

## Research Article



## Interchromosomal Insertion 46,XY, ins(1;2) (p31; p13p23) in an Algerian Patient with Spermatogenic Failure.

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### ABSTRACT

In this study we describe clinical and cytogenetic findings in a male referred to our laboratory for cytogenetic investigation. Karyotyping was performed on peripheral blood lymphocytes according to standard methods. Genomic DNA was isolated from blood and PCR was carried out with a set of 6 AZFa, AZFb and AZFc STS markers to detect the Y microdeletions. The patient's age was 36 at the time of referral. Severe oligoasthenoteratozoospermia was confirmed on semen analysis. The karyotype of peripheral blood showed 46, XY, ins(1;2) (p31; p13p23). To the best of our knowledge this is only the four report in literature of infertile patient with chromosomal insertion but, there is no previous report of this specific chromosome rearrangement. Such an autosomal anomaly may lead to the disruption of genes responsible for spermatogenesis or impaired synaptic complex pairing during meiosis resulting in reproductive failure.

**Keywords:** Karyotype. Chromosomal insertion. Male infertility. Oligoasthenoteratozoospermia.

### INTRODUCTION

Genetic anomaly is the most predominant factor involved in human infertility. For the infertile males, chromosomal aberration is the most prevalent defects.<sup>1</sup>

The incidence of chromosomal aberration is 10–15.2% in azoospermic patients, and 3–5% in oligozoospermic ones.<sup>2</sup> Increases in chromosomal aberrations have been clearly demonstrated to increase proportionally with increasing severity of the infertility.<sup>3</sup>

Chromosomal abnormalities can be of two types: numerical and structural. The structural aberration describes altered chromosomes that occur either as intrachromosomal or interchromosomal events. The most frequent structural aberrations are translocations.<sup>4</sup>

Among the chromosomal structural abnormalities, insertions are rare rearrangements resulting from a three breaks mechanism.<sup>5</sup> Insertions can be intra- or inter-chromosomal. In an inter-chromosomal insertion or an insertional translocation, as in the present case a portion of one chromosome deleted from its normal location and inserted into another non-homologous chromosome. Because of three breaks involved, it is classified as complex chromosomal rearrangements (CCR).

According to a previous review, interchromosomal insertions (ICIs) occur with an estimated frequency of 1:80,000.<sup>6</sup> Although all chromosomes participate in these rearrangements, two insertions of 1q have been described<sup>7,8</sup> but no insertion in 1p has been reported.

To the best of our knowledge, this is the first report on an inter-chromosomal insertion that cause spermatogenic

failure and is associated with oligoasthenoteratozoospermia.

### MATERIALS AND METHODS

#### Patient

A 36-year-old man was referred for evaluation of subfertility to our laboratory with a 5-year history of primary infertility. He was born from a full term natural delivery with no apparent complication.

Semen analysis was carried out in accordance with the World Health Organization Laboratory Manual for the Examination of Human Semen.<sup>9</sup>

The patient was found to have a severe oligospermia with a volume of 2.5 ml, (sperm count = 4.3 million/ml), asthenozoospermia (grade a+b sperm motility 30%) and teratozoospermia (78% with atypical forms). Clinical examination and Gonado-trophin concentrations were normal (6.07 mU/ml; 3.26 mU/ml; 2.92 ng/ml) for FSH, LH and Testosterone, respectively.

Three fertile men, having at least one healthy child, were recruited as normal controls for this study, and similar tests were conducted on them.

The patient and controls gave informed consent to their participation in the study.

The study protocol was approved by the Local Ethics Committee.

#### Methods

##### Cytogenetic analysis

Chromosomal analysis was performed on peripheral lymphocyte cultures using standard cytogenetic methods.



Briefly, peripheral blood lymphocytes were cultured in 6.5 ml TC 199 (SIGMA), 1.5 ml of fetal bovine serum (SIGMA) and 10 µg/ml phytohaemagglutinin (SIGMA) were added and incubated at 37°C. After 72 h of incubation, 150 µl colcemid (10 µg/ml, SIGMA) was added. The cells were incubated at 37°C for about 20 mins. The suspension was centrifuged, and the pellet was resuspended in 6–10 ml KCL (0.075 M) for about 20 mins at 37°C. After centrifugation the cells resuspended in fixative (3v methanol: 1v acetic acid, SIGMA). The fixative was changed at least 3 times. After dropping on slides, the chromosomes were treated by RHG banded. At least 30 metaphase cells were analyzed, and 10 metaphases were photographed to determine the patients' karyotype that was named according to ISCN.<sup>10</sup>

### Genetic testing of Y-chromosomal microdeletions

Genomic DNA was extracted from the peripheral blood of the patient. Six STSs (sY84, sY86 for AZFa, sY127, sY134 for AZFb, and s254, sY255 for AZFc) were used. This primer set was suggested by Simoni (2004).<sup>11</sup> and is prescribed by the European Academy of Andrology and European Molecular Genetics Quality Network. SRY gene (sY14) was used as internal control, and the genomic DNA from fertile male and female were served as positive and negative controls respectively. PCR was carried out as described.<sup>12</sup>

The PCR products were electrophoresed on 2% agarose gel and visualized under ultraviolet light.

## RESULTS

### Karyotyping

Karyotyping on R-banded metaphases of peripheral blood lymphocytes revealed a constitutional chromosomal insertion 46,XY, ins(1;2) (p31; p13p23) in all the cells (Fig. 1).

The result revealed a DNA fragment that breaks off from chromosome 2 and inserts into chromosome 1 (Fig. 2)



**Figure 1:** Karyotypeshowing a novel insertion: [46,XY,ins(1;2)(p31;p13p23)] (arrows indicate the points of rupture)



**Figure 2:** Schematic diagram indicating breakpoints in chromosome 1 and 2, showing the banding patterns of the 2 derived chromosomes 2p and 1p

### Detection of Y-chromosomal microdeletions

To make sure that the insertional translocation described above is not an accidental coincidence but the real causative anomaly for the subfertility, we performed a genetic testing to detect Y-chromosomal microdeletions using PCR. Six STSs were used from AZFa, AZFb, and AZFc regions. It was found that the patient did not have any genomic deletions in the AZFa, AZFb and AZFc regions on the long arm of the Y chromosome.

## DISCUSSION

Chromosome aberrations are found in 2–7% of couples with fertility problems.<sup>13</sup> A relationship between autosomal rearrangements and infertility has been reported.<sup>14,15</sup> These disorders are more frequent among infertile men in comparison with the general population.<sup>16</sup> Although chromosomal insertions are rarely detected, several different insertions have been documented, and are among the factors causing human male infertility.

The insertion ins (1;2) (p31; p13p23) detected in the cells of the presented OATS patient is to the best of our knowledge a novel chromosomal abnormality. A systematic review of the literature did not reveal any previous reports on ins (1;2) patients with male infertility.

Similar result was also found in an earlier report by Rao (2005) from the South Indian population, reported for the first time this novel pattern in a woman with 46,XX,ins (12;6)<sup>17</sup> Iyer (2007) also reported an interchromosomal abnormality of insertion in a husband with karyotype 46,XY, ins (1;13)<sup>18</sup> and De la Fuente-Cortés (2009) found a karyotype of 46,XX,ins (15;8)(q26;p11p23).<sup>19</sup> Li (2014) described an infertile, azoospermic who inherits an aberrant karyotype 46,XY, inv ins (18,7) (q22.1; q36.2q21.11) from his mother. Intensive NGS discovered two disrupted genes, DPP6 and CACNA2D1 at breakpoints of 7q36.2 and 7q21.11<sup>20</sup>, whereas interchromosomal

insertions are rarely reported with male infertility. The mechanism for this chromosome breakage is unknown.

An increased number of carriers of structural chromosomal disorders has been reported among severely oligozoospermic and azoospermic men and the most common chromosome involving in these rearrangements was chromosome 1<sup>21,22</sup>, suggesting that some genes on chromosome 1 may play important role in spermatogenesis.

Bache. (2004) studied 464 infertile male with balanced rearrangements and found that the break-point at chromosome 1 is most predominant.<sup>23</sup> These data are consistent with our case.

In fact, several genes closely related to spermatogenesis have been located in chromosome 1, such as SCP-I, MSH4 and MMP-23 gene.<sup>24</sup>

The causal relation between chromosomal rearrangements and spermatogenesis failure has been suggested to be a structural effect related to alterations in the process of chromosome synapsis during meiosis.<sup>25</sup> Another mechanism might be related to the loss of a few genes during translocation between two non-homologous by inter chromosomal insertion.

## CONCLUSION

In conclusion, this study provides the evidence that chromosomal insertion cause spermatogenesis failure probably by disturbing meiosis in the patient.

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