Case report

A Rare De Novo Complex Chromosomal Rearrangement (CCR) Involving Four Chromosomes in An Oligo-asthenosperm Infertile Man: Case report

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Abstract: Complex chromosomal rearrangements (CCR) are rare events involving more than two chromosomes and more than two breakpoints. They
are usually associated with infertility or sub fertility in male carriers. Here we report a novel case of a CCR in a 30 year oligoasthenosperm man with a history of Varicocelectomy, normal testes size and normal endocrinology profile, who referred for chromosome analysis to genetic laboratory of Royan reproductive biomedicine research center. Chromosomal analysis was performed from peripheral blood lymphocyte cultures and analyzed by GTG banding. Additional tests such as C-banding and multicolor fluorescence in situ hybridization (FISH) procedure for each of the involved chromosomes were performed to determine the patterns of the segregations. Y chromosome microdeletions in the azoospermia factor (AZF) region were analyzed with multiplex polymerase chain reaction. To identify the history and origin of this CCR, all the family members were analyzed. The case had a complex chromosomal rearrangement. No micro deletion in Y chromosome was detected. The same \textit{de novo} reciprocal exchange was also found in his monozygous twin brother. The other siblings and parents were normal. CCR are associated with male infertility as a result of the disruption of spermatogenesis due to complex meiotic configurations and the production of chromosomally abnormal sperm. These chromosomal rearrangements might have influence in decreasing the number of sperms.
**Keywords:** Complex Chromosomal Abnormality (CCR), Infertility, Karyotype, FISH

**Introduction:**
Complex chromosomal rearrangements (CCRs) are balanced or unbalanced structural aberrations that are characterized by three or more breakpoints, located on more than two chromosomes (1). CCRs are very rare events in the human population (2). About only 250 patients with CCRs have been reported in the literature, although this number will likely increase owing to the application of molecular cytogenetic techniques (3). The balanced CCRs range from simple three-way exchanges between three chromosomes to highly complex translocations involving four or five chromosomes with multiple breaks, inversions, and insertions. Up till now, CCRs have been classified according to whether they are inherited or de novo, and according to the number of chromosomes or the number of breaks involved (4). In almost 70% of the cases (2), especially in most of the familial cases, the phenotype is normal in the apparently balanced carriers but they may have a significant risk of reproductive failure (2, 5). Most de novo CCRs are originated from spermatogenesis and cause mental retardation in high incidence, whereas most familial CCRs are of maternal origin and usually have three to four breakpoints (6-9). Most of the patients with CCRs are women who have been identified because they give birth to malformed children or have repeated spontaneous abortions (9), while vast majority of the males with CCRs were found in men showing infertility problems (10). According to the literature, the
complexity of meiotic configurations may cause hypospermatogenesis or spermatogenic arrest in CCRs carrying patients (2). A review of published reports revealed that 13.7% of azoospermic men and 4.6% of oligozoospermic men have an abnormal karyotype. In the azoospermic group, sex chromosome abnormalities predominate, mainly 47,XXY. In the oligozoospermic group, autosome anomalies, such as Robertsonian and reciprocal translocations, are the most frequent karyotypic abnormalities (11).

The interpretation of CCRs by conventional banding techniques alone may be impossible, particularly when deletions, insertions or inversions as well as reciprocal translocations occur. Fluorescent in-situ hybridization (FISH) with chromosome-specific DNA probes allows exploring chromosome rearrangements in greater detail and is a useful tool for an accurate diagnosis.(12)

The present case represents the new case of CCR in a man presenting with a spermatogenic defect. We report here the case of an oligospermic male with a CCR, where four chromosomes are involved (13, 14, 16 and 18) and five breakpoints were observed.

**Case Report:**
A 30-year-old man, who suffered from infertility for three years, underwent cytogenetic examination. For this research, a written informed consent for use of the results of examinations was obtained from the patient. There were no mental retardation, no malformation, no gynecomastia, no erectile dysfunction, no thromboembolic disease, and no reduced muscle strength. Testes volumes were normal. His parents were not related. Although, two of his brothers had children, his monozygotic twin brother was infertile too.

Serologic analysis revealed serum levels of FSH, LH, prolactin, and testosterone were in normal ranges. Clinical assessment verified the presence of varicocele grade-I on the left side. The semen analysis indicated total volume (3.7 ml), normal pH (7.8), low concentration (0.6 millions/ml) and normal color (white-Gray), with reduced sperm motility; sperm total motility (5%) together with a low yield of progressively motile sperm and teratozoospermia (98% of spermatozoa showed abnormal morphology) and low viability (44%).

Histological assessment of testis biopsy specimen showed incomplete spermatogenic arrest with sign of sloughing. Few spermatozoa were seen. For more investigation, cytogenetic tests were proposed (13). Cytogenetic analysis was performed according to standard methods on phytohemagglutinin (PHA)-stimulated peripheral lymphocyte cultured cells from the patient, his brother, his sister and his parents’ peripheral blood. Briefly, cells were cultured in complete RPMI 1640 (GIBCO) for 72 h. The Colcemid arrested cells were spun and the pellet was resuspended in 5–10 ml hypotonic solution for about 20 mins at 37°C. After centrifugation the cells were fixed with Carnoy’s fixative. Fixed cells were used for slide preparation. The procedure was followed by FISH, GTG as well as C-banding techniques.
At least 20 GTG banded metaphases from the cases were analyzed at resolution 550 bands. The latest ISCN guidelines for chromosome nomenclature were followed (14).

Karyotype of peripheral blood revealed a de novo complex chromosomal rearrangement;

46,XY,der(13)t(13;18)(q22;q21.2)ins(13;14)(q22;q24q32.1),del(14)(q24q32.1),
der(16) t(16;13)(p12.3; q22),der(16;18)(p12.3;q21.2)(Figure 1).

According to the karyotype and judging from the figures 2, the following is supposed to have happened: a segment of 14q (bands between q24 and q32.1) has inserted to the long arm of chromosome 13 at band q22. A segment from the same chromosome 13 from q22 has moved to the short arm of 16(p12.3), the segment from 16p has moved to the long arm of 18 at band q21.2 and the segment from 18 has moved to the long arm of 13. This would be a CCR involving four chromosomes with five breakpoints.

Additional multicolor fluorescence in situ hybridization (FISH) procedure on metaphase chromosomes was performed on prepared slides to determine the exact patterns of this CCR according to the standard cytogenetic protocols (15, 16). Appropriate DNA probes (Vysis, Abbott Molecular, USA) for involved chromosomes were applied. For this purpose, a well-tune fluorescent microscope (Olympus BX51, Japan) equipped with necessary and optimum filter sets (Spectrum Orange/ Spectrum Green/ DAPI single band pass filter sets, Abbott Molecular, USA) and an image acquisition and processing software (Cytovision V4.0, Applied Imaging, Genetix, UK) were used (Figure 2).
His monozygotic twin brother had the same karyotype. De novo translocation was confirmed by the normal karyotype of the patient’s parents. His sister had normal karyotype too.

Yq microdeletion screening was done for the patient too. Genomic DNA was extracted from peripheral blood samples using the Genomic DNA Extraction Kit (Bioneer, Korea). Detection of microdeletions on the Y-chromosome was based on three multiplex PCRs (17). Molecular analysis showed no microdeletions in the Y chromosome.

Discussion:

Male infertility may be attributed to chromosomal alterations that usually involve the sex and autosomal chromosomes (18, 19). It had been reported that a substantial cases of infertile men have constitutional chromosomal abnormalities, including 47,XXY, Robertsonian and reciprocal translocation of autosomes (11). Infertile men with spermatogenesis impairment are 10 times more likely to have structural chromosome abnormalities than the normal population (5.1% compared to 0.5%, respectively) (20). Whilst the consequences of a simple reciprocal translocation for male fertility have been well studied, CCRs are generally considered to lead to severe reproductive disturbance (21). Infertility in such situations is usually related either to disturbance in meiosis or to the generation of unbalanced gametes through chromosome malsegregation (20).
The CCR presented here theoretically represents a heptavalent structure at meiosis I (Figure3). It has been assumed that spermatogenic arrest occurs as a consequence of the complex meiotic configurations during meiosis.(7) As few sperms were found in seminal analysis in this patient, it can be concluded that a deep impairment of spermatogenesis occurred at the late pachytene stage which caused most of the spermatocytes’ cell death and eventually perturbation of spermiogenesis. Only few spermatocytes could escape pachytene apoptosis and were able to deal with a heptavalent in the metaphase I spindle and pass to anaphase I.

The origin of pachytene apoptosis has been attributed to a ‘pachytene checkpoint’(22) which detects failures in chromosome synapsis and recombination. In the present case, the long stretches of asynaptic regions in the heptavalent at pachytene could initiate the apoptotic process. Such an explanation may justify the greatly reduced sperm parameters and severe oligospermia observed in this carrier. Interestingly, the lower frequency of unbalanced sperms than expected was found by Kirkpatrick and Ma in a carrier of a rare CCR. They hypothesized that a great amount of unbalanced chromosome complements are in fact produced during segregation but selection during spermatogenesis preferentially selects spermatogonia which contains balanced/normal chromosome complements.(7)
Cytogenetic studies (different banding techniques) and FISH were performed on this patient because he was supposed to undergo intracytoplasmic sperm injection (ICSI) procedure. As the risk of chromosome aberration transmission always exists in oligo- or azoospermic males, cytogenetic study prior to ICSI is of great importance (2). In the present case, the use of FISH technique was necessary for the correct diagnosis of this CCR. The availability of specific DNA probes and chromosomal libraries have made FISH clinically applicable. Clinical application of FISH has shown that CCRs may be more common than initially considered. Although it is suggested that the more complexity of a CCR, the more severity in the spermatogenic impairment is, according to the literatures, neither the origin and the complexity nor the number of breaks can be used to predict whether a certain CCR will lead to infertility in the male (4, 23). Surprisingly, the normal phenotype of this patient suggests that the breakpoints in involved chromosomes do not inactivate functional genes or gene regions with regulatory functions, whose disruption could produce phenotypic alterations. Nevertheless, other disruptions at the molecular level cannot be disregarded. Spermatogenesis dysfunction in translocation carriers can now be bypassed by ICSI. However, ICSI is not considered a solution for infertility in male carriers of CCRs because of the low percentage of balanced sperm availability (3, 4, 24). Imbalanced sperms can lead to reproductive
impairments, such as; fetal abnormalities and repeated spontaneous miscarriages. The incidence of having normal healthy babies in CCR carriers is very low. Though, CCR carriers still have a limited chance of having a healthy child (1).

Preimplantation genetic diagnosis (PGD) with FISH has been applied successfully to detect chromosomal imbalances in preimplantation embryos before being transferred into the mother’s uterus. Although PGD-FISH is difficult to diagnose the embryos which are CCR carriers due to the limited availability of FISH probes, it may be useful for selecting balanced embryos in such patients.(1, 25)

**Conclusion**

This is another study that emphasizes on the importance of probing techniques (e.g. FISH) as an ideal confirmatory method in cytogenetic studies. Moreover, this study also focuses on this fact that, although vast majority of people with CCR, may apparently seem normal despite having high extent of genetic alterations, they are vulnerable to gametogenesis defect.

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Fig1. Karyotype of GTG banded chromosomes of the patient showing CCR involving chromosomes 13, 14, 16 and 18.
Fig2. FISH of mitotic lymphocytes with whole chromosome or segmental paints:

(A and B) 18 Centromere probe (Aqua), 14q Telomer probe (Orange), 16p Telomer probe (Green); (C) 16 Centromere probe (Aqua), 18q Telomer probe (Orange), chromosome 13 and 18 paint (Green). The arrow shows the inserted segment from 14q to 13q; (D) chromosome 13 paint (Green), 13q Telomer probe (Orange), 18 Centromere probe (Aqua); (E) chromosome 18 paint (Green), 18q Telomer probe (Orange); (F) 16 Centromere probe (Aqua), 14q Telomer probe (Orange).
Fig3. This schematic figure shows the heptavalent pachytene configuration adopted at meiosis I by complex chromosome rearrangements (CCR). In this example, the configuration allows the efficient synopsis of the eight chromosomes involved in this complex.