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CASE REPORT

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Identification of novel translocation between short arm of chromosome 4 and long arm of chromosome 6 in an infertile man using Interphase Chromosome Profiling (ICP)

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Summary

Conventional cytogenetics has always been a favourite to detect chromosomal aberrations. Carriers of chromosomal translocation are often phenotypically normal but are infertile. Couples are often advised to go for karyotyping, but culture failure or improper metaphase spread with poor banding often makes the analysis difficult. We report here a novel translocation between short arm of chromosome 4 and long arm of chromosome 6 in an infertile man using an advanced molecular cytogenetic technique of Interphase Chromosome Profiling (ICP).

KEYWORDS
chromosome translocation, infertility, interphase chromsome profiling

1 | INTRODUCTION

Balanced translocations are the type of chromosomal abnormalities that alter the chromosomal structure and can lead to the primary infertility or secondary infertility in the carrier. To detect chromosomal abnormalities, various techniques can be utilised such as conventional GTG banding, chromosomal painting, single nucleotide polymorphism (SNP) array, comparative genomic hybridization (CGH) out of which conventional cytogenetics has always been a favourite of diagnostic laboratories due to low cost involved (Dutta, 2016). But, culture failure, small specimen size and low band resolution have always been a big limiting factor for this technique. The detection of genomic changes <5 Mb and telomere region is not possible by this technique (Flint et al., 1995; Vissers et al., 2003). To overcome these drawbacks, high-resolution technique fluorescent in situ hybridization (FISH) was introduced in early 1980s for targeted regions where RNA that was directly labelled on the 3′end with fluorophore was used as a probe for specific DNA sequences (Bauman, Wiegant, Borst, & Van Duijn, 1980). The procedure takes less than 48 hr and involves three basic steps—denaturation, hybridization and heating. The technique can target rearrangement of almost 3 Mb size (Halder, Jain, Chaudhary, & Varma, 2012). Next-generation sequencing is another high-throughput molecular method, but it has lacked its applications in identifying the structural abnormalities such as copy number variants (CNVs) and structural variants (SVs) (Tattini, D'Aurizio, & Magi, 2015).

A novel cytogenetic method, interphase chromosome profiling (ICP), has been introduced in recent years which can correctly identify structural rearrangements and accurate localisation of break points (Babu & Koduru, 2015). The technique utilises a panel of FISH probes (three fluorophores) that target DNA sequences in an equidistant fashion along the entire length of the chromosome. The technique can be applied in the interphase nuclei when chromosomes are in the uncondensed state. In this paper, we discuss the confirmation of a novel chromosomal translocation involving chromosome 4 and 6 in an infertile man through ICP and application of ICP in routine diagnostics.

2 | CASE REPORT

2.1 | Subject

A couple with history of repeated spontaneous abortions (three times) reported in our laboratory. Upon the recommendations of clinicians, lymphocyte culture and GTG banding were performed which identified a novel translocation between chromosome 4 and 6 (Figure 1).

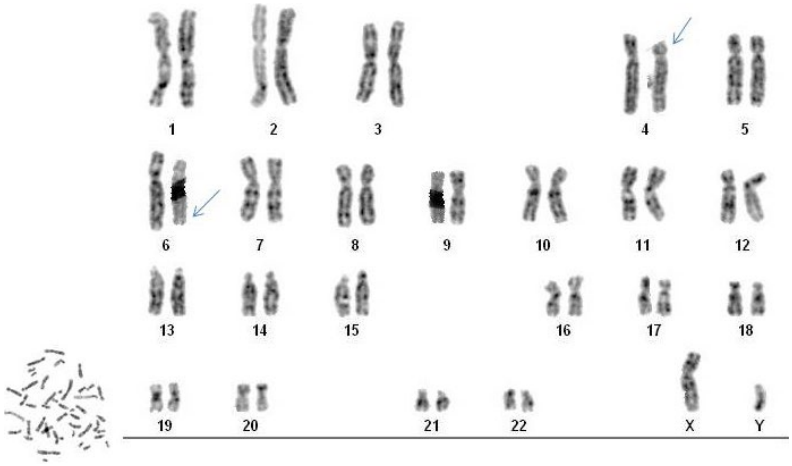

FIGURE 1 Karyotype of a man indicating translocation between short arm of chromosome 4 and long arm of chromosome 6

The semen quality parameter was as follows: 55% motility, 1 ml semen volume, 40-min liquefaction time.

2.2 | Methods

2.2.1 | Molecular cytogenetic analysis using ICP by standard resolution ASR probe for chromosome 4 and 6

Slides prepared from lymphocyte culture were treated with 1XPBS twice, Pepsin–HCL solution (0.005%) and again with 1XPBS at RT. It was codenatured with ICP probes by leaving in Thermostat at 80°C. Hybridisation was performed at 37°C overnight. Afterwards, slides were then put in 2X SSC at RT to prevent the drying of the slides. The slides were given washing with 0.3% NP40 at 74°C and then with 0.1% NP40 at RT. The slides were air-dried and the Slow Fade Gold Antifade was added to the concerned area. Interphase chromosome profiling probes used were standard resolution ASR probes for chromosome 4 (Catalog #: CHR-4 SR-ASR) and 6 (Catalog #: CHR-6 SR-ASR). The details of the fluorophore along with its cytogenetic position are mentioned in Table 1. Slides were scanned using microscope Olympus BX53 and ASI software.

TABLE 1 Details of fluorophore for its cytogenetic position

Probe ID	Cytogenetic position	Fluorophore
IG CHR4-p1	4p16.3	Fluorescein-12
IG CHR4-q1	4q11	Cyanine 555
IG CHR4-q6	4q35.2	CF 594
IG CHR6-p1	6p25.3	Fluorescein-12
IG CHR6-q1	6p11.1 and 6p11.2	Cyanine 555
IG CHR6-q6	6q27	CF 594

3 | RESULTS

The three colour probes are observed quite near to each other in control (Figure 2a), whereas the signals are displaced in Figure 2(b) indicating translocation (4; 6) (p16;q27). Around thirty cells were analysed and translocation was confirmed in 100% of the cells. To the best of our knowledge, this is the first case of translocation involving these two chromosomes in an infertile man.

4 | DISCUSSION

We have reported the first case of translocation between involving short arm of chromosome 4 and long arm of chromosome 6 in an infertile man and its reconfirmation using a molecular cytogenetic technique-ICP by standard resolution ASR probe for chromosome 4 and 6. In spite of our best efforts, we could not get very good G-banded chromosome and hence were not sure about the translocation identified through conventional method. Although expensive, the technique is much easier to perform in comparison with conventional cytogenetics. The technique is quick and apparently error-free. ICP can detect both numerical and structural aberrations including characterisation of marker chromosomes. The technique seems to be very useful in prenatal diagnostic studies where individual nuclei can be analysed (Babu et al., 2017).

Fluorescent in situ hybridization has been used for the cytogenetic analysis of cells without culture (Thompson & Gray, 1993), and thus the results are quick. Chromosome rearrangements can be characterised in interphase nuclei at 600-band resolution (Babu, Ernesto, Stephen, Srikanthi, & Sarah, 2016). Interphase chromosome profiling is a modified form of FISH where three fluorophores are used for each chromosome covering centromere and telomere bands and sub-bands. Also, using this technique, the molecular karyotype of any tissue using interphase cells can be assessed, and numerical, most balanced and unbalanced structural aberrations including all Robertsonian translocations can be identified (Babu & Koduru, 2015). The technique involves focussing of individual chromosomes in their uncondensed state in an interphase nucleus using a panel of FISH probes. The DNA sequence is targeted in an equidistant fashion along the entire length of the chromosome using only three fluorophores.

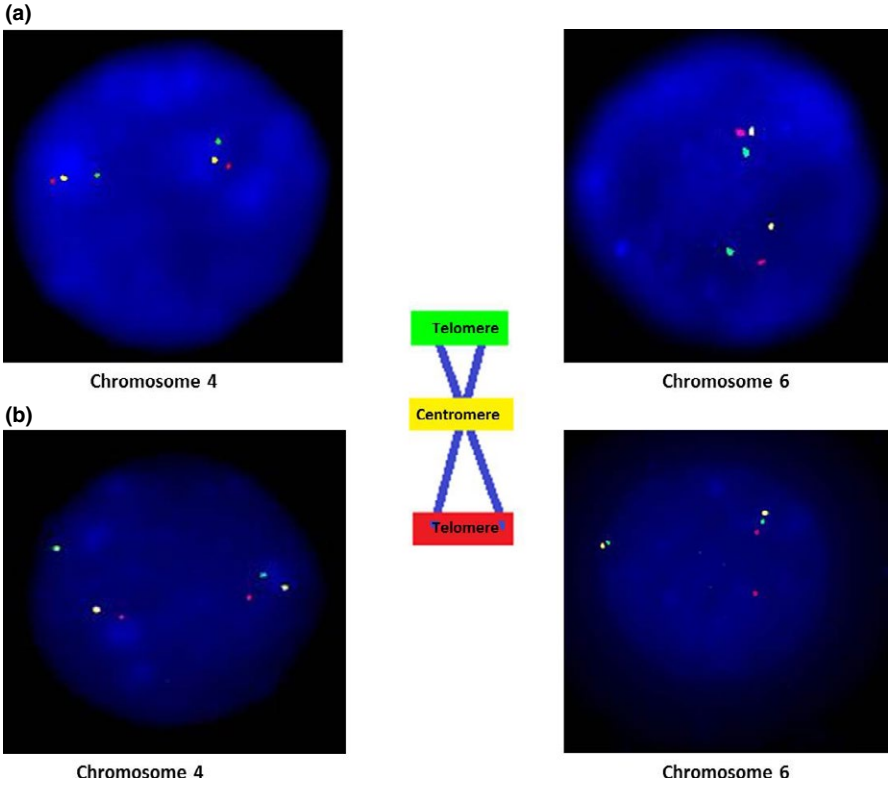

FIGURE 2 (a) Indicating signals from both 4 and 6 chromosomes at normal position (b) Displaced signals indicating translocation between chromosome 4 and 6 (4; 6) (p16;q27)

Cytogenetic aberrations are one of the important genetic causes responsible for infertility (Punab et al., 2017). Chromosomal abnormalities are the chief genetic cause of oligozoospermia and nonobstructive azoospermia (Shamsi, Kumar, & Dada, 2011). The rearrangements may alter the functionality of genes resulting in defective spermatogenesis and abnormal semen parameters. (Ching, Ko, Hecht, Smith, & Sabanegh, 2012). However, semen parameters cannot be indications for chromosomal screening as semen with normal quality has been reported in many cases of structural aberrations (Vozdova et al., 2013).

Cytogenetic analysis can play a decisive role for the infertile couples and is necessary in infertile couples before infertility treatment by assisted reproduction techniques (Pylyp, Spinenko, Verhoglyad, Kashevarova, & Zukin, 2015). Molecular cytogenetics is more sensitive and accurate and is applied in cases where only small samples are available or only interphase cells are present. The technique can also be used for the reconfirmation of the structural variants.

Hence, ICP with simultaneous usage probes for all the chromosomes can be applied to identify the aberrations and result can be made available within a day. Although the technique is useful, cost will remain a limiting factor for many laboratories.

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