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ORIGINAL ARTICLE

The Importance of Cytogenetics and Associated Molecular Techniques in the Management of Patients Carrying Robertsonian Translocation and Their Pregnancy Outcome by Intracytoplasmic Sperm Injection

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About the Author

Dr. Bibhas Kar Ph.D. in Life Science, has 20 years of research experience in the field of human genetics. He has been honored with many prestigious international and national fellowship and awards which include Stevens Shapiro Memorial Fellowship from UK and International Union of Biochemistry and Molecular Biology Young Scientist Award from the USA and Indian Science Congress Association Young Scientist Award from India for his work in the field of human genetics. He has published over 44 original research and review articles in peer-reviewed journals. He has served on the editorial boards of several journals. He is also a life member of various professional bodies. He worked earlier at Sankara Netralaya as Genetic Scientist and Apollo Hospitals as Consultant Geneticist and Head.

Abstract

Objective The present study outlines three cases of a Robertsonian translocation and the consequences for the initiation of pregnancy by intracytoplasmic sperm injection (ICSI). Three case histories are presented documenting structural chromosome abnormalities in infertile males.

Materials and Methods Semen analysis was performed according to the World Health Organization guidelines. Chromosome analysis was performed using G-banding. Y chromosome microdeletions were detected by multiplex polymerase chain reaction assays.

Results Cytogenetic analysis revealed Robertsonian translocation 45,XY,der(14;21)(q10;q10) in a male with severe oligoasthenoteratozoospermia (SOAT) after three subsequent ICSI treatments were unsuccessful. The second case involved a Robertsonian translocation 45,XY, der(13,14)(q10;q10) with SOAT detected in a male after one pregnancy loss. Third case involved a Robertsonian translocation 45,XY,der(13,14)(q10;q10) with SOAT.

Conclusion This case series emphasize the necessity of cytogenetic analysis of couples with primary infertility and recurrent miscarriages before any assisted reproductive technology is performed. For couples in whom one or more

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partners have a translocation, prenatal genetic diagnosis/preimplantation genetic diagnosis is recommended.

Keywords Infertility · Cytogenetics · Robertsonian translocation · Intracytoplasmic sperm injection

Introduction

Robertsonian translocations are the most common structural chromosomal abnormalities observed in humans with an incidence of 1.2 per 1000 live births. The great majority of Robertsonian translocations involve two non-homologous chromosomes and occur between chromosomes 13 and 14 or chromosomes 14 and 21 with an estimated frequency of 0.97 and 0.20, respectively [1]. Most couples where one partner is a Robertsonian translocation carrier do not have fertility problems. In carrier men, 10–15% of the sperm may be chromosomally unbalanced, and in addition, it is thought that the translocation chromosome may block the creation of sperm [2]. As even men with low sperm counts produce some sperm, it should still be possible to achieve pregnancy using intracytoplasmic sperm injection (ICSI). The present study outlines three cases of a Robertsonian translocation and the consequences for the initiation of pregnancy by ICSI.

Case Presentation

Case 1

A couple with a 13-year history of infertility presented to Institute of Reproductive Medicine and Women's Health. The ages of the male and his female partner were 41 and 38 years, respectively. Semen analysis according to World Health Organisation guidelines showed 8×10^5 sperm cells/mL, a progressive motility of 22% and normal morphology in 2%. Serum hormone concentration of free thyroxine (FT4), testosterone (T), thyroid-stimulating hormone (TSH), prolactin (PRL) and follicle-stimulating hormones (FSH) were 12.9 Pmol/L, 8.80 nmol/L, 2.51 mIU/L, 29.7 ng/mL and 4.89 mIU/mL, respectively. The infertile couple underwent ICSI because of severe oligoasthenoteratozoospermia (SOAT). ICSI was performed three times with over a 2-year period with 6–8 oocytes injected, 6 oocytes fertilized normally and three to four embryos transferred at the 8-cell stage per treatment cycle. Three ICSI procedures did not result in a clinical pregnancy. To know the cause of the failure, the couple was referred to Centre for Genetic Studies and Research for chromosomal analysis. For a conventional cytogenetic study, 5 mL peripheral blood (PB) from the couple was collected into a heparinized vacutainer. Lymphocyte cultures were initiated according to standard protocol [3]. The karyotype of the couple was prepared by G-banding technique [4]. Images of well-banded metaphases were analyzed by CytoVision software (Leica, Germany) at 400–550 band resolution. Karyotyping of 30 metaphases was performed and analyzed. Karyotyping of each couple was carried out according to the International System for Human Cytogenetics Nomenclature (ISCN) 2009 [5]. After three ICSI treatments, chromosome analysis of PB from the male and female showed 45,XY,der(14;21)(q10;q10) and 46,XX, respectively. Screening for Y chromosome microdeletion was performed on a routine basis for male infertility using a standard protocol [6]. DNA was extracted from the given sample and used to perform two multiplex polymerase chain reaction (PCR) tests. These indicate the presence or absence of 2 particular STS sequences in each of the three different azoospermic factors (AZFa, AZFb and AZFc) of the Y chromosome. Results indicate the presence of all the three regions of the Y chromosome tested. The couple underwent pre-in vitro fertilization (IVF) genetic counseling especially on the aspect of low success rate using the husband's own sperm. So they were recommended to use donor sperm for her next cycle to achieve pregnancy. They did not prefer to go ahead using donor sperm.

When the family history was taken and the pedigree (Fig. 1) was analyzed, it was found that he was the only

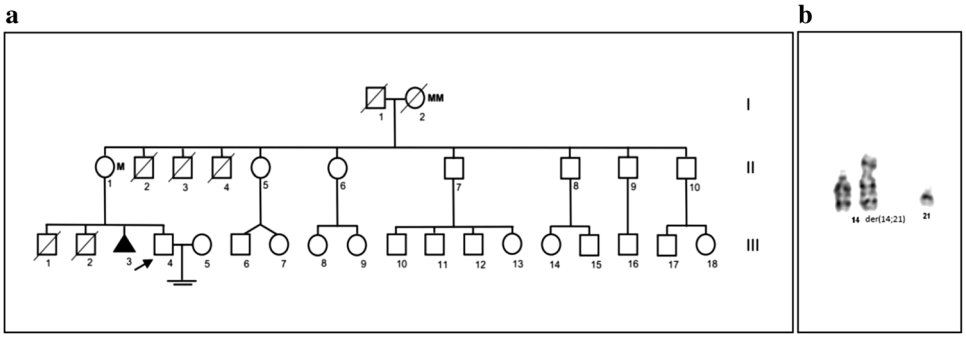

Fig. 1 **a** The pedigree analysis of the family (case 1). **b** G-banded partial karyotyping of the proband showing der(14;21)

child of his parents, and there was a history of 2 intrauterine death (IUD) and 1 miscarriage to his mother (M) (Fig. 1; III.1–3). The chromosomal analysis of both the parents was also done. Cytogenetic results of M showed the same 14/21 translocation. She had six siblings, and none of them had a history of any infertility or fetal deaths. Patient's maternal grandmother (MM) (Fig. 1; I.2) had a history of 3 IUD. Chromosomal analysis of the maternal grandparents could not be studied as none of them were alive.

Case 2

A 29-year-old male presented with his wife for secondary infertility, who conceived within 4 months after marriage and pregnancy ended with spontaneous abortion at 8 weeks of gestation (Fig. 2; IV.3). Male semen analysis shows 2.5×10^5 sperm cells/mL, a progressive motility of 60 and 4% normal morphology. Serum hormone concentration of FT4, T, TSH, PRL and FSH was 11.1 Pmol/L, 6.02 nmol/L, 2.30 mIU/L, 10.5 ng/mL and 2.39 mIU/mL, respectively. ICSI was planned, and pregnancy was established by ICSI because of SOAT. Seven oocytes were injected, and 5 were normally fertilized. Three embryos were transferred at the 4-cell stage, and no pregnancy resulted. Due to that, the couple was referred for chromosomal analysis. The karyotype results showed balanced Robertsonian translocation 45,XY,der(13;14)(q10;q10) in male and normal karyotype 46,XX in female. Y chromosome microdeletion result on the three different AZF (AZFa, AZFb and AZFc) regions indicated the presence of all the three regions of the Y chromosome tested. The couple had pre-IVF counseling who later on had agreed to use donor sperm. Following ICSI of four oocytes, three embryos were transferred on day 2. A pregnancy test was taken 2 weeks later, and the result was positive. Gestation is still ongoing. The translocation was found to be de novo as the parental karyotypes were normal (Fig. 2).

Case 3

A couple with a 3-year history of primary infertility presented for treatment. Infertility was treated with ICSI because of SOAT in the male showing 5×10^5 sperm cells/mL, a progressive motility of 8 and 2% normal morphology. Serum hormone concentration of FT4, T, TSH, PRL and FSH was 16.4 Pmol/L, 8.13 nmol/L, 2.68 mIU/L, 12.2 ng/mL and 9.20 mIU/mL, respectively. Chromosome analysis revealed a normal karyotype 46,XX in the female and a Robertsonian translocation 45,XY,der(13;14)(q10;q10) in the male. Y chromosome microdeletion result on the three different AZF (AZFa, AZFb and AZFc) regions indicated the presence of all the three regions of the Y chromosome tested. The Couple was counselled by infertility specialist and medical geneticist about the risk of reproductive outcomes using husband sperm. After extensive counseling, the couple still opted to use the husband's sperm only for ICSI procedure. Informed consent was obtained, and ICSI was performed. Six to eight oocytes were injected, four to six oocytes fertilized normally, and two embryos transferred per treatment cycle. Serum β-hCG concentration was measured 12 days after transfer. Clinical pregnancy was defined as the presence of a fetal heartbeat on vaginal ultrasound at 4–6 weeks after

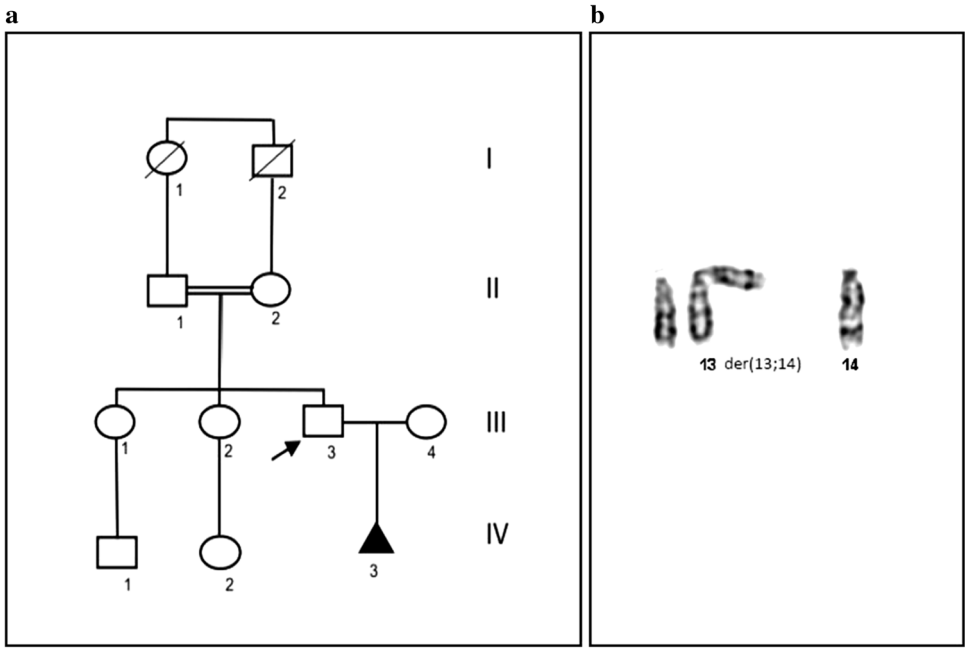

Fig. 2 **a** The pedigree analysis of the family (case 2). **b** G-banded partial karyotyping of the proband showing der(13;14)

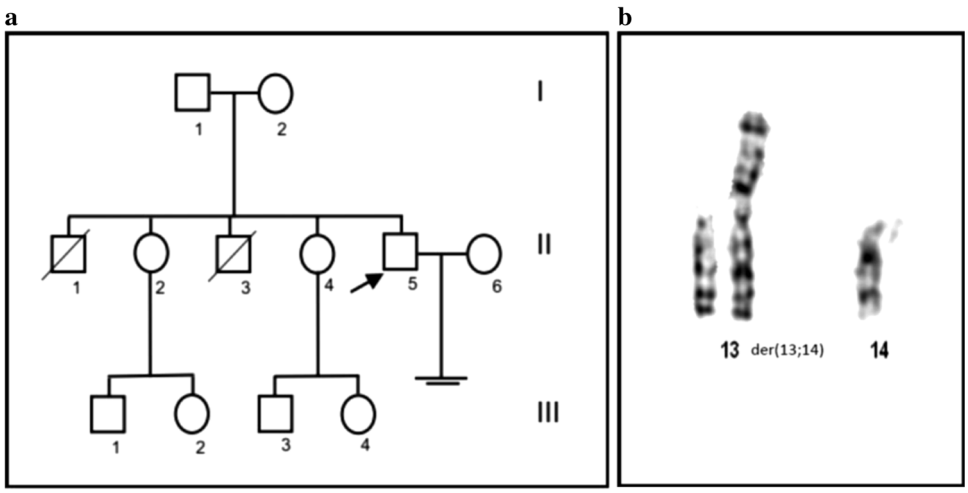

Fig. 3 **a** The pedigree analysis of the family (case 3). **b** G-banded partial karyotyping of the proband showing der(13;14)

embryo transfer. In order to rule out chromosomal abnormalities in the fetus, the couple was recommended to undergo first-trimester prenatal genetic testing using chorionic villus sampling at 8 weeks of gestation or amniotic fluid at 16 weeks of gestation. The patient opted genetic testing using amniotic fluid: 15–30 mL amniotic fluid was aseptically collected via transabdominal amniocentesis. The fetal cells obtained in the fluid were cultured, and a chromosome analysis was performed. Fetal karyotype was 45,XY,der(13;14)(q10;q10) which is inherited from father (Fig. 3). A healthy male was born at 36 weeks of gestation with birth weight 2.870 kg. Parental karyotype of case 3 was performed, and his mother was found to be the carrier of Robertsonian translocation 13/14.

Discussion

Chromosomal abnormalities in infertile men have been found within the range of 2.2–15.2% compared to the normal population. A total of 3.7% of these involve the sex chromosomes and 1.3% involves autosomes. Robertsonian translocations were found in 0.9% of the oligozoospermic and 0.3% of the azoospermic patients [7]. In the present study, all the male partners exhibited a balanced Robertsonian translocation, with 45 chromosomes. The observed translocation in case 2 was de novo, whereas in case 1 and case 3, they were transmitted from carrier parents. In general, carriers of Robertsonian translocations have a normal phenotype. However, they may have spermatogenesis alterations expressed by oligozoospermia or azoospermia, and they may be affected by reproductive failure owing to imbalances in chromosome meiotic segregation [8].

A trivalent configuration in metaphase I of meiosis could have resulted in a monosomic or trisomic condition. During pachytene stage in meiosis I, homologous pairing of Robertsonian translocation is achieved by the formation of a trivalent structure. If an alternate segregation occurs, then all gametes are potentially viable with balanced chromosomes. Nevertheless, adjacent segregations result in gametes, which are nullisomic or disomic for one of the chromosomes involved in the rearrangement and consequently a zygote with trisomy or monosomy for one of the involved chromosomes. Zygotes with monosomy are not compatible with life and most translocated trisomy concepti are expected to result in early or first-trimester losses [9]. The couple (case 1) had gone through an extensive series of fertility treatments and had attempted assisted fertilization. The cytogenetic result of the couple makes it evident that repeated ICSI failed because of an embryo with either a monosomy or trisomy, which is non-viable.

Studies have indicated that deletions on the long arm of the Y chromosome involving a particular and consistent segment might lead to azoospermia and sometimes to severe oligospermia [10]. In our all the three cases, Y chromosome microdeletion results indicates the presence of all the three AZF regions.

During genetic counseling, other family members at risk of being translocation carriers were advised to undergo chromosomal analysis. Although the situation was explained in detail, the family members refused to participate in the cytogenetic study.

A treatment option for a carrier of Robertsonian translocation includes expectant management, donor sperm and IVF with preimplantation genetic diagnosis (PGD). Couples must consider that each treatment option has associated costs, risks, and success rates. Whereas expectant management has the lowest treatment-related costs, there is a higher risk of miscarriage, reaching 50% for most translocations [11]. Sperm donation can eliminate the risk associated with the translocation, but has higher costs of treatment and does not maintain a genetic link to both parents, which is extremely important to some couples.

That is the reason our first case did not prefer to go ahead using donor sperm, but the second case opted donor sperm.

Prenatal diagnosis has been available to carriers of Robertsonian translocations for many years. However, termination of pregnancy in the event of translocation trisomy is not an acceptable option for some couples and for carriers of these translocations; there is growing interest in PGD in conjunction with assisted conception using IVF or ICSI. Screening the embryo prior to implantation assures that only those embryos with appropriate numbers of chromosomes are implanted. For couples in whom one or more partners have a translocation, PGD reduced the frequency of spontaneous abortions to 12.5% and increased the live birth rate to more than 80% [12].

Conclusion

Increasing evidence of a genetic involvement in infertility and the biological plausibility of transmitting genetic disorders to the offspring mandates a genetic evaluation of the couple with clinical examination. This case series emphasizes the necessity of cytogenetic analysis of couples with primary infertility and recurrent miscarriages before any ART procedure is performed in order to exclude the probable presence of any chromosomal rearrangements which are transmitted for many generations without detection in healthy individuals with a normal phenotype.

Authors' contributions BK conceived and designed the research article. He performed genetic evaluation and clinical review of the family. He also provided genetic counseling to the patients and their families. Sample collection, processing and genetic analysis were done by SS. BK and SS wrote the draft of the manuscript. SK and TRV referred the families for genetic studies and provided medical management for the patients. All authors participated in revising the manuscript for publication.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Consent for Publication Written informed consent was obtained from the patient's/legal guardian(s) for publication of this case report.

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