Indian J. Genet., 78(4): 515-518 (2018)
DOI: 10.31742/IJGPB.78.4.14

Short Communication

Pericentric inversion of chromosome 7 in human associated with recurrent ICSI failure: A case report

Ruchi Shah, Sonali Verma, Amrita Bhat, Ghulam Rasool Bhat, Kosheen Kamal, Rima Dada[1] and Rakesh Kumar*

School of Biotechnology, SMVDU, Katra 182 320, J&K; [1]Laboratory for Molecular Reproduction and Genetics, Departmentof Anatomy, All India Institute of Medical Science (AIIMS), New Delhi 110 029

(Received: May 2018; Revised: September 2018; Accepted: October 2018)

Abstract

Assisted reproductive techniques is specially a boon for severe male factor infertility with non-obstructive azoospermia (NOAZ) and severe oligospermia (<5 million/ml). To study the unusual pericentric inversion in a recurrent ICSI failure case, cytogenetic analysis was done from peripheral blood lymphocytes. Semen analysis revealed that the man had Oligoasthenoteratozoospermia with sperm count of 0.9 million/ml. A karyogram revealed pericentromeric inversion of Chromosome 7. It could be accommodated on models that location of the breakpoint harbours critical genes for spermatogenesis (*swm*, *swm2* and *STAG3*). Pericentromeric Inversion for chromosome 7 is a rare finding. It may be the etiopathogenic for partial spermatogenetic arrest and recurrent ICSI failure. The children born to such couples must also be analyzed and kept under observation for any clinical indications.

Key words: ICSI, inversion, oligoasthenoteratozoospermia, primary infertility, swm, CFTR

Assisted reproductive techniques have revolutionized the management of infertility. ICSI is major advancement in ART whereby a sperm is injected into ova (Stephens et al. 2013). Through these techniques, not only are these genetic abnormalities transmitted to offspring but also results in fertilization failure or post implantation failure which adds insult to injury (Stuppia et al. 2015).

Male sterility is a multifaceted uncontrolled disorder with extremely varied phenotypic appearances, from wide-ranging nil spermatozoa in the testes to different modifications of sperm superiority (Krausz and Riera-Escamilla 2018). Genetic factors are an important cause of spermatogonia arrest leading to infertility (Dada et al. 2006; Harton and Tempest 2012). Pericentric inversions have a frequency of 1-2% (Collodel et al. 2006; Tayebi and Khodaei 2011). It was hypothesized that inversion alters sperm morphology, impaired motility. It also leads to impaired meiotic segregation. It is possible that inversion in chromosome 7 may be the reason for the compromised semen status of the patient and our study seems to be in perfect agreement with this hypothesis (Collodel et al. 2006).

In the present study, to the best of our knowledge, we describe the first case of pericentric inversion of chromosome 7 found in a primarily infertile male with severe Oligoasthenoteratozoospermia.

Case report

A 29-year old man was referred to our lab for cytogenetic analysis after ART failure. He has been married for 6 years. His wife (26 Years) was normal on clinical and cytogenetic investigation. They were unable to bear a child naturally for 5 years. However, when this couple sought treatment, they experienced recurrent ART/ICSI failure, which seemed to be at the post implantation period within the first month following ART. In this study we aimed to investigate the cause of infertility with recurrent ICSI failure in 29-year-old

*Corresponding author's e-mail: kumar.rakesh@smvdu.ac.in
Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

male. In initial years of marriage, the wife had conceived thrice but the pregnancies were spontaneous aborted in the first trimester.

The patient underwent routine physical/testicular examination, hormonal evaluation, and semen analysis for finding the cause of infertility. Vas deferens was normal and volume of left testis (11 ml) was smaller than right (14 ml). Serum concentrations of LH, FSH, PRL, and testosterone were normal. Semen analysis was done thrice at an interval of 1 month after abstinence period of 7-8 days, which confirmed Oligoasthenoteratozoospermia (sperm count upto 1 million/ml) with motility (grade^{a+b}) less than 30%. About 80% spermatozoa had coiled tails and impaired linear progressive motility.

Chromosome preparation

After recording informed consent, 4-5 ml of heparinized venous blood from patients was drawn and kept in upright position at room temperature. The plasma lymphocyte solution (PLS) was added to glass cultural vial containing 5ml of RPMI-1640 and 0.2ml of phytohemagglutinin (Invitrogen). After 70 hours of incubation, the 0.1 ml (0.2%) of colcemid (Invitrogen) was added to arrest the cells at metaphase and washed after 2 hours. The cell suspension was centrifuged at 1000 rpm for 10 minute and resuspended in pre-warmed (37°C) hypotonic solution (0.56% KCl) for 20 minutes. After hypotonic treatment, pellet was suspended in carnoys fixative (Methanol: Acetic Acid 3:1). A minimum of three changes of fixative were done and sample was left for 24hours at 4°C. 2-5 µl of suspended cells were dropped from an arm's length into the clean and wet slides (Bluestar frost plus).

G-banding was developed by Giemsa staining (Invitrogen) of slides after trypsin treatment. The unstained matured 3days old prepared slides were flooded with 0.25% trypsin and immediately washed in phosphate buffer saline. Chromosomes stained by these protocol exhibit light and dark stained regions along their length. The time of exposure to trypsin was standardized for each set of slides. Following, this the slides were rinsed in normal saline (0.9% NaCl solution) and stained in 2% Giemsa solution for 5 minutes. The excess stain was washed off by rinsing the slides in distilled water. The slides were then air dried and observed under a bright field microscope. The slides were also mounted with DPX for permanent storage.

The chromosome preparations were screened using Olympus Axioplan bright field microscope under 10X objective. Well spread metaphase was further analyzed under 100X oil immersion objective. A minimum of 50 well spread Giemsa banded metaphase was analyzed.

The karyogram revealed a pericentric inversion of chromosome 46, XY;inv7(p12;q31) (Fig. 1). Couple

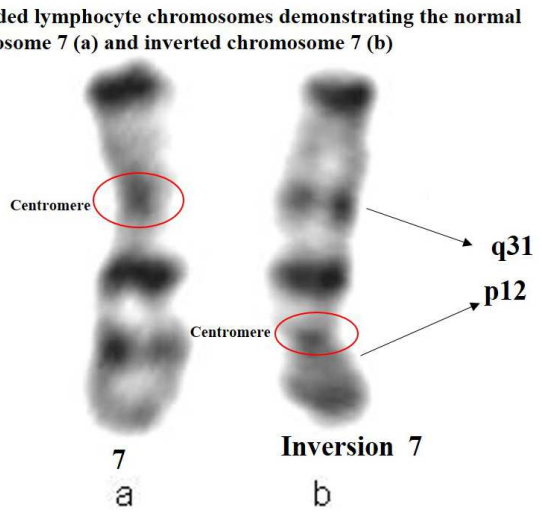

Fig. 1. (a) G-Banded lymphocyte chromosomes demonstrating the normal counterpart of chromosome 7. (b) G-Banded lymphocyte chromosomes demonstrating the pericentromeric inversion of chromosome 7 karyotype 46, XY inv(7) (p12-q31)

underwent ICSI thrice but failed. Blastocyst failed to grow post implantation and was spontaneously aborted within the first month following ICSI. Somatic chromosome abnormalities are found in 13.7% azoospermia and 4.6% in oligospermic infertile men. Although sex chromosome abnormalities are predominant (93%) in azoospermia men; however, autosomal structural abnormalities predominate (67%) in men with sperm count less than 20 million/ml (Dada et al. 2006). The probability of finding inversion (paracentric and pericentric) is 0.16% among infertile men (CC and CG 2014) (Van Assche et al. 1996). It was reported that the inversion of chromosomes were directly correlated to infertility (Dana and Stoian 2012). Although this is first case report regarding the pericentromeric inversion in a ICSI failed Oligoasthenoteratozoospermia man, we also found few other case reports in literature (Anonymous 1986; Matsuda et al. 1992; Navarro et al. 1993b). Oligospermic males and males opting for ICSI have

an inversion frequency of about 0.3 and 0.2%, respectively (Kohn et al. 2015) (Mau-Holzmann 2005). Pericentric inversion of chromosome 1 appears to carry a special risk of male infertility regardless of the breakpoint position (Chandley et al. 1987). Recent study also reported that carriers of chromosome 7 translocations suffering from infertility of both presentational and gestational infertility in females (R.X. Wang 2016). Navarro J et al (Navarro et al. 1993a) reported a sub fertile man with pericentromeric inversion of chromosome 7 but in a heterozygous condition. Chromosome 7 inversions in the human male could be accommodated on models that link defective germ cell maturation to failure of synapses and these chromosomal imbalances produce unstable gametes in offspring's (Kochhar and Ghosh 2013) (Chandley et al. 1987). Pericentric inversion of chromosome 7 appears to carry a special risk of male infertility regardless of the breakpoint position (Zhang et al. 2015) (Pettenati et al. 1995).

There are few important genes involved in testicular function that are located on chromosome 7. One is CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene and another is TES that is expressed in testis (Cocuzza et al. 2013). Both these genes have been mapped to 7q31 (Ichioka et al. 2005). Lessard C et al (Lessard et al. 2006) has marked the *swm2* (sperm without motility) gene on proximal portions of chromosome 7. Sperm from mutant males for the *swm* gene failed to fertilize oocytes in vitro (Ward et al. 2003) (Stanfield and Villeneuve 2006). The swm series of mutations (Sperm without motility) causes spermatogenetic defects. The mutants show either absence or greatly diminished numbers of mature sperm. Stromal in 3 (STAG3) is another protein that is expressed specifically in testis and mapped to 7q22. STAG3 is associated to the synaptonemal complex. Six human *STAG3*-related genes have also been mapped: two at 7q22 near the functional gene, one at 7q11.22, and three at 7q11.23 (Pezzi et al. 2000). In our case, cytogenetic analysis shows pericentric inversion of chromosome 7, i.e., 46; XY, inv 7(p12; q31), which exactly disrupts the three genes discussed above except the CFTR gene. The presence of intact CFTR is indicated by presence of normal vas deferens and no respiratory ailment as in our case. Even the disruption of the single gene of the three (TES, swm, and STAG3) is enough for failure of the spermatogenesis of the affected male.The presence of intact CFTR is indicated by presence of normal vas deferens and no respiratory ailment as in our case. These deletions and duplications present in the meiotic recombinant chromosomes might be lethal and create higher rate of fertilization or implantation failures after ICSI, and the rate of spontaneous abortions might be elevated as well. It is possible that such offspring, if conceived, may also be infertile, as these anomalies are germline.

Through present study, it is clear that chromosomal analysis should be conducted prior to ART/ICSI, which will prevent patients from financial and emotional distrust. In addition, these chromosomal abnormalities can provide a path for deciphering the genomic changesresponsible for the variable phenotypes of infertility associated with Oligoasthenoteratozoospermia.

Authors' contribution

Conceptualization of research (PK, RD); Designing of the experiments (RK); Contribution of experimental materials (RD); Execution of field/lab experiments and data collection (RK); Analysis of data and interpretation (RS, SV, GR, AB); Preparation of manuscript (RS, RV, KK).

Declaration

The authors declare no conflict of interest.

Acknowledgement

Rakesh Kumar acknowledge Department of Science and Technology (DST-SERB) with sanction no. YSS/2014/000659 for young scientist grant.

References

Anonymous. 1986. Paracentric inversions in man. A French collaborative study. Groupe de Cytogénéticiens Français. 169-76.

CC Y. 2014. CG E: The largest paracentric inversion, the highest rate of recombinant spermatozoa. Case report: 46, Xy, Inv (2)(Q21. 2q37.3) and literature review. Balkan J. Med. Genet., **17**: 55-61.

Chandley A., McBeath S., Speed R., Yorston L. and Hargreave T. 1987. Pericentric inversion in human chromosome 1 and the risk for male sterility. J. Med. Genet., **24**: 325-334.

Cocuzza M., Alvarenga C. and Pagani R. 2013. The epidemiology and etiology of azoospermia. Clinics, **68**: 15-26.

Collodel G., Moretti E., Capitani S., Piomboni P., Anichini C., Estenoz M. and Baccetti B. 2006. TEM, FISH and molecular studies in infertile men with pericentric inversion of chromosome 9. Andrologia, **38**: 122-127.

Dada R., Gupta N. and Kucheria K. 2006. Cytogenetic and molecular analysis of male infertility. Cell Biochem. Biophys., **44**: 171-177.

Dana M. and Stoian V. 2012. Association of Pericentric Inversion of Chromosome 9 and Infertility in Romanian Population. Mædica, **7**: 25-29.

Harton G. L. and Tempest H. G. 2012. Chromosomal disorders and male infertility. Asian J. Androl., **14**: 32.

Ichioka K., Yoshimura K., Honda T., Takahashi A. and Terai A. 2005. Paracentric inversion of chromosome 7 (q22–31) associated with nonobstructive azoospermia. Fertility Sterility, **83**: 455-456.

Kochhar P. K. and Ghosh P. 2013. Reproductive outcome of couples with recurrent miscarriage and balanced chromosomal abnormalities. J. Obst. Gyna. Res., **39**: 113-120.

Kohn T. P., Clavijo R., Ramasamy R., Hakky T., Candrashekar A., Lamb D. J. and Lipshultz L. I. 2015. Reproductive outcomes in men with karyotype abnormalities: Case report and review of the literature. Canadian Urological Association J., **9**: E667.

Krausz C. and Riera-Escamilla A. 2018. Genetics of male infertility. Nature Reviews Urology.

Lessard C., Lothrop H., Schimenti J. and Handel M. 2006. Mutagenesis-generated mouse models of human infertility with abnormal sperm. Human Reprod., **22**: 159-166.

Matsuda T., Horii Y., Ogura K., Nonomura M., Okada K. and Yoshida O. 1992. [Chromosomal survey of 1001 subfertile males: incidence and clinical features of males with chromosomal anomalies]. Hinyokika kiyo Acta urologica Japonica, **38**: 803-809.

Mau-Holzmann U. 2005. Somatic chromosomal abnormalities in infertile men and women. Cyto. Genome Res., **111**: 317-336.

Navarro J., Benet J., Martorell M., Templado C. and Egozcue J. 1993a. Segregation analysis in a man heterozygous for a pericentric inversion of chromosome 7 (p13; q36) by sperm chromosome studies. Amer. J. Human Genet., **53**: 214.

Navarro J., Benet J., Martorell M. R., Templado C. and Egozcue J. 1993b. Segregation analysis in a man heterozygous for a pericentric inversion of chromosome 7 (p13;q36) by sperm chromosome studies. Amer. J. Human Genet., **53**: 214-219.

Pettenati M., Rao P., Phelan M., Grass F., Rao K., Cosper P., Carroll A., Elder F., Smith J. and Higgins M. 1995. Paracentric inversions in humans: a review of 446 paracentric inversions with presentation of 120 new cases. Amer. J. Med. Genet. Part A, **55**: 171-187.

Pezzi N., Prieto I., Kremer L., Jurado LAP., Valero C., Del Mazo J., MartÍnez-a C. and Barbero J. L. 2000. STAG3, a novel gene encoding a protein involved in meiotic chromosome pairing and location of STAG3-related genes flanking the Williams-Beuren syndrome deletion. The FASEB J., **14**: 581-592.

R. X. Wang HGZ., Pan Y., Zhu J. H., Yue F. G., Xue L. T. and Liu R. Z. 2016. Chromosome 7 translocation breakpoints in male carriers: Clinical features and implications for genetic counseling. Received July 6, 2016, Accepted August 15, 2016, Published October 17, 2016.

Stanfield G. M. and Villeneuve A. M. 2006. Regulation of sperm activation by SWM-1 is required for reproductive success of *C. elegans* males. Curr. Biol., **16**: 252-263.

Stephens S. M., Arnett D. M. and Meacham R. B. 2013. The use of in vitro fertilization in the management of male infertility: What the urologist needs to know. Reviews in Urology, **15**: 154.

Stuppia L., Franzago M., Ballerini P., Gatta V. and Antonucci I. 2015. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. Clinical Epigenetics, **7**: 120.

Sumner A. T. 1982. The nature and mechanisms of chromosome banding. Cancer Genet. Cytogenet., **6**: 59-87.

Tayebi N. and Khodaei H. 2011. A rare case of pericentric inversion, inv (21)(p12; q22) in repeated pregnancy loss: a case report. Oman Med. J., **26**: 441.

Van Assche E., Bonduelle M., Tournaye H., Joris H., Verheyen G., Devroey P., Van Steirteghem A. and Liebaers I. 1996. Cytogenetics of infertile men. Human Reprod., **11**: 1-26.

Ward J. O., Reinholdt L. G., Hartford S. A., Wilson L. A., Munroe R. J., Schimenti K. J., Libby B. J., O'Brien M., Pendola J. K. and Eppig J. 2003. Toward the genetics of mammalian reproduction: induction and mapping of gametogenesis mutants in mice. Bio. Reprod., **69**: 1615-1625.

Zhang M., Fan H., Zhang Q., Wang X., Yang X., Tian W. and Li R. Genetic screening and evaluation for chromosomal abnormalities of infertile males in Jilin Province, China. Genet. Mol. Res., **14**: 16178-16184.