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Case report

Ring Y chromosome, review and case report

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Chromosomal aberrations seem to emerge as a cause of idiopathic oligo-atheno-teratozoospermia. Ring Y chromosome – as one of these aberrations- though rare, should be considered or excluded in cases of ambiguous genitalia, severe oligozoospermia, morphologic abnormalities and especially before Intra-Cytoplasmic Sperm Injection (ICSI) in suspicious cases. We report a 34 year old male patient, completely androgenized but short, complaining of failure of conception for 7 years. Medical treatment was tried and varicocelectomy were done but in vain. ICSI was tried 7 times; one of them resulted in pregnancy which was aborted 40 days later for an unexplainable cause. Karyotyping revealed 46Xr(Y)/45X genotype and Polymerase Chain Reaction (PCR) for Azoospermic Factors (AZFs) microdeletion revealed preserved genes. We concluded that other genes that are responsible for spermatogenesis which could cause infertility could be deleted during ring formation; and the lethal ring Y chromosome may be the cause of failure of ICSI. So, sex selection of the X bearing sperms during ICSI may give hope of fecundity.

Key words: Ring Y chromosome, ICSI, oligozoospermia.

INTRODUCTION

Chromosomes are often regarded merely as static containers for genetic information. However, it is now becoming increasingly clear that chromosomes are highly dynamic structures with a tightly regulated organization.

A ring chromosome is a chromosome whose arms have fused together to form a ring. Ring chromosomes may form in cells following genetic damage by mutagens like radiation (acquired), they may also arise spontaneously during development constitutiona (Schmidt et al., 1981).

The first mechanism supposed in the formation of a ring chromosome occurs by two DNA breaks, one in each arm of the same chromosome, followed by fusion of the proximal broken ends **(Figure 1).** (Gisselsson et al., 1999). The causes of these DNA breaks are usually unknown and so is the mechanism behind ligation of the ends. It is possible that the non-homologous end-joining machinery plays a role in this process. A ring can also be formed by fusion of two breakpoints in the same chromosome arm. However, only few examples of such rings have been described. Most probably, this is because they are acentric and lack attachment point for the cell division machinery. Unless there is a different anchorage sequence for the kinetochore complex they will be lost in subsequent mitoses. Such "neocentromere" sequences have, however, been described in rare cases of constitutional (Slater et al., 1999) and acquired ring chromosomes (Gisselsson et al., 1999).

The second mechanism may occur by fusion of dysfunctional telomeres from the same chromosome **(Figure 2)**. Several in vitro and animal models have shown that shortening of telomeric DNA repeats leads to the detachment of protective proteins from the chromosome ends. This renders the chromosome ends prone to recombination with DNA either from other chromosomes leading to formation of a dicentric or with the other arm of the same chromosome leading to formation of a ring (Counter et al., 1992).

Most ring chromosomes arise de novo and ≤1% of all ring chromosomes are inherited (Kosztolányi et al., 1991). Ring chromosomes most frequently transmitted are chromosomes 20, 21 and 22 (Palmer et al., 1977; Stoll and Roth, 1983; Hertz, 1987; Back et al., 1989; Kennerknecht et al., 1990).

Constitutional ring chromosomes occur in 1/50,000 human fetuses (Jacobs et al., 1975). In most instances, these rings are formed by breakpoints in both arms, followed by fusion of the proximal ends into a ring with loss of the distal material. Such rings may thus result in clinical features mimicking terminal deletion syndromes. Alternatively, congenital ring chromosomes are supernumerary, that is, they occur together with two

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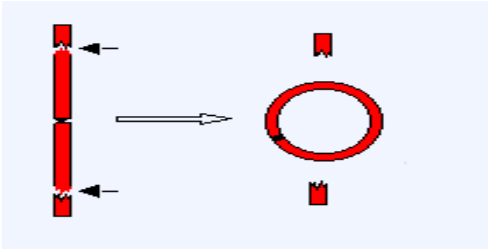

Figure 1. Double DNA breaks (Gisselsson et al., 1999).

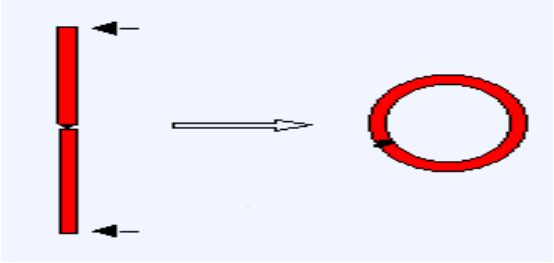

Figure 2. Telomere dysfunction. (Counter et al., 1992)

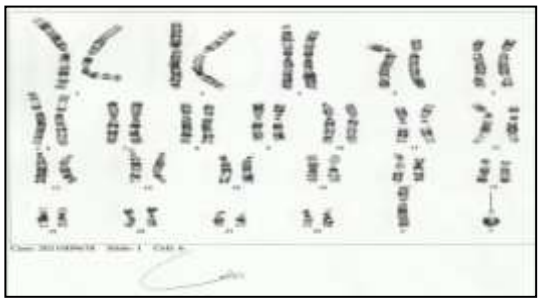

Figure 3. Karyotyping showing ring Y chromosome in most of the cell line.

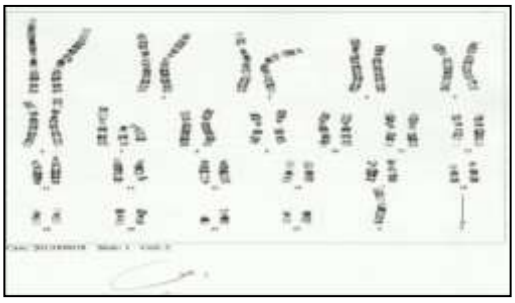

Figure 4. Karyotyping (the same sample) showing lost Y chromosome in some of the cell line.

normal homologues of the corresponding chromosome, and the consequences will be similar to partial trisomies or duplications. The ring syndromes are thus a very heterogeneous group, with different characteristics depending, not only, on which chromosome is involved, but also on the position of breakpoints within the chromosome (Anderlid et al., 2001). Compared to constitutional rings, the acquired ring chromosomes linear marker chromosomes by capturing telomeric sequences from other chromosomes (Gisselsson et al., 1999).

The mechanism behind the great ring chromosome variability in some neoplastic cells is not completely understood. Several studies have shown that the normal DNA damage response is disrupted in many malignant

A thirty-four year old male patient who complained of failure of conception for 7 years since his marriage was presented to our andrology unit. The patient has a well stable, not interrupted, sexual life with his 29 year old wife who is gynecologically free. The patient has one brother and a sister who are fertile with no other family history of fertility problems. He stopped smoking for 5 years after 7 years of heavy smoking. No urine manifestation or history of other chronic diseases was found. The patient height is 146 cm, span 146 cm, upper segment/ lower segment is 70/76. Secondary masculine sex characters are well presented with no gynecomastia. Testicular volume is average, Tanner V penis and scrotum. Repeated semen analyses revealed severe oligo- athenozoospermia. The results of the patient's

occurring in some diseases, which are often tumors, are highly unstable. The rings are rarely lost. Instead, they are frequently present in more than one copy and there is wide variability in ring size and structure within each case (Gisselsson et al., 1998) and chromosome bridges occur frequently at mitosis. There is also evidence that the rings may break up into large

tumors. There is evidence that one common mechanism behind the deficient response to DNA damage is inactivation of the TP53 protein by point mutations (Stark, 1993; Gisselsson et al., 2000).

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hormonal profile are shown in (Table 1). Digital rectal examination and Expressed prostatic secretion revealed normal prostate. Scrotal duplex US revealed normal size *(*Rt 4.5*2.3*3 & Lt 4.2* 2.5*2.5) and echogenecity of both testes with right moderate hydrocele. Testicular biopsy revealed severe hypospermatogenesis. The patient underwent 7 times of TESE and ICSI, one of which had resulted in pregnancy which was aborted after 40 days of gestation but unfortunately, the patient does not have any documents about these trials and the cause of abortion. Many courses of tamoxifen and Clomifene citrate were taken and varicocelectomy was done. As usual, in our protocol in the management of idiopathic cases of severe oligo-thenozoospermia, Karyotyping and cytogenetic

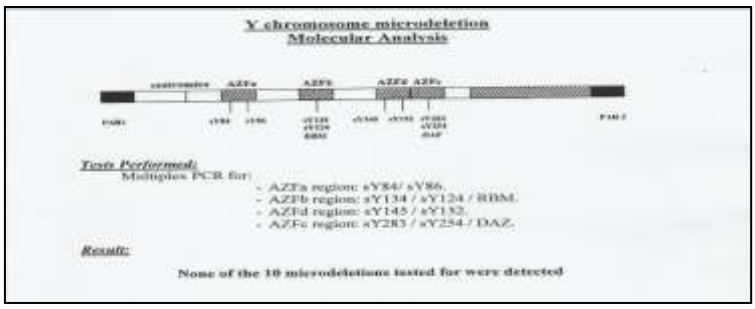

Figure 5. AZF microdeletion study by PCR showing preservation of these genes

Table 1. Hormonal profile of the patient.

Hormone	Patient result	Reference range
FSH	7.5 mIU/ml	1-18 mIU/ml
LH	8.1 mIU/ml	2-18 mIU/ml
Total Testosterone	470 ng/dl	270-1100 ng/dl
Prolactin	15 ng/ml	< 20 ng/ml
Estradiol	34 pg/ml	10 - 60 pg/ml

studies were done. Karyotyping revealed 46X r(Y) in 65% of the cell line and 45X in 35% of the cell line denoting mosaic form of the r(Y) chromosome syndrome (Figures 3 & 4). AZFs microdeletion study by PCR revealed no deletion of any of the AZFs (Figure 5).

METHODS

Karyotyping: (ISCN, 1985) principle

Karyotypes evolve with continued lymphocyte culture. This technical procedure has been reported to produce banding patterns on metaphase chromosomes. A band is defined as that part of a chromosome which is clearly distinguishable from its adjacent segments by appearing darker or lighter. The chromosomes are visualized as consisting of a continuous series of light and dark bands. A G-staining method resulting in G-bands uses a Giemsa dye mixture or Leishman dye mixture as the staining agent. What follows is a brief description of the steps involved in assembling a karyotype.

Time required

15-30 minutes to cut, arrange, glue and interpret one metaphase spread on a karyotype sheet.

Procedure

1. Counting the number of chromosomes. Solid stained chromosomes or chromosomes treated with a trypsin and giemsa stain can be counted with a microscope having a 100X magnification. It is necessary to photograph and print the chromosome spreads. Two prints should be made of each spread. One will be cut for karyotyping; the uncut print serves as a reference.
2. Each individual chromosome is cut out and on arranged a karyotype sheet. Chromosomes are ordered by their length, the position of the centromere, the position of the chromosome bands, and the relative band sizes and 3. In the distributions.
construction of the karyotype, the autosomes are numbered 1 to 22, in descending order of length. The sex chromosomes are referred to as X and Y.
4. Chromosomes are secured in place with glue. Then paired closely together and the centromeres are aligned. A description of the karyotype is recorded on the karyotype sheet. First the number of chromosomes is recorded, including the sex chromosomes, followed by a comma (,). The sex chromosome constitution is given next. Any structural rearrangements and additional or missing chromosomes are listed next.

Analysis of the AZF genes (Yq11) by PCR-sequence-tagged sites

Analysis of microdeletions in the AZF (Yq11) region was performed on our patient. Genomic DNA was extracted from the peripheral blood of the patient using standard techniques. The DNA was amplified by multiplex PCR

using 28 Y chromosome-specific sequence-tagged sites (STS) according to the method described by Henegariu et al. in (1994). Reaction products were separated on 3% agarose gel (Metaphor, FMC Bioproducts, Rocklands, ME, USA) and stained with ethidium bromide. The deletion of one or more PCR fragments was confirmed by analyzing the corresponding patient genomic DNA in single-primer pair PCRs, using the same experimental conditions and primer pairs from the missing amplification products of the multiplex PCR. This analysis was performed at least three times for each microdeleted sample.

DISCUSSION

Ring chromosomes involving both the autosomes and the sex chromosomes have been described (Pezzolo et al., 1993). Disorders arising from the formation of a ring chromosome include ring chromosome 20 syndrome where a ring formed by one copy of chromosome 20 is associated with epilepsy. Ring chromosome 14 and 13 syndrome are associated with mental retardation and dysmorphic facial features; ring chromosome 15 is associated with mental retardation, dwarfism and microcephaly. Ring formation of an X-chromosome causes Turner syndrome (Schmidt et al., 1981). Ring Y chromosomes are known to be unstable during mitosis, and this is the reason why usually a mosaicism with a 45X cell line is detected in most reported patients (Henegariu et al., 1997).

Patients with ring Y chromosome can present a wide spectrum of sex phenotypes including patients with Ullrich-Turner syndrome, patients with ambiguous genitalia, and patients with a complete masculinization. The phenotypic sex of the patients with r(Y) may be related to the presence/deletion of certain genes/loci that are essential for sexual development in humans and to the proportion of 45X mosaicism (Robinson et al., 1999). It has been shown that long-arm deletions of Y chromosome encompassing the Azoospermic Factor (AZF) regions are related to sperm count anomalies and that about 8.2% of males with oligospermia or azoospermia have deletions in AZF loci (AZFc 60%, AZFb 16%, AZFa 5%)(Vogt, 1998). The main presentation in our patient is severe oligoathenozoospermia. However, there was no deletions in the azoospermic factors as shown in fig 3. This may be due to deletion of other different genes related to spermatogenesis and harbored in other regions, such as *USPY9* and *DBY*, *RBMY1*, and *DAZ* (Foresta et al., 2000). However, other Yq genes have been isolated and their contribution to the AZF phenotype is still unknown (Tilford et al., 2001).

Therefore, infertility which is the main effect of r(Y) chromosome may be due to the loss of Y genes directly implicated in spermatogenesis, the presence of a 45X cell line or the lack of sex chromosome pairing during meiosis.

The SRY gene (Yp11.3), which is involved in gender determination, is located close to the telomeric region. Its accessibility and regulation could be disturbed by the ring conformation **(**Mouaffak et al., 2007)**.** This gene may not be affected in our patient as he shows complete masculine features denoting normal androgenization. The SYBL1 and NLGN4Y genes both map to the Yq pseudoautosomal region and encode proteins that are essential for functional synapses. Variants of those genes have been found to be associated with bipolar (Muller et al., 2002) and autism spectrum disorders (Jamain et al., 2003), respectively. Deletions in the later regions were hypothesized to be responsible for gender identity disorders in a case reported by Mouaffak and his colleagues in 2007.

Our patient is too short in relation to the average height of both his parents and also his bother and sister. It is hypothesized that this phenotypic character may be explained by the deletion in the Growth Controlling Region (GCR) on the Y chromosome which has occurred during ring formation. This region is located close to the DYS11 marker but no specific gene has been identified yet (Carvalho et al., 2007).

Up to our knowledge, only 16 cases of ring Y chromosome were reported in literature as follows: Wilson et al., (1976), Taillemite et al., (1978), 2 cases by Tzancheva et al., 1999, Blanco et al. (2003), 2 cases by Lin et al.(2004), 2 cases by Bertini et al.(2005), Carvalho et al.(2007), Mouaffak et al.(2007), Lopez-Valdes et al. 2009 and Chen et al.(2011). The other 3 cases were reported to occur during ICSI (Bofinger et al., 1999), (Spinner et al., 2004) and (Arnedo et al., 2005). Our patient has tried TESE and ICSI 7 times and only one of them had resulted in pregnancy which was spontaneously terminated at 40 days of gestation with no apparent cause of abortion. The theory of lethal ring Y chromosome stated by Stone in (1982) who studied the ring Y chromosome and its lethality on the new offspring of Drosophila Melanogaster may explain failure of ICSI in such patients and also may explain the rarity of such cases without mosaicism. Cytological observations of inviable embryos have revealed that the ring-Y chromosome causes gross disorganization of the cleavage nuclei **(**Stone, 1982).

We think that, during ICSI, it is worthy to try sex selection using sperms bearing X chromosomes, the point that was not published or discussed before in the literature. If so, we will avoid transmission of this aberration to the coming offspring which will be always a female. However, this family may need strict counseling as there may be a great risk of transmission of not only sex transmitted diseases but autosomally transmitted ones as well.

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CONCLUSION

Ring Y chromosome is a rare chromosomal abnormality

which should be excluded not only in cases of unexplained male infertility but in cases of ambiguous genitalia, abnormal growth and gender identity disorders. Therefore, karyotyping and cytogenetic studies for gene deletions may be mandatory especially before ICSI to avoid transmission of these aberrations to the offspring and to save money for hopeless cases such as those with AZFa deletions. Also, sex selection may be tried in infertile males seeking for conception by ICSI using X bearing sperms.

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