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Azoospermia and paternal autosomal ring chromosomes: case report and literature review

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Abstract Two men of the same family presented with ring chromosome 22 and azoospermia. The literature on all autosomal ring chromosomes and semen abnormalities was reviewed. Autosomal ring chromosomes were often associated with a low sperm count. This is probably as a result of gamete instability at meiosis due to the ring chromosome which leads to an increased breakdown. In addition, ring chromosomes transmitted from the parents may manifest quite differently in the progeny. Prior to treating these patients with assisted reproduction, appropriate counselling should be offered, in view of the varying phenotypic manifestations of ring chromosomes in the resulting progeny, and prenatal diagnosis or preimplantation diagnosis must be considered.

KEYWORDS: azoospermia, cryptozoospermia, genetic counselling, male infertility, oligozoospermia, ring chromosomes

Case report

A 28-year-old man was referred for evaluation of subfertility. Azoospermia was detected. Clinical examination and gonadotrophin concentrations were normal. Karyotyping revealed a ring chromosome 22—46,XY,r(22) (p12q13.3).ishr22 (bcr+, qter+) — in all the cells. G banding showed that the ring had all the bands present in a normal chromosome 22. There were no Y chromosome microdeletions.

Coincidentally the man's brother (aged 30 years), was also referred for azoospermia and had the same abnormality at karyotyping (no mosaicism) with no Y deletion. He had undergone testicular sperm extraction 2 years previously (from both testes) and the histology showed an incomplete maturation arrest. Rare tubules contained spermatids and even rarer mature spermatozoa. The brother's serum gonadotrophin and inhibin B concentrations were normal.

A family history was taken. They were a family of four brothers and two sisters. Both the sisters were fertile. Both the proband and his brother had no identifiable dysmorphic features and were working as a security guard and a salesman, respectively. The other two brothers had not yet tried

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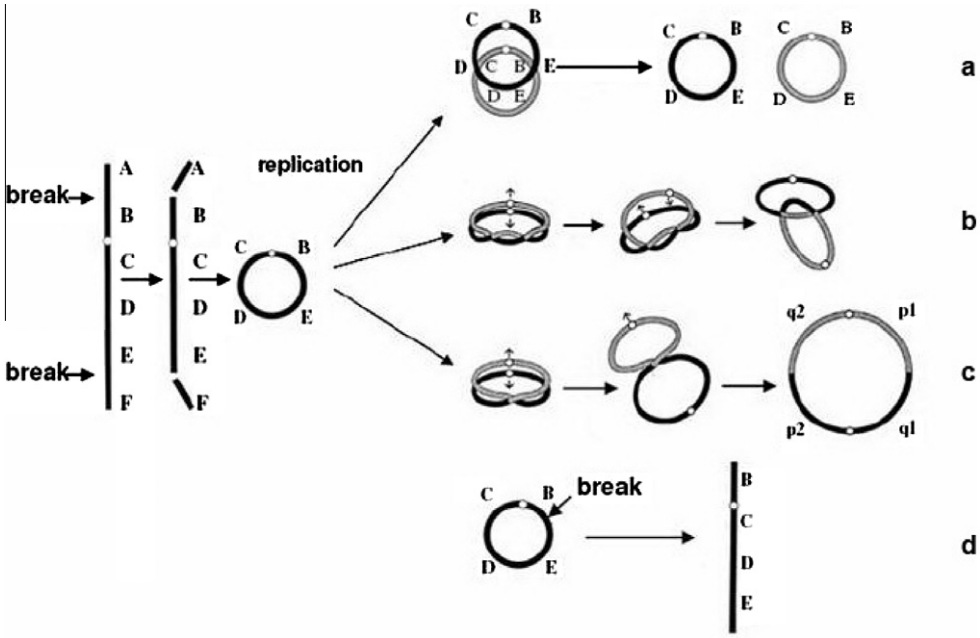

Figure 1 Scheme of ring formation and instability after replication, arising from chromatid exchanges or breaks, and originating: (a) two monocentric rings; (b) interlocked rings; (c) a double-sized dicentric ring; and (d) a broken or open ring. Reproduced with permission from Sodre et al. (2010).

to start a family. There was no family history of subfertility or recurrent miscarriages. The other family members looked apparently normal. The suggestion of karyotyping for the rest of the family was not possible due to cultural issues. The proband was referred for genetic counselling.

Approximately 2% of infertile males have somatic chromosomal abnormalities and this incidence increases as the count decreases (Chandley, 1998). Structural karyotypic abnormalities associated with azoospermia include Klinefelter syndrome 47,XXY, Y microdeletions and chromosomal translocations. In the context of these two brothers presenting with ring chromosomes and azoospermia, the literature on ring chromosomes and azoospermia was reviewed.

Review

Ring chromosomes have been identified for all human chromosomes. They commonly result from terminal breaks in both chromosome arms, followed by fusion of the broken ends. This may either result in no loss of genetic material and the formation of a complete ring chromosome, or the loss of genetic material which may result in subtelomeric microdeletion syndrome. A ring chromosome may also result from telomere-to-telomere fusion of an inherently unstable chromosome prone to circularization (Pezzolo et al., 1993). Transmission of these unstable chromosomes may lead to de-novo ring formation in the second generation, in addition to the presence of a normal cell line, accounting for a mosaic karyotype in both the parent and the offspring (Kosztolanyi et al., 1991).

During mitosis, the ring chromosome may duplicate and assort regularly to the daughter cells resulting in transmission of the ring. Alternatively sister chromatid exchange between rings may result in a double-sized ring or two interlocking rings (**Figure 1**). Such interlocking or double-sized rings may: (i) be lost from both cells resulting in a monosomic cell with possible serious effects on cell survival if the ring was autosomal, but with much less effect if of sex chromosomal origin; (ii) result in non-disjunction with production of two cell lines, one without the ring (monosomic) and the other with a double ring (trisomic); or (iii) result in symmetrical or asymmetrical breakage of the resulting ring leading to deletions and duplications in the resulting cells. The broken ends of the chromosomes may rejoin as rings of variable size or the sister chromatids of the two ends may join across, with the cycle restarting at the next cell division (Paul, 1964). Hence, an individual with a ring chromosome may have a varying chromosomal constitution in his somatic cells. This is termed dynamic tissue mosaicism.

Sodre et al. (2010) had evaluated the stability of different ring chromosomes during mitosis in lymphocyte cultures. A ring chromosome is considered 'stable' when secondary aberrations are found in 0—5% of the mitoses and 'unstable' when the aberrations are more than 5% in number. They found that cells showing ring chromosome instability carried between them different ring chromosomes (ring 4, 14, 15 and 18). In addition, two of their patients with the same ring karyotype (ring 14) showed a different frequency of metaphase cells with the ring at culture, thereby suggesting that mosaicism varies in different individuals with the same ring chromosome. They found no correlation between ring size and stability, and no difference between complete rings and rings with genetic material deletion. As karyotyping normally involves haematopoietic tissue, tissue mosaics may not be quantified. Phenotypes will vary depending on the extent of euchromatin deletion, presence of secondary aneuploid cells due to ring instability, ring stability and the degree of tissue mosaicism (Kosztolányi, 1987).

Table 1 Azoospermic men with ring chromosomes.

Study	Chromosome	FSH, LH, T (IU/l, IU/l, ng/ml)	Semen analysis	Patient characteristics
McIlree et al. (1966)	Ring autosome 21—22	Not mentioned	Azoospermia	Normal
Moreau and Teyssier (1982)	93% 46,XY,r(15); 2% 45,XY; 5% 46,XY	14, 5, 2.7	Azoospermia/severe oligospermia	Normal, brother childless; no karyotyping obtained from family
Huret et al. (1985)	94% 46,XY,r(21); 6% 45,XY	5.3, 7, 9.7	Azoospermia	Normal phenotype, brother azoospermic; no karyotyping obtained from family
Dallapiccola et al. (1986)	46,XY,r(21)	Normal, low, low	Azoospermia	Normal
Martin et al. (2008)	85% 46,XY,r(12); 15% 46,XY	29.5, not known, normal	Azoospermia/severe oligospermia, used donor spermatozoa after counselling	Small head, some developmental delay
Hammound et al. (2009)	88% 46,XY,r(21); 5% 45,XY,—21; 7% 46,XY	Not available	Cryptozoospermia	
Zuccarello et al. (2010)	Blood: 93% 46,XY,r(22); 7% 45,XY Buccal: mosaic 97% 46,XY,r(22); 3% 45,XY,—22	3, 3.3, 3.51	Azoospermia, complete spermatogonial arrest	Normal
Patients (n = 2)	46,XY,r(22) (p12q13.3).ishr22 (bcr+, qter+); brothers	7.2, not done, 15.8; index case 7.2, 6.1, 22.4; brother	Azoospermia, incomplete maturation arrest at the stage of secondary spermatocytes	Normal, brothers

Ring characteristics during spermatogenesis are further complicated by a reduction of the chromosomal complement at meiosis. Men with ring chromosomes have been shown to have severe sperm count abnormalities resulting in oligospermia and azoospermia (**Table 1**; McIlree et al., 1966, Dallapiccola et al., 1986; Hammound et al., 2009; Huret et al., 1985; Martin et al., 2008; Moreau and Teyssier, 1982; Zuccarello et al., 2010). While low sperm counts have been reported for individual ring chromosomes, it is believed that this spectrum can be extrapolated to all ring chromosomes, because of the process of pairing and separation at meiosis. Females with ring chromosomes are often unaffected at gametogenesis (Dallapiccola et al., 1986).

McIlree et al. (1966) suggested that the azoospermia in relation to a ring chromosome was due to pairing failure during meiosis which resulted in chromosomal degeneration and breakdown of spermatogenesis. However Jobanputra et al. (2009) have shown that pairing and exchange may occur between a ring and a normal chromosome during meiosis in a female. Theoretically this can be extrapolated to male meiosis and could result in spermatogenesis. Crossing over of the ring may, however, lead to gamete instability resulting in low or absent sperm counts. Salvage of the ring may be possible with double crossover and spermatogenesis may rarely occur (Palmer et al., 1977). Alternatively if the ring is due to an unstable chromosome, it is postulated that it could open out during meiosis resulting in some spermatogenesis, accounting for paternal transmission. Speevak et al. (2003) suggested that such an opening of a ring chromosome fused at telomeric sequences could result in the DNA repair pathway capping the chromosome, rather than closing the ring. This linearized ring is compatible with meiotic pairing and gametogenesis. Such a linearization, stabilization and normalization of the ring chromosome may impact favourably on the chromosomal constitution in individual tissues.

Although spermatogenesis is possible, the majority of patients with autosomal rings reported in literature present with azoospermia, reflecting the extensive gamete loss at meiosis (**Table 1**). Any autosomal ring chromosome karyotype could lead to diminished or absent sperm counts. Histological assessment at testicular biopsy in such men would show complete to incomplete maturation arrest, depending on the individual's load of ring chromosome, its stability, the degree of mosaicism in the testes and the unpredictable consequences at meiosis. Men with unstable rings and mosaic rings may have varying degrees of spermatogenesis.

Table 2 Paternal ring transmissions and pregnancies.

Study	*Chromosome*	*Fertility*	*Familial characteristics*
Burden et al. (1973)	46,XY,r(17) in both father and son	Fertile, with four children: the last child had the ring chromosome	Father: normal. Fourth child: microcephaly, developmentally delayed with bilateral ectopic testes
Stoll and Roth (1983)	Daughter: 50% 46,XX; 50% 46,XY,r(22). Father: 60% 46,XY; 40% 46,XY,r(22)	Seven children (five girls, two boys): all but one having a normal karyotype	Father: normal. Other children: normal. Daughter: dysmorphic features, mild mental retardation and microcephaly
Crusi and Engel (1986)	Case 1: Father: 46,XY,r(22) with the ring more marked in some cells than others. Child: 46,XY,r(22) in 11/14 cells	Four pregnancies: two miscarriages, one child with a normal karyotype and one child with ring chromosome	Father and child normal
	Case 2: Child: 46,XY,r(21) ring in 21/31 mitoses studied. Father: similar genotype in 16/19 mitoses. Grandfather: similar ring	No mention at the time to conception. However the couple had two miscarriages >10 weeks, followed by two healthy children being born before this pregnancy. There was a family history of Down's syndrome in a cousin, who was born to a 42-year-old mother	Father, child and grandfather normal

All of the chromosomes were likely to be unstable. None of these patients had reports of hormonal evaluations.

The literature review identified nine men with predominantly azoospermia. Of these, four men had non-mosaic rings 21 or 22 and the rest had mosaic rings 12, 15, 21 and 22. The majority of the men were phenotypically normal. There was no correlation between serum gonadotrophins and sperm characteristics.

Only four cases of paternal transmission of the ring chromosome have been reported so far, as shown in **Table 2** (Burden et al., 1973; Crusi and Engel, 1986; Stoll and Roth, 1983). Stoll and Roth's patient's pregnancy could be explained by testicular mosaic tissue. Alternatively all these pregnancies could be explained by an unstable paternal ring chromosome which tended to linearize at meiosis resulting in adequate gametogenesis as shown by normal karyotypic offspring in all of them. A more permanent ring is more likely to be associated with interference with normal gametogenesis and infertility or an increased likelihood of sperm abnormalities. Further studies are essential to determine factors affecting stability and the exact nature of the ring chromosome in the individual.

The children born to these men had karyotypes varying from normal to different degrees of mosaicism for the ring chromosome, suggesting the presence of an unstable chromosome rather than a persistent ring. Paucity of cases suggesting paternal transmission of the ring does suggest that normal sperm counts are probably uncommon though it could reflect a publication bias. In addition, if patients with the ring chromosome have a normal count and fertility, they are unlikely to be karyotyped in the absence of a child with a suggestive phenotype.

The karyotype of spermatozoa from these individuals is uncertain. Hammound et al. (2009) have analysed the meiotic segregation of spermatozoa in a cryptozoospermic man with mosaic ring chromosome 21, and concluded that he showed a preferential meiosis of normal spermatogonia (from a count of 169 spermatozoa suitable for intracytoplasmic sperm injection (ICSI)). However there was an incidence of 7.7% of spermatozoa with an abnormal karyotype (6.5% carrying a ring chromosome and 1.25% carrying a ring chromosome 21 and its normal homologue-trisomy 21) whose potential at fertilization is unknown and whose segregation at ICSI currently impossible to predict. They postulated that there was preferential meiosis of normal spermatogonia and did not recommend preimplantation diagnosis to their patient, as the risk of trisomy 21 due to the ring chromosome was low. However, Kosztolanyi et al. (1991) have stated that the phenotypic manifestations of the inherited ring chromosome may be more severe in one-third of the offspring than the parent, regardless of whether the child was mosaic or non-mosaic for the ring chromosome. Extrapolating to Hammoud's study, this would imply that the 7.7% of spermatozoa fertilizing an embryo might result in a child with manifestations of trisomy or the ring phenotype. Preimplantation genetic diagnosis (PGD) should be offered to these couples at IVF in the absence of current techniques to determine chromosome complement at ICSI. An alternative would be prenatal genetic diagnosis post conception.

In an investigational PGD for a female carrier of a ring/deletion 22, no completely normal embryos were found after two PGD cycles and two natural pregnancies. All possible segregational patterns were seen with no preferential mode and post-zygotic errors were widespread in all preimplantation embryos, resulting in a high degree of mosaicism. The only balanced carrier embryo accumulated post-zygotic errors by day 5 (Mantzouratau et al., 2009). Any of these embryos could have produced a viable unbalanced pregnancy with partial trisomy or monosomy. Hence PGD is suggested for all paternal ring chromosomes rather than for

only viable ring chromosomes due to the unstable nature of ring chromosomes and the varied phenotypic variations of the same ring in different individuals. Counselling these couples would be challenging given the range of mosaicism and the possible phenotypes, in addition to the possibility of no suitable embryos for transfer. However, women with autosomal ring chromosomes are often fertile though at an increased risk for aneuploidy and spontaneous abortions (Dallapiccola et al., 1986).

In conclusion, this study wishes to highlight the high incidence of sperm count abnormalities in men with autosomal ring chromosomes. In this era of ICSI with microsurgical sperm retrieval, these men have to be adequately counselled about the prognosis in their offspring and PGD should be an option.

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