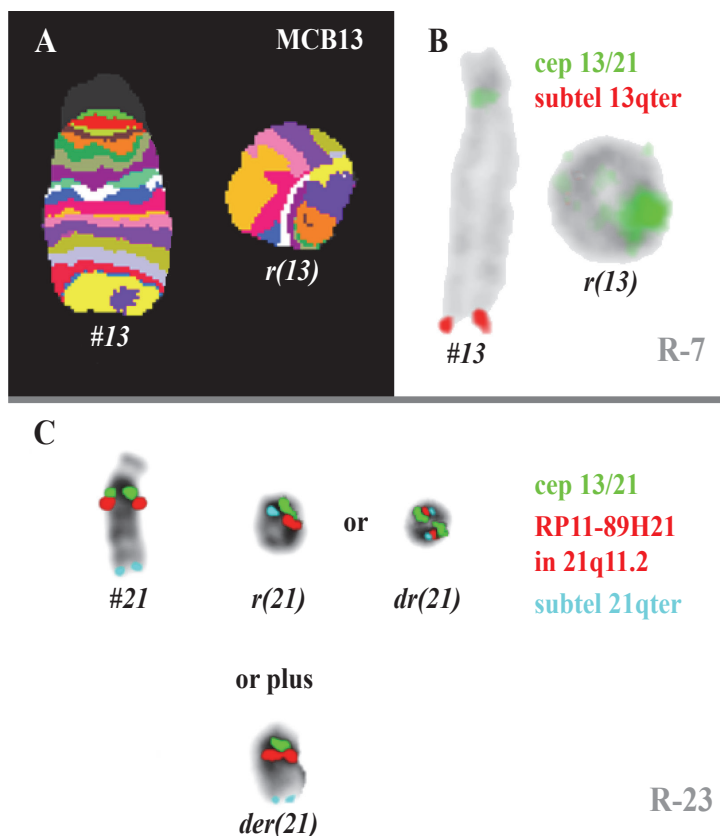
In 29 cases with ring chromosomes, the chromosomal origin and content could be determined using molecular cytogenetics. The rings were derived from chromosome 4 (one case), 10 (one case), 13 (five cases), 14, (three cases), 18 (two cases), 21 (eight cases), 22 (three cases), X (five cases) and Y (one case). The exact breakpoints and mosaic states are summarized in Table 1 and examples of the FISH results are shown in Figure 1. In the following data, the obtained results were compared with the literature by chromosomal origin; afterwards,

the chromosomal imbalances were analyzed, and finally, a conclusion was drawn.

**Analyzed Rings by Chromosomal Origin.**

Numerous cases for *ring chromosomes 4* have been reported previously [12-14]. Interestingly, those cases fall into two cytogenetic groups: one group where the ring is stable and the other group where it is unstable within the studied cells, as in case R-1. Further studies are necessary to rule out where this instability comes from, and what the clinical impact is. To the best of our knowledge, no clinically normal ring chromosome 4 case has yet been reported.

In case R-2, the first ever seen balanced ring formation involving *chromosome 10* formed by the McClintock mechanism [2] is reported. The rearrangement was connected with clinical problems, as the ring was lost in ~20.0% of the cells. Ring chromo-



**Figure 1.** Representative results for the molecular cytogenetic characterization of the studied ring chromosomes. A) In case R-7, a ring chromosome derived from chromosome 13 [r(13)] was present as well as a normal chromosome 13 (#13); breakpoints were characterized by MCB as 13p1?2 and 13q34. B) Application of a subtelomeric probe for chromosome 13qter (subtel 13qter) together with a centromeric probe for chromosomes 13 and 21 (cep 13/21) confirmed a partial deletion in 13qter. C) Application of a centromeric probe for chromosomes 13 and 21 (cep13/21) with a subtelomeric probe for chromosome 21qter (subtel 21qter) in combination with a subcentromeric probe in 21q11.2 revealed the presence of three derivatives of a chromosome 21 in case R-23; a ring [r(21)], a double ring [dr(21)] and a shortened derivative of chromosome 21 [der(21)] were observed. For final karyotype results, see Table 1.

somes derived from chromosome 10 are rare (only about 10 cases) and were recently reviewed [15].

Martin *et al.* [16] suggested the existence of a **ring chromosome 13** syndrome and gave an incidence of 1/58,000 in live births. Here, five cases with ring chromosomes 13 were studied (R-3 to R7), all of them were clinically abnormal.

Similarly to chromosome 13 derived rings, **chromosome 14** is also suggested as a specific syndrome [17]. In concordance with the literature, all three ring chromosome 14 cases studied here (R-8 to R10) had an abnormal phenotype.

**Ring chromosomes 18** were present in the prenatally studied cases R-11 and R-12. Here too, a recognizable syndrome was suggested [18]. Similar to chromosome 4, for rings derived from chromosome 18, cytogenetically stable (*e.g.*, present two cases) and unstable rings [19] are reported.

Eight cases with **ring chromosomes 21** were characterized in the present study (cases R-13 to R-20). While cases R-13 to R-15 were unbalanced and led to clinical signs, two of the cases just detected were due to infertility (R-17 and R-20). As reported in [20], most, if not all ring chromosome 21 cases are mosaic, as the ones here described. A ring chromosome 21 syndrome was also postulated [21].

The three **ring chromosome 22** cases were either cyto-genetically stable (R-21 and R-22) or unstable (R-23). The reason for the cytogenetic study was available only for case R-21; it was infertility, and in the literature there are several similar cases reported [22].

Turner syndrome is cytogenetically characterized by karyotype 45,X; in ~5.0% of the cases, this main cell line is accompanied by a second one having 46 chromosomes due to an additional derivative X- or Y-chromosome [23]. Here, six such cases were characterized in more detail, as they had a ring derived from the **X-chromosome** (cases R-24 to R-28) or the **Y-chromosome** (R-29). Interestingly, all cases were detected during adulthood and only two of them due to a suspicion of Turner syndrome (R-24 and R-26). The majority of the cases were referred due to infertility.

**Ring Chromosome-Induced Imbalances.** In all 29 studied ring chromosome cases (Table 1), eukaryotic imbalances were present except for cases R-7, R-21 and R-22. In the latter, clinical data was available only for case R-21, and infertility was the

only clinical problem observed there. Primarily, case R-2 did not have any imbalance due to a ring chromosome, but double ring formation and loss of the ring chromosome led to a partial tri- or monosomy in 23.0% of the patient's cells overall.

Imbalances were exclusively induced by the ring chromosome formation in case R-10. Moreover, in all the remaining 24 cases, imbalances were also caused by secondary effects of the ring chromosome formation: **i)** double ring formation: R-1, R-3 to R-5, R-8, R-15, R-16, R-18 to R-20, R-23, R-28 and R-29; **ii)** ring doubling: R-1, R-15, R-26 and R-28; **iii)** complex changes of the ring itself: R-1, R-15 to R-17 and R-23; **iv)** ring opening (including further rearrangements): R-1, R-8, R-11, R-13, R-15, R-17, R-19, R-20 and R-23; **v)** loss of the ring: R-1, R-4, R-6, R-8, R-9, R-12 to R-16, R-19 and R-24 to R-29.

Similar observations were also made for other ring chromosomes 22. The idea that there might even be a "ring syndrome" irrespective of the chromosomal origin of the ring [24] might be due to the gross imbalances induced by these secondary changes [25].

## CONCLUSIONS

In general, this report supports the idea that ring chromosome patients fall into two groups: a larger one with (severe) clinical signs and symptoms due to the ring chromosome and a smaller one with no obvious clinical problems apart from infertility. The latter can be due to gamete instability at meiosis due to the ring chromosome which leads to an increased breakdown [22]. This cytogenetic study of 29 rings also shows that chromosomal imbalances are secondary inducings in ~85.0% of the cases by loss of the ring (68.0%), double ring formation (52.0%), ring opening (36.0%), ring doubling (16.0%) and complex changes of the ring itself (16.0%).

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