

## CASE REPORT

# Pre- and Postnatal Genetic Evaluation reduce the Reproductive Risk of nonhomologous Robertsonian Translocation Carrier Couple

<sup>1</sup>Puspal De, <sup>2</sup>Sudipa Chakravarty, <sup>3</sup>Amit Chakravarty

## ABSTRACT

**Purpose:** Spontaneous abortion has been reported in 15 to 20% of all diagnosed pregnancies. The most common cause of spontaneous abortion is chromosomal abnormalities of the embryo. Robertsonian translocation (RT) is one of the major chromosomal rearrangements with a prevalence rate of 0.1% of the general population and 1% of the infertile population. Robertsonian translocation carriers, especially 21-14, are the most common balanced rearrangement among the carrier couples with a history of spontaneous abortion.

**Materials and methods:** Cytogenetic analysis was carried out based on phytohemagglutinin-stimulated peripheral blood lymphocyte cultures and without phytohemagglutinin-stimulated amniocyte culture. Lymphocyte and amniocyte culturing and GTG banding were performed following standard protocols as described by the Association of genetic technologists (AGT) Cytogenetics Laboratory Manual.

**Results:** Cytogenetic evaluation of both the partners and the child revealed that the child had translocated Down's syndrome and the mother was a carrier of balanced RT of 14q;21q. Amniocentesis of the next pregnancy and detection of chromosomal abnormality in the fetus was done by fluorescence *in situ* hybridization (FISH) analysis of the amniotic cells with 13,18,21,X,Y probe mix found normal chromosomal constituent in the fetus.

**Conclusion:** The present study shows that genetic counseling, cytogenetic evaluation, prenatal diagnosis by amniocentesis, and FISH together help couples with nonhomologous RT and history with syndromic child and repeated abortions to get normal offspring.

**Keywords:** Balanced chromosomal rearrangement, Chromosomal abnormality, Genetic counseling, Robertsonian translocation, Spontaneous abortion.

**How to cite this article:** De P, Chakravarty S, Chakravarty A. Pre- and Postnatal Genetic Evaluation reduce the Reproductive

Risk of nonhomologous Robertsonian Translocation Carrier Couple. *Int J Infertil Fetal Med* 2017;8(1):36-40.

**Source of support:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. All the research work was done by the affiliated institution's funding.

**Conflict of interest:** None

**Date of received:** 5-9-2016

**Date of acceptance:** 10-10-2016

**Date of publication:** May 2017

## INTRODUCTION

Generally, the couples planning for their first pregnancy remain unaware of any reproductive problems. Every sixth couple experience difficulties in pregnancy outcome worldwide.<sup>1</sup> Around 15 to 20% of all pregnancies in humans end in spontaneous abortions, and the risk in future pregnancy reached up to 33% in patients with recurrent miscarriage.<sup>2</sup> So, clinicians feel that patients deserve evaluation even after two recurrent miscarriages, though historically defined recurrent spontaneous abortion was three or more consecutive pregnancy loss before 20 to 22 weeks of gestation.<sup>3</sup>

According to studies, the prevalence of chromosomal aberration is approximately 8% in cases suffering reproductive problems, and the frequency rises as high as 50% in case of spontaneous recurrent abortion.<sup>4</sup> The cause is unknown in many instances, but a parental chromosomal abnormality has been taken as one of the possible causes for recurrent miscarriage within the first 3 months of pregnancy.

In the advancement of modern cytogenetics, prenatal cytogenetic testing is very common for the detection of chromosomal abnormality in the fetus. It helps to determine prenatally whether the fetus has certain hereditary or spontaneous genetic disorders. Some of these noninvasive tests, such as ultrasonography and certain blood tests are often part of routine prenatal care. These tests are safe and sometimes help to determine whether more invasive prenatal genetic tests (chorionic villus sampling, amniocentesis, and percutaneous umbilical blood sampling) are needed. Usually, more invasive tests are done when

<sup>1</sup>Assistant Professor, <sup>2</sup>Vice Principal, <sup>3</sup>Director

<sup>1</sup>Department of Genetics, Institute of Genetic Medicine and Genomic Science, Kolkata, West Bengal, India

<sup>2</sup>Department of Genetics, Institute of Genetic Engineering Kolkata, West Bengal, India

<sup>3</sup>Department of Biotechnology, Institute of Genetic Engineering Kolkata, West Bengal, India

**Corresponding Author:** Puspal De, Assistant Professor Department of Genetics, Institute of Genetic Medicine and Genomic Science, Kolkata, West Bengal, India, Phone: +9103325260051, e-mail: puspal.dey@gmail.com

couples have an increased risk of having cytogenetically unbalanced zygote.

In the present observation, we studied a couple with history of an abnormal child and three repeated spontaneous abortions. Cytogenetic evaluation of both the partners and the child revealed that the child has translocated Down's syndrome and the mother was carrier of balanced Robertsonian translocation (RT) of 14q;21q. Amniocentesis of the next pregnancy at 16 weeks was done and fluorescence *in situ* hybridization (FISH) analysis of the amniotic cells with 13,18,21,X,Y probe mix to find out the presence of any chromosomal abnormality in the fetus. The present study shows that genetic counseling, cytogenetic evaluation, prenatal diagnosis by amniocentesis, and FISH together help couples with nonhomologous RT and history with syndromic child and repeated abortions to get normal offspring.

## CASE REPORT

A nonconsanguineous couple, 30 years male and 27 years female, with their 5-year-old mentally retarded child came to our cytogenetic laboratory for the evaluation of genetic disorder of their child. The child had dysmorphic faces with delayed development, such as delayed neck holding and walking. They had a history of three repeated abortions in the past 2 years. The first abortion was in the fourth month from pregnancy and the second was at third month of pregnancy. The third was a missed abortion from a 2-month pregnancy. There were no such histories of repeated abortions in any other family member. The cytogenetic evaluation of the fetuses after abortion was not done. The biochemical and hormonal profile of the female partner was in normal range and the ultrasonography reports did not show any structural abnormality in the internal organs. The semen analysis profile of the male partner was found to be normal. In the present status, they wanted to know the possibilities of getting normal child in future.

## MATERIALS AND METHODS

### Cytogenetic Analysis

In our laboratory, we first collected 2 mL of peripheral blood from both partners and the child in heparinized tubes and harvested white blood cells for karyotyping.<sup>5,6</sup> Twenty five metaphases were analyzed and the karyotype was interpreted using the Applied Imaging software (Cytovision 3.92). The chromosomes were identified and classified according to the guidelines by the International System for Human Cytogenetic Nomenclature (ISCN, 1995). The chromosome profile (loss and gain analysis) of the abnormal chromosome was done with the help of the same software.

### Prenatal Diagnosis

In this procedure, a sample of the fluid that surrounds the fetus (amniotic fluid) is removed. Amniocentesis is usually done at 15 weeks of pregnancy or later; in our case, the amniocentesis was done at 16 weeks of gestation. Before the procedure, ultrasonography was done to evaluate the heart of the fetus, to confirm the length of the pregnancy, to locate the placenta and amniotic fluid, and to determine how many fetuses were present. The fluid contains cells that have been shed by the fetus. These cells are grown in a laboratory in amniomax with fetal bovine serum. The harvested cells were selected for FISH. The five probe mix (13,18,21,X,Y) for interphase nucleus (Vysis AneuVysion probe) was used to determine the abnormality in the fetus.<sup>7,8</sup> Fifty cells were observed and interpreted.

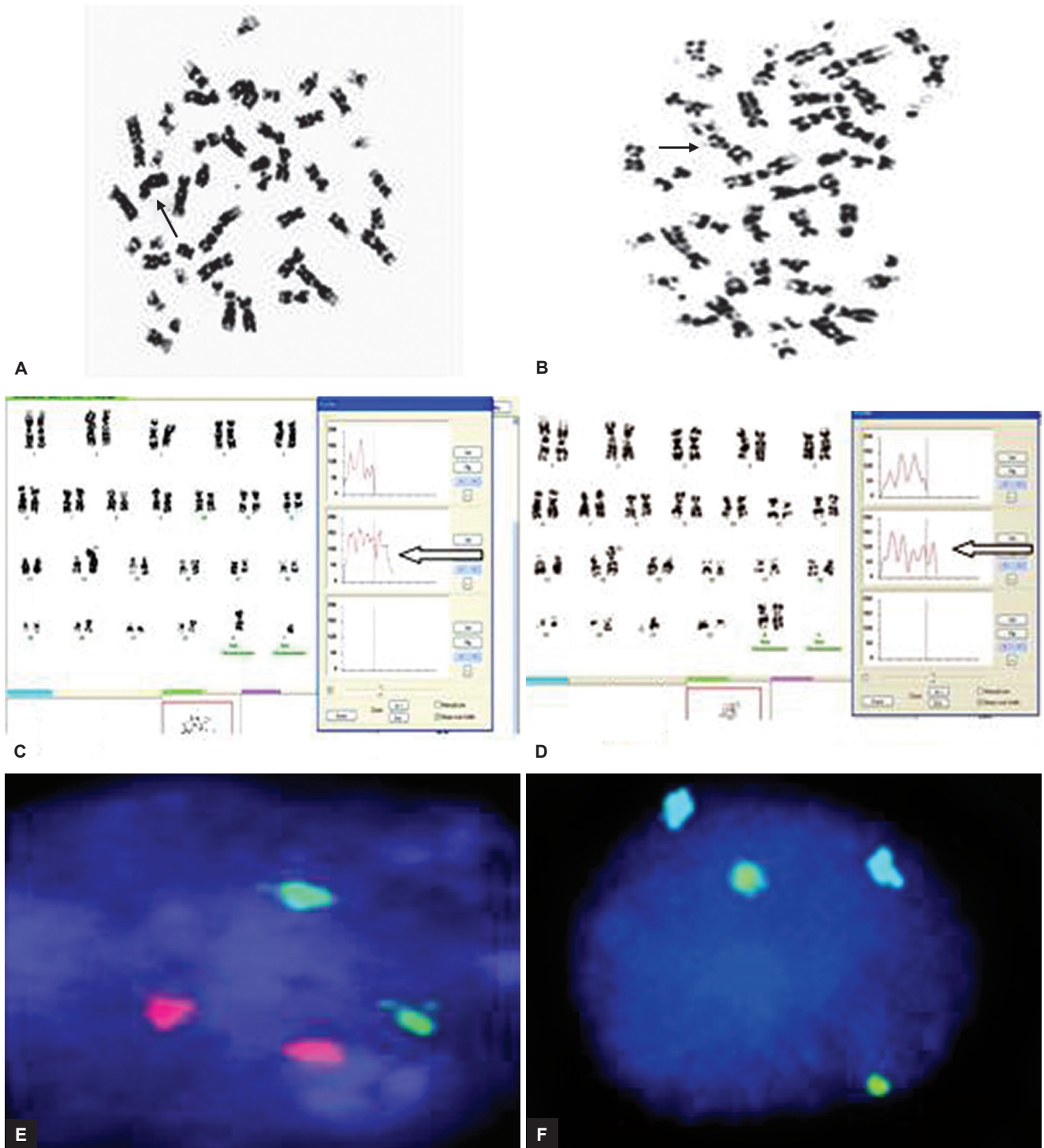
## RESULTS

Chromosomal analysis revealed an abnormality in the female partner with 45, XX, robs (14; 21) chromosomal constitution. The female karyotype revealed 45 chromosomes with missing chromosomes of 14 and 21, along with an additional chromosome that did not fit into any group of the chromosomes in the karyotype. The banding pattern of the short and long arms of the additional chromosome was similar to chromosome 14 and 21, thereby indicating the presence of a nonhomologous RT. Thus, karyotype was confirmed as 45, XX, rob (14q;21q) as depicted in Figures 1A and C. Chromosomal analysis of the male partner showed normal 46,XY karyotype. Chromosomal analysis revealed 46,XY, rob(14q;21q) in the child. The karyotype of the child also showed 46 chromosomes, with missing chromosome of 14 along with an additional chromosome whose banding pattern was similar to the nontranslocated chromosome present in his mother as well as chromosome 14 and 21. So, the child had translocated Down's syndrome (Figs 1B and D).

The FISH result of amniocytes in interphase nuclei shows presence of two green and two orange signals in first hybridization locus-specific probe and two aqua and two green signals in second hybridization chromosome enumeration probe. Two green and two orange signals confirm the presence of one homologous pair of chromosome no 13 and one homologous pair of chromosome no 21 in first hybridization, and two aqua signals and two green signals confirm the presence of one homologous pair of chromosome no 18 and one pair of sex chromosome in second hybridization. The test proves the presence of normal numerical count of specified chromosome only (Figs 1E and F).

## DISCUSSION

The potential factors, such as genetic, anatomic, endocrine, infectious, and immunological factor influenced



**Figs 1A to F:** (A) Metaphase spread of affected child. Arrow indicates the translocated t(14q,21q) chromosome; (B) metaphase spread of the female individual with balance translocation. Arrow indicates the translocated t(14q,21q) chromosome; (C) karyotype with chromosome profile of affected child. Arrow indicates the gain of materials in chromosome 14q in respect of normal chromosome 14; (D) karyotype with chromosome profile of the female individual with balance translocation. Arrow indicates the gain of materials in chromosome 14q in respect of normal chromosome; (E) two green and two orange signals confirm the presence of one homologous pair of chromosome no 13 and one homologous pair of chromosome no 21 in first hybridization; and (F) two aqua signals and two green signals confirm the presence of one homologous pair of chromosome no 18 and one pair of sex chromosome in second hybridization

recurrent spontaneous abortion in varying degrees. But the proper etiology is often unclear. So, it is necessary to consider each of these contributing factors in repeated spontaneous abortion. Cytogenetic analysis of the couple and if possible of the abortos was the

preferential area to the clinician for the detection of chromosomal makeup.<sup>3</sup>

Chromosomal anomalies are mainly of two types: numerical and structural. Both the anomalies affect approximately 50% cases of recurrent spontaneous abortion.



Numerical anomalies like trisomy, X chromosome monosomy, and polyploidy especially triploidy are the main factors and they altogether cause 95% chromosomal abnormality in recurrent miscarriage cases.<sup>9</sup> On the contrary, structural anomalies like chromosomal translocation are observed in highest percentage. Half of the structural chromosomal abnormalities may be inherited from parent carrying a balanced translocation, which increases the risk of having children with abnormal cytogenetic makeup.<sup>10-12</sup> At prenatal diagnosis, this risk percentage is considerably higher than the theoretical risk because most cytogenetically abnormal embryos would have failed to establish a pregnancy. So, it is expected that cytogenetic screening of embryo before birth may increase the chance of successful pregnancy. Translocation are of two types: Reciprocal and Robertsonian. In reciprocal translocation, the exchange of chromatin blocks between chromosomes is involved in abnormal rearrangements. But the exchange does not affect the gene action. In RT, two acrocentric chromosomes are fused at their centromeric region and lose their short arm, resulting in balanced karyotype, which has only 45 chromosomes including the translocated one. The incidence in newborn is 1.25/1,000.<sup>13</sup> The person with it is called carrier. As carriers are healthy and have normal lifespan, it is difficult to judge their unusual chromosomal rearrangements. In fact, this translocation can be passed down in families for many generation without anyone discovering it. It is noticed only after a baby is born with chromosomal disorder. Only a minor percentage of babies have one parent as carrier, either the translocation produced *de novo* at gametogenesis. These *de novo* unbalanced gametes with RT have an increased risk of pregnancy loss. According to studies, babies with trisomy 14, 15, or 22 usually miscarry in the first 12 weeks instead of trisomy 13 or 21. So, it has been suggested that some carriers are particularly prone to pregnancy loss.<sup>14</sup>

In the present study, the couple under observation showed normal anatomical, endocrinal, and immunological background. The previous blood reports for hormonal and immunological profile of the couple, ultrasonography report of the female, and semen analysis report of the male partner supported the above statement. Not only that, they do not have any history of infections in pre, post, or during pregnancy. Cytogenetic evaluation revealed normal chromosome profile in male partner with karyotype of 46,XY but RT carrier in female partner with karyotype of 45,XX,rob(14q:21q). The cytogenetic evaluation of the child showed translocated Down's syndrome with karyotype of 46,XY,rob(14q:21q). The chromosomal analysis confirmed that the translocation was inherited from the carrier mother to child. The couple had a history of three repeated abortions without any chromosome analysis report of aborted fetuses. We could not have

any confirmatory idea about these repeated abortions. We think that the translocation derivative chromosome during segregation may cause nonviable pregnancy in these cases.

As one of the parents is normal and other is balance translocation carrier, the possibility of getting normal offspring is 50%. But the present couple had a history of three repeated abortions and a child with translocated Down syndrome. In this status, it is important to know the chromosomal constituent of their present fetus. For this reason, we performed chromosomal analysis of the fetus by amniocentesis. The amniocytes are selected for FISH analysis with 13,18,21, X,Y probe mix, and the result showed normal chromosomal constituent without any numerical as well as structural chromosomal abnormality of the fetus, which suggested carrying the pregnancy till birth.

## CONCLUSION

Most couples where one of the partner is a RT carrier usually do not face any major fertility problems, but in case if it appears to be so, it is possible to get help through proper genetic counseling, cytogenetic evaluation, prenatal diagnosis, or using assisted reproduction. In carrier men, 10 to 15% of the sperms may be chromosomally unbalanced, and in addition, it is thought that the translocation chromosome may block the spermatogenesis. As even men with low sperm counts do produce some sperm, it should still be possible to achieve pregnancy using intracytoplasmic sperm injection. In carrier female, prenatal diagnosis or in countries where this is permitted, preimplantation genetic diagnosis may also offer to ensure that implanted embryos are chromosomally balanced. In case there is more than one affected pregnancy or had repeated miscarriages caused by unbalanced chromosomes with or without affected child, the above-mentioned are the options that should be considered.

## ACKNOWLEDGMENTS

Puspal De acknowledges the Institute of Genetic Engineering (IGE) for funding and affiliation. Authors would like to thank laboratory members and associated persons of IGE for their enthusiastic participation, Dr Madhumita J Mukhopadhyay for her unending cooperation, enthusiasm, and valuable suggestion and also Mr. Dipak Kundu for his computational assistance.

## REFERENCES

1. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. *Fertil Steril* 2010 Jan;93(1):1-12.

2. Middeldorp S, Goddijn M. Recurrent miscarriage and thrombophilia. *Ned Tijdschr Geneesk* 2006 Jan 28;150(4):189-193.
3. Dubey S, Chowdhury MR, Prahlad B, Verma IC. Cytogenetic causes for recurrent spontaneous abortions an experience of 742 couples (1484 cases). *Indian J Hum Genet* 2005 Jul;11(2):94-98.
4. Düzcan F, Atmaca M, Cetin GO, Bagci H. Cytogenetic studies in patients with reproductive failure. *Acta Obstet Gynecol Scand* 2003 Jan;82(1):53-56.
5. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res* 1960 Sep;20:613-616.
6. Seabright M. A rapid banding technique for human chromosomes. *Lancet* 1971 Oct 30;2(7731):971-972.
7. Verp MS, Gerbie AB. Amniocentesis for prenatal diagnosis. *Clin Obstet Gynecol* 1981;24:1007.
8. Shaffer LG, Bui TH. Molecular cytogenetic and rapid aneuploidy detection methods in prenatal diagnosis. *Am J Med Genet C Semin Med Genet* 2007 Feb 15;145C(1):87-98.
9. Ananthapur V, Avvari S, Tella S, Nallari P, Akka J. A Robertsonian translocation rob (14;15) (q10;q10) in a patient with recurrent abortions: a case report. *J Reprod Infertil* 2010 Oct;11(3):197-200.
10. Tsui KM, Yu WL, Lo FM, Lam TS. A cytogenetic study of 514 Chinese couples with recurrent spontaneous abortion. *Chin Med J (Engl)* 1996 Aug;109(8):635-638.
11. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol* 2000 Oct;14(5):839-854.
12. Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil Steril* 2000 Feb;73(2):300-304.
13. Gardner RJM, Sutherland GR. Chromosome abnormalities and genetic counseling. 2nd ed. Oxford: Oxford University Press; 1996.
14. Scriven PA, Flinter FA, Braude PR, Ogilvie CM. Robertsonian translocations – reproductive risks and indications for preimplantation genetic diagnosis. *Hum Reprod* 2001 Nov;16(11):2267-2273.