A de novo complex chromosome rearrangement involving three chromosomes (2, 13, and 18) in an oligospermic male

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Objective: To determine an unusual complex chromosome rearrangement found in a man with oligospermia with a normal phenotype.

Design: Case report with a review of the literature.

Setting: Academic research environment.

Patient(s): A man with oligospermia but otherwise apparently healthy.

Intervention(s): Peripheral blood lymphocytes were used for karyotyping, and metaphases were analyzed by the fluorescence in situ hybridization (FISH) procedure. Further characterization of the karyotype was done by using multicolor banding (MCB) probes.

Main Outcome Measure(s): Physical examination, semen analysis, GTG banding, FISH, MCB.

Result(s): The semen analysis revealed oligospermia. The lymphocytic karyotype detected an unusual complex chromosome rearrangement involving chromosomes 2, 13, and 18 determined by banding cytogenetics. The karyotype was established as 46,XY,t(2;13;18)ins(2;13)(2qter/2p25.1::13q13/13q22::18q12.3/18qter;13pter/13q13::2p25/2pter;18pter/18q12.3::13q22/13qter) after MCB analysis.

Conclusion(s): The association of an unusual complex chromosome rearrangement with three recurrent spontaneous abortions was reported. (Fertil Steril 2009;92:391.e9–e12. ©2009 by American Society for Reproductive Medicine.)

Key Words: Complex chromosome rearrangement (CCR), oligospermia, recurrent spontaneous abortion, multicolor banding (MCB)

Complex chromosome rearrangements (CCRs) are structural aberrations that involve exchange of genetic material between two or more chromosomes, and they are classified depending on the origin, number of breakpoints, or number of chromosomes involved (1, 2). CCRs are rare events; to our knowledge, 156 cases of CCRs have been reported up to the time of writing (3–21). Despite the fact that there are familial cases reported, most CCRs are de novo. The transmission of the familial cases occurs mainly via the maternal line. However, the origin of de novo cases can also be of paternal origin (3). According to Kouseff et al. (22), CCRs can be categorized as group I, including those with ≤4 breaks, and group II, including >4 breaks. CCRs are usually associated with congenital anomalies and mental retardation. The resulting abnormal phenotype may be due to the disrupting of genes at the breakpoints or the presence of submicroscopic deletions or duplications. Chromosomal rearrangements resulting in gene inactivation at the breakpoints have been reported in a number of cases (2, 19, 23, 24). On the other hand, CCRs can also occur in phenotypically normal persons, and in such cases they are referred to as truly balanced. The regulatory sequences can also be affected at the balanced chromosomal rearrangements, which are usually associated with severe reproductive impairment through meiotic disturbance or chromosomal imbalance in gametes in men and recurrent miscarriages in women (2, 7, 25).

It is difficult or even impossible to identify precise breakpoints of CCRs using only conventional cytogenetic techniques. Recently developed multicolor banding (MCB), based on overlapping region-specific microdissection libraries, is a powerful molecular cytogenetic method which allows the characterization of chromosomal breakpoints at the GTG sub-band level (26).

In the present study, we report a patient with oligospermia who was found to have a de novo CCR involving the three chromosomes 2, 13, and 18.

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MATERIALS AND METHODS
Clinical Case Report

The patient was a 28-year-old man working in a restaurant, from nonconsanguineous parents, who was married for 4 years and whose wife had three consecutive spontaneous abortions each within the first trimester. The first abortion was within 3 months, the second within 2 months, and the third within 1 month. His physical examination revealed a normally androgenized male with an unexpected volume difference between testes. His body mass index was 24.49 kg/m², height 175 cm, and weight 92 kg. When his testes were investigated by using color Doppler ultrasound, it was found that the left one measured 40 × 21 × 25 mm, 11 mL in volume, and the right one measured 44 × 25 × 30 mm, 18 mL in volume. He had bilateral varicocele with grade 2. The FSH, LH, and T values were within the normal ranges.

Semen analysis was performed three times according to the World Health Organization (27) guidelines for semen analysis within an interval of three months. Semen analyses revealed oligospermia with only 1.6 mL in volume, sperm concentration 20.2 × 10⁶/mL, forward motility 35% at 1 hour, and after 4 hours, 80% immotile and 92% normal morphology. Total ejaculate contained only 32.3 × 10⁶/1.6 mL on the average.

Ethical Considerations

Informed consent was obtained from the family before the study. The Institutional Review Board approved the study.

Banding Cytogenetics

Cytogenetic analysis was performed by standard methods using cultured peripheral blood lymphocytes from the patient, his parents, one of his unmarried brothers, and his wife. Although the patient had two brothers and one sister, for this study only his unmarried brother was available. Twenty metaphases were analyzed from each subject by GTG banding technique. His sister and other brother were married with children.

Molecular Cytogenetics

The MCB probes for chromosomes 2, 13, and 18 and subtelomeric probe for 2pter (Abbott) and whole chromosome probe were performed to define the exact breakpoints of rearranged chromosomes (26). Metaphase spreads were analyzed by using a fluorescence microscope (Axioplan 2 mot, Zeiss)
DISCUSSION

In the present case, three chromosomes were involved in CCRs: 2, 13, and 18. As a result, three derivative chromosomes were formed. The four breakpoints were located in 2p25.1, 13q13 and 13q22, and 18q12.3. To the best of our knowledge, this is the first time a patient with such an apparently balanced translocation associated with three recurrent consecutive spontaneous abortions has been described. Compared with rearrangements up to 15 breakpoints (9), this case with CCR is considered to be at the low level of complexity.

Lurie et al. (28) found that some chromosomes have more recurrent involvement in CCRs than others; among frequently involved chromosomes, 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, and 21 are usually altered, chromosomes 3 and 7 most frequently. Batanian and Eswara (3) reviewed 114 cases with CCR and proposed a hot spot on 18q21. Because there are two breakpoints on chromosome 18, they concluded that their case contradicts the general suggestion that only large chromosomes are susceptible to more than one break; this was also contradicted by a case with nine breaks in chromosome 21 (10). Battisti et al. (29) confirmed the hot spot on 18q21 and showed that chromosomes 3, 4, 8, 13, and 18 were involved in CCRs. The present study doesn’t confirm this hot spot on 18q21, but two breakpoints present on chromosome 13 confirm the suggestion by Batanian and Eswara that not only large chromosomes are prone to more than one breakpoint.

The fact that the present case is an oligospermic man raises the question of whether the involvement of autosomal chromosomes is the reason, because it is known that sex chromosome abnormalities are predominant in azoospermic men (12.6%), whereas autosomal abnormalities are more frequent in patients with oligospermia (3%) (30). Chromosomal rearrangements alter chromosome synopsis during meiosis, and this might cause impaired sperm production (31). In mice, asynapsed regions may trigger the meiotic checkpoint machinery to eliminate spermatocytes (32). Association of chromosomal abnormalities with deficient spermatogenesis might be explained by a similar mechanism (33). Although the mechanism of CCRs is unknown, molecular and cytogenetic analyses indicate that familial cases are due to the mistakes in female gametogenesis but most of de novo CCRs are of paternal origin occurring during spermatogenesis (7, 19). De Gregory et al. (17) suggested that some cells escape the mechanism for proper crossing-over during spermatogenesis in which chaotic breaks allow reunion of several chromosomes resulting in CCRs.

Owing to the malsegregation of derivative chromosomes or the generation of a recombinant chromosome, the carrier of CCR has a risk for abnormal conception. Malsegregation follows the general principles as set forth for the simple translocation, but naturally the range of unbalanced combinations is greater. For the three-way CCR, the broad categories of malsegregation are 3:3 and 4:2, and theoretically 5:1 and 6:0. Recombination is rare indeed, and only eight examples have been recorded (34).

There are several possibilities for a higher incidence of abnormal pregnancy outcome in our case. At the pachytene stage, the normal and derivative chromosomes 2, 13, and 18 are likely to have two possible configurations: 1) as a hexavalent with chromosomes 2, 13, and 18 and the derivative der(2)t(2;13;18); or 2) tetravalent between chromosomes 13 and 18 and the derivatives der(13)t(2;13) and der(18)t(13;18) in addition to bivalent configuration. Either the tetravalent or the hexavalent configurations will give rise to many possible combinations of rearranged chromosomes in the embryos. Consequently, the unbalanced segregations are most likely to produce much greater genotypic imbalance and are expected to be nonviable, with all of them resulting in early pregnancy loss (35).

Precise definition of CCRs is not possible by classic banding cytogenetics. Identification of the exact breakpoints requires using molecular methods such as fluorescence in situ hybridization (FISH) with locus-specific probes or array CGH (36). Development of banding techniques based on FISH allows characterization of chromosomal subregions (37). The recently developed MCB technique is a powerful molecular cytogenetic method that produces changing fluorescence intensity ratios along the chromosome, by which it is possible to identify the exact breakpoints in the chromosomes at the band and subband level (38). We performed the MCB technique, following Weise et al. (26), as a very suitable approach to clarify the breakpoints and other chromosomal changes in CCRs to define exact breakpoints in the present case.

The CCR presented here may be the cause of oligospermia and is, with high probability, responsible for the consecutive recurrent spontaneous abortions in the corresponding partnership. It is very important to characterize exact breakpoints in CCRs to understand the mechanism underlying the formation of CCRs and to provide correlation between phenotype and chromosomal rearrangement. It is also important to give adequate genetic counseling for the patients. The occurrence of CCRs is rare, but the number of reported cases has increased over the recent years, and therefore accumulating data may help to reveal the mechanism of CCRs.
REFERENCES


