Live birth from a patient with a three-way balanced translocation t(5;8;12)

A Ramdeo, MB BCh, MRCOG; **K Naidoo**, N Dip Med Tech; **T Ernest**, BMedSci Hons; **K Pluta**, MSc, PhD

C.A.R.E. Clinic (Centre for Assisted Reproduction and Endocrinology), Westville, Durban, South Africa

Corresponding author: *A Ramdeo (pcrlab.careclinic@gmail.com)*

Background. Three-way balanced translocations are unusual and can lead to infertility as well as abnormal embryos. In this case report, we describe a couple who experienced repeated miscarriages as a result of the male partner being a carrier of a three-way translocation t(5;8;12).
Objectives. Array comparative genomic hybridisation (array-CGH) was used to screen embryos for chromosome imbalances.
Methods. Embryo biopsy, preimplantation genetic diagnosis using a 24sure+ kit to detect translocations in embryos.
Results. Of 10 embryos tested, 2 were found to have an unbalanced translocation, 4 were aneuploid, 2 failed to amplify and 2 were euploid. Transfer of the two euploid embryos resulted in a singleton pregnancy and subsequent birth of a baby.
Conclusion. Array-CGH in conjunction with a 24sure+ kit should be used as a routine screening method for embryos of balanced translocation carriers, as it can decrease the time to pregnancy and prevent repeated miscarriages.

S Afr J Obstet Gynaecol 2015;21(1):18-20. DOI:10.7196/SAJOG.922

Chromosome translocation, also known as chromosome rearrangement, is an abnormality caused by exchange of parts between non-homologous chromosomes. Translocations can be balanced (when exchange of material occurs with no genetic information extra or missing) or unbalanced (where the exchange of chromosome material is unequal, resulting in extra or missing genes). Carriers of balanced translocations resemble a normal phenotype, while those who have unbalanced translocations may represent abnormal phenotype and functional disability.[1]

Further subdivision of translocation types can be made according to the exchange of chromosomal material and are classified as: (*i*) reciprocal, when segments from two different chromosomes have been exchanged; or (*ii*) robertsonian, in five acrocentric chromosomes (13, 14, 15, 21 and 22), where long arms fuse to form a single chromosome with a single centromere. The short arms also join to form a reciprocal product, which typically contains non-essential genes and is usually lost within a few cell divisions. Translocations subdivided in groups regarding the number of chromosomes involved are: (*i*) one-way translocation with one-way transfer of a chromosomal segment to another chromosome; (*ii*) two-way translocation with two-way transfer of a chromosomal segment to another chromosome; and (*iii*) the most common group of complex chromosomal rearrangements (CCRs), with three or more chromosomes involved in the exchange.[2] This type of rearrangement takes place during meiosis I and involves formation of a hexavalent configuration that allows the full synapsis of homologous segments.

Chromosomal translocations can be formed *de novo* or can be inherited through so-called 'familial transmission'.

Carriers of chromosomal translocations are known to have reduced fertility. In these patients, loss of fertility is mainly caused by the high prevalence of gametes that have lost or gained chromosome material as a result of the rearrangement of the derivative chromosomes or a generation of a recombinant chromosome.

It has been reported that for translocation carriers, *in vitro* fertilisation combined with preimplantation genetic diagnosis (PGD) is a faster method of conceiving a child than natural conception.[3]

Case report

This report describes a couple who experienced five spontaneous abortions and one elective abortion due to an abnormal fetus. The couple had managed to have one spontaneous pregnancy resulting in a healthy child before experiencing recurrent miscarriages.

Peripheral blood analysis of the male partner revealed a modal number of 46, karyotype 46, XY, t(5;8;12)(5pter-5q33::8q24.1-8qter; 8pter-8q34.1::12p13-12pter;12qter-12p13::5q55-5qter). This meant that the male partner was a carrier of a balanced translocation involving chromosomes 5, 8 and 12. The mother of the male patient had the same balanced chromosome rearrangement, while his sister had an unbalanced form of this translocation with only derivative 5 and derivative 12 present, and not derivative 8, resulting in mental retardation. Two cousins of the same family were diagnosed with an unbalanced karyotype resulting in mental retardation. The same unbalanced karyotype was present in the fetus of the investigated couple, which had prompted them to undergo an elective abortion previously.

Semen analysis revealed a normal pH, volume, viscosity, liquefaction, total count, progression motility and morphology. The female partner successfully responded to hormonal stimulation and 15 oocytes were collected, including 7 MII (metaphase II), 2 MI (metaphase I) and 6 GV (germinal vesicle). Of these, ten oocytes were injected with sperm and all fertilised. Ten of the fertilised oocytes resembled normal phenotype and had the following grades: 3 were hatched blastocysts, 4 were hatching blastocysts and 3 were at the blastocele stage. All ten embryos were subjected to array comparative genomic hybridisation (array CGH). The trophectoderm cells were lysed, and genomic DNA and negative control were amplified using the SurePlex DNA Amplification System (BlueGnome, UK) according to the manufacturer's instruc-

tions. DNA samples and references were then labelled and hybridised using arrays designed for translocations (24sure+, BlueGnome, UK). Slides were washed, scanned with InnoScan710 AL (INNOPSYS, France) and processed using Bluefuse Multi Software (BlueGnome, UK).

Of the 10 embryos subjected to array CGH, 2 failed to amplify, 2 were euploid, 4 were aneuploid and 2 had unbalanced translocations.

Embryos 2 and 8, despite being graded as morphologically 'good embryos' showed an unbalanced complement of the translocation. In embryo 2, a partial loss of the long arm of chromosome 5 from 5q33.3-5q35.3 (24,951,204 bp), a partial loss of the long arm of chromosome 8 from 8q24.1-8q24.3 (23,417,117 bp) and a partial gain of the short arm of chromosome 12 spanning 12p13.1-12p13.3 (5,727,495 bp) was observed (Fig. 1). In embryo 8, a partial gain of the long arm of chromosome 5 from 5q33.1-5q35.3 (29,602,079 bp) and a partial loss of the short arm of chromosome 12 spanning 12p13.31-12p13.33 (6,129,127 bp) was observed (Fig. 1).

On the basis of the array CGH results, two euploid embryos were transferred on day 6 post oocyte retrieval resulting in a singleton pregnancy. A normal healthy baby was born at 35 weeks by caesarean section. Cytogenetic analysis revealed a normal karyotype of the baby.

Discussion

This report describes a selection process of embryos originating from a balanced three-way reciprocal translocation carrier using array CGH. It proves that phenotypically normal embryos originating from a chromosomal translocation carrier may be carrying chromosomal imbalances.[4]

The male patient was diagnosed with a balanced three-way reciprocal translocation after his female partner suffered repeated miscarriages and an elective abortion due to the fact that the embryo was affected with an unbalanced translocation. It has been reported that balanced translocation carriers have an increased risk of abnormal conceptions and miscarriages,[5] caused by either malsegregation of the derivative chromosomes or the generation of a recombinant chromosome.[2] The mother and sister of our male patient had been diagnosed with balanced and unbalanced chromosomal translocations, respectively, highlighting that this was a familial chromosomal rearrangement.[6] The same type of reciprocal balanced three-way translocation involving chromosomes 5, 8 and 12 was previously reported in an Indian family from KwaZulu-Natal.[7] Several members across three generations of this family were affected. There were 13 adults with a balanced karyotype, 3 children with an unbalanced karyotype presenting with severe intellectual disability and dysmorphic characteristics, and a history of 3 miscarriages and 4 neonatal deaths.[7]

Basic semen analysis of the male patient and the fact that he had previously fathered a healthy child indicated that his fertility was not affected by his chromosomal translocation, contrary to previous literature reports stating that male balanced translocations carriers are prone to sterility.[8,9]

Array CGH was utilised to assess chromosomal imbalances in the embryos. This method allows screening of all 23 chromosomes simultaneously, including the sex chromosomes (X and Y), making it more accurate than the recently used fluorescence *in situ* hybridisation (FISH) method, which only allows screening for a limited number of chromosomes.[10]

Two out of ten screened embryos inherited an unbalanced version of the father's translocation. It has been reported that three-way chromosomal rearrangements are particularly familial and can be transmitted from generation to generation.[9]

Balanced chromosomal translocations have been found in approximately 4% of couples who experienced recurrent spontaneous abortions.[11] When present in par-

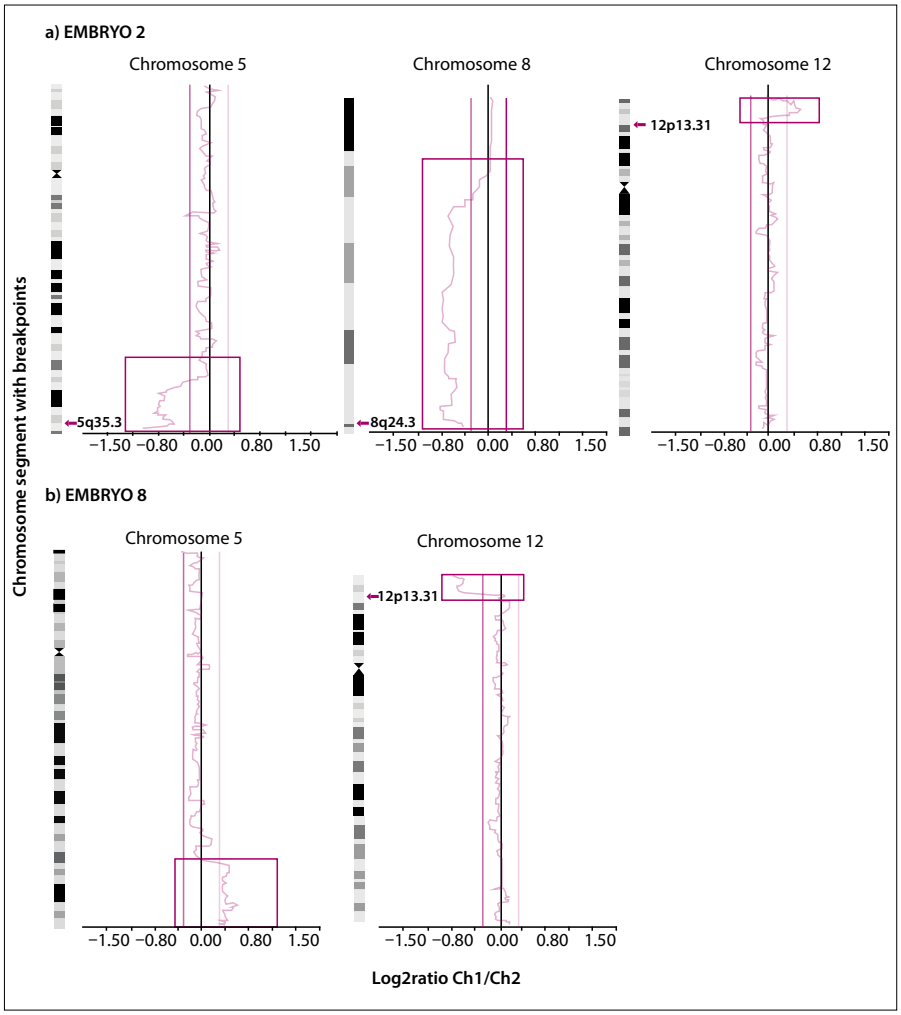


Fig. 1. Chromosomes affected by unbalanced translocation in embryos 2 (a) and 8 (b). (a) Embryo 2 showing partial loss of the long arm of chromosome 5 from 5q33.3-5q35.3 (24,951,204 bp), a partial loss of the long arm of chromosome 8 from 8q24.1-8q24.3 (23,417,117 bp) and a partial gain of the short arm of chromosome 12 spanning 12p13.1-12p13.3 (5,727,495 bp). (b) Embryo 8 showing partial gain of the long arm of chromosome 5 from 5q33.1-5q35.3 (29,602,079 bp) and a partial loss of the short arm of chromosome 12 spanning 12p13.31-12p13.33 (6,129,127 bp).

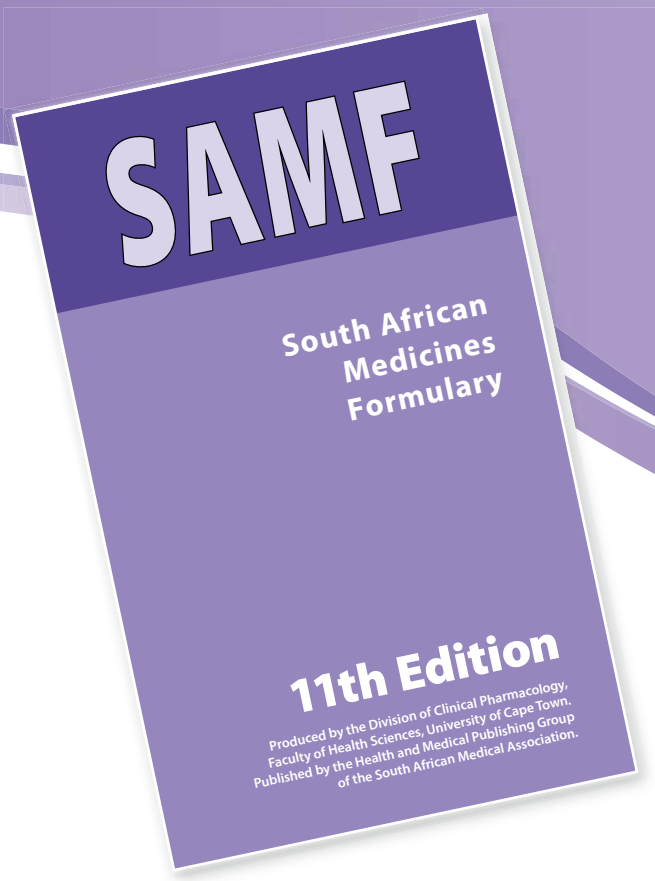
ents, these chromosomal rearrangements can later lead to chromosome irregularities in offspring and may also be a cause of stillbirth and fetal malformations.

Using the array CGH technique in this patient reduced the risk of recurrent miscarriage and the associated emotional distress, and gave the patient a viable pregnancy. By the same method we may offer the chance of a viable pregnancy to other members of this family who are planning to conceive.

The limitations of array CGH is that it cannot detect balanced translocations or polyploidy, as it only detects copy number variation. Further karyotype testing of patients is advised.

In conclusion, chromosomal screening of couples with recurrent abortions and decreased fertility can enable these couples to achieve a healthy pregnancy in a shorter period of time. We advise that PGD be a part of the investigation of these patients.

1. Snustad DP, Simmons MJ, Jenkins JB, et al. Principles of Genetics. New York: John Wiley, 2000.
2. Gardner RJM, Sutherland GR, Shaffer LG. Chromosome abnormalities and genetic counselling. Oxford: Oxford University Press, 2004.
3. Otani T, Roche M, Mizuike M, et al. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. Reprod Biomed 2006;13(6):869-874. [http://dx.doi.org/10.1016/s1472-6483(10)61037-1]
4. Farcas S, Belengeanu V, Popa C, et al. Role of chromosomal translocations in recurrent spontaneous abortion. Timisoara Med J 2007;2:117-121.
5. Rai R, Regan L. Recurrent miscarriage. Lancet 2006;368(9535):601-611. [http://dx.doi.org/10.1016/s0140-6736(06)69204-0]
6. Pellestor F, Anahory T, Lefort G, et al. Complex chromosomal rearrangements: Origin and meiotic behavior. Hum Reprod Update 2011;17(4):476-494. [http://dx.doi.org/10.1093/humupd/dmr010]
7. Winship WS, Beighton P. Genetic disorders in the Indian community of South Africa. S Afr Med J 2011;101(7):481-484.
8. Bartels I, Starke H, Argyriou L, et al. An exceptional complex chromosomal rearrangement (CCR) with eight breakpoints involving four chromosomes (1;3;9;14) in an azoospermic male with normal phenotype. Eur J Med Gen 2007;50:133-138.
9. Coco R, Rahn MI, Estanga PG, et al. A constitutional complex chromosome rearrangement involving meiotic arrest in an azoospermic male: Case report. Hum Reprod 2004;19:2784-2790.
10. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med 2007;357(1):9-17. [http://dx.doi.org/10.1056/nejmoa067744]
11. Alfarawati S, Fragouli E, Colls P, et al. Embryos of robertsonian translocation carriers exhibit a mitotic interchromosomal effect that enhances genetic instability during early development. PLoS Gen 2012;8(10):e1003025. [http://dx.doi.org/10.1371/journal.pgen.1003025]



The ESSENTIAL MEDICAL REFERENCE for every healthcare professional!

The convenient pocket-sized design enables you to fit it comfortably into your hospital bag or coat pocket, so it can always be at hand for ready reference. South African Medicines Formulary (SAMF), produced by the Division of Clinical Pharmacology of the University of Cape Town, provides easy access to the latest, scientifically accurate information, including full drug profiles, clinical notes and special prescriber's points. The thoroughly updated 11th edition of SAMF is your essential reference to the rational, cost-effective and safe use of medicines.

Please direct all order queries to: Edward –

Fax: 086 600 6218

email: edwardm@hmpg.co.za

Tax invoice to be posted on dispatch of order.