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Case Report

Preimplantation genetic testing for a complex chromosome rearrangement, case report of a cryptic translocation detected on pre-PGT workup

Abstract

Pre-implantation genetic testing (PGT) is an established alternative to prenatal diagnosis of inherited disorders at the chromosome or gene level. Different molecular techniques are now available for detection of 24 chromosome aneuploidy and translocations. However, fluorescence *in situ* hybridization (FISH) is still used for certain telomeric and subtelomeric translocations and inversions. A non-consanguineous Indian couple had a son with an unbalanced translocation t (16;17)(p13.3;q25.3) detected after microarray showed deletion 16p13.3 and duplication 17q25.3. Prenatal diagnosis during the second pregnancy showed a similarly affected fetus. The husband was a carrier of the balanced translocation. We then recommended IVF-ICSI with PGT. During pre-PGD workup with FISH probes for telomeres 16p, 17q and centromere 16 on metaphases from the husband's lymphocyte culture, it was found that one signal of 17q was present on a C group chromosome instead 16p. G banding of fresh metaphase spreads followed by reflex-FISH on the same metaphases showed that the signal of 17q was located on 9q. This 3-way translocation was further confirmed on other G banded metaphases using a 9q34 probe, which showed that this region was translocated to 16p. Thus the karyotype of the husband was revised as 46,XY,t(9;16;17)(q34;p13.3;q25.3) due to the cryptic translocation seen only by FISH. PGT by FISH was carried out for the couple during the IVF-ICSI cycle on three embryos which reached the blastocyst stage on day-5. A few trophectoderm cells were biopsied and fixed on slides. FISH was set up using probes for chromosomes 16 and 17 in the first round and for 9q in the second round. Only 1 of 3 embryos showed a normal or balanced status. The third round of FISH on the same cells showed no aneuploidy for chromosomes 13, 18, 21 X and Y. The lady got pregnant after a frozen embryo transfer in the next cycle.

Keywords: fluorescence *in situ* hybridization, preimplantation genetic testing, cryptic, translocation, chromosomal microarray, embryo, karyotype, pre-PGT workup

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Abbreviations:

PGT, Preimplantation Genetic Testing; FISH, Fluorescence *in situ* hybridization; IVF, *In vitro* fertilization; ICSI, Intracytoplasmic sperm injection; CMA, Chromosomal microarray; NGS, Next Generation Sequencing; PGS, Preimplantation Genetic Screening; PGD, Preimplantation Genetic Diagnosis; Tel, Telomere; CEP, Centromere enumeration probe; ISCN, An International System for Human Cytogenetic Nomenclature; pter, Terminal region of short arm 'p' of chromosome; qter, Terminal region of long arm 'q' of chromosome; ESHRE, European Society of Human Reproduction and Embryology; aCGH, array comparative genomic hybridization; SNP, single nucleotide polymorphism

Introduction

Pre-implantation genetic testing (PGT) is an established alternative to prenatal diagnosis of inherited disorders at the chromosome or gene level. It involves selecting unaffected embryos from a cohort generated by intracytoplasmic sperm injection (ICSI). Recent techniques of chromosome microarray (CMA) and next generation sequencing (NGS) have largely replaced fluorescence *in situ* hybridization (FISH) to check for segmental aneuploidies of all 24 chromosomes by preimplantation genetic screening (PGS). However, when the breakpoint of the chromosome rearrangements is in the telomeric or subtelomeric region, FISH is still the method of choice for separating out embryos with unbalanced rearrangements which result in implantation failure, early pregnancy losses or a child with

developmental delay.[1] Transfer of normal/balanced embryos results in high pregnancy rates. Prior to taking up a case for preimplantation genetic diagnosis (PGD), the couple needs to undergo a pre-PGD workup. In cases of a balanced translocation detected in one partner by karyotyping, FISH on the couple's metaphases with centromere and subtelomere probes for the chromosomes involved occasionally picks up an additional cryptic anomaly involving a third chromosome. We earlier reported a successful twin pregnancy using PGT by FISH for inversion 12 with a cryptic translocation involving 9q detected by reflex FISH during the workup of the husband.[2] In such cases, PGT on each embryo has to be carried out for all the chromosomes involved and common aneuploidies, in multiple rounds on the same biopsied cells, which needs expertise.

Case reports

Herein we report the second case from India of an ongoing pregnancy after preimplantation genetic testing (PGT) by fluorescence *in situ* hybridization (FISH) in a couple, where the husband was a carrier of a balanced 3-way translocation t(9;16;17)(q34;p13.3;q25.3) in which the cryptic involvement of 9q34 was detected only during pre-PGD workup.

A nonconsanguineous Indian couple had a male child with delayed milestones and developmental delay. Chromosomal Microarray (CMA) of the proband done elsewhere, showed an unbalanced translocation with deletion 16p13.3 and duplication 17q25.3, i.e.

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arr 16p13.3(,0-509,189)x1,17q25.3(73,881409-78,774,742)x3. The deleted region on the short arm of chromosome 16 carries 2 of 4 alpha globin genes. Deletion of these 2 genes led to alpha minor (0) thalassemia in the proband. Hemoglobinopathy testing done elsewhere confirmed the heterozygosity for alpha-0-Thalassemia (type;-,-/alpha, alpha). Chromosomal investigation and hemoglobinopathy testing were also done elsewhere for the couple, where the wife showed normal results for both the tests, while the husband showed a reciprocal translocation t(16;17)(p13.3;q25.5) and heterozygosity for the alpha 4.2 deletion (silent carrier) of alpha-Thalassemia (type;-,alpha/alpha, alpha). Prenatal diagnosis by CMA on a chorion villus sample of the next pregnancy also showed the same abnormality and the pregnancy was terminated. Subsequently, they were referred to us for genetic counseling. The couple was advised to undergo IVF-ICSI followed by PGT to eliminate unbalanced translocations in the next pregnancy.

Pre-PGD work up was carried out in our laboratory by FISH on metaphases from peripheral blood lymphocyte cultures of the couple using the required centromeric (CEP) 16 and subtelomeric probes for 16p and 17q. It was incidentally found that the translocation in the husband involved a third chromosome. Reflex FISH was set up on G banded metaphases and the cryptic translocation was confirmed to involve the 9q34 region. Subsequently, the couple underwent one IVF-ICSI cycle followed by day-5 trophectoderm biopsy on 3 embryos. FISH was set up on fixed trophectoderm cells in multiple rounds. Only one embryo showed signals for a normal or balanced translocation and was further tested for aneuploidies for chromosomes 13, 18, 21 and sex chromosomes. This embryo was transferred back to mother's uterus in the subsequent cycle and the pregnancy was confirmed by beta-HCG test and ultrasonography.

Material and methods

Pre-PGT work-up

FISH was carried out on metaphase and interphase nuclei of the couple obtained from PHA stimulated lymphocyte cultures, using Vysis (Abbott Molecular) probes for 16p (green), 17q (orange) and CEP 16 (aqua) to test the probes, confirm the loci and check for signal polymorphisms of size and intensity. Subsequently, the BCR-ABL probe for 9q34 (orange) and 22q11.2 (green) available with us was used, for confirmation of the three ways cryptic translocation detected on metaphase FISH.

PGT

The wife underwent a routine IVF-ICSI protocol where hormonal stimulation was carried out to increase the number of follicles and oocytes were collected under anesthesia. For PGT, 4-5 trophectoderm cells were biopsied from each of the three blastocyst embryos on day 5 and tested by FISH using the protocol standardized in our laboratory.

Around 4-5 trophectoderm cells biopsied from each of the three embryos were treated with hypotonic solution and fixed on glass slides with a 3,1 mixture of methanol and acetic acid, under a Leica stereomicroscope. The nuclei were then observed under 10X phase contrast of the Zeiss epi-fluorescent microscope Axioskop-2 and the co-ordinates were noted for relocation after hybridization and washing.

FISH was carried out in three rounds on the same fixed trophectoderm cells according to the manufacturer's protocol. The first round was set up using probe mixture for chromosomes 16 (Tel16p-Green, CEP16-Aqua) and 17 (Tel17q-Orange) from Vysis (Abbott). After recording the results and capturing the images with the Metasystems *isis* software, the slides were washed to strip the probes and a mixture of probes for 9q (Orange) and 22q (Green) (from Vysis-Abbott) was used for overnight hybridization in round-two. For testing common aneuploidies, the Multivysion PGT probe mixture (Vysis-Abbott) for chromosomes 13 (Red), 18 (Aqua), 21 (Green), X (Blue) and Y (Gold) was used in round-three on embryo no. 2. Thus a total of 9 probes were checked in three days. Institutional ethics committee approval and patient's consent was taken for the procedure.

Results

Pre-PGT workup

FISH on metaphase spreads of the couple with a mixture of probes for chromosomes 16 and 17 in three colours (16p in green, 16 centromere in aqua and 17q in orange) showed presence of a 3-way cryptic translocation. The 16p green signal was visible on normal 16 with presence of 1 aqua signal on the same chromosome. In this case the signal of 17q (orange) was expected to be present on derivative 16 along with the aqua signal for centromere 16. Instead, derivative 16 had only 1 aqua signal and no 17q orange signal. The normal 17 showed presence of 17q orange signal. Derivative 17 showed presence of 16p green signal confirming the translocation of 16p at 17q region. A cryptic translocation was inferred from the fact that the orange signal of 17q was not adjacent to the aqua signal on derivative 16, but was on a third chromosome, resembling a sub-metacentric chromosome from 'C' group, on inverted DAPI. Fresh G-banded metaphases were captured (Figure 1A) and subsequent FISH was set up on the same metaphases. The results showed presence of an orange signal (17q) at the telomere of 9q (Figure 1B). Subsequent FISH on the other metaphases (Figure 1C) using a probe for 9q34 in orange showed the presence of one orange signal on the normal 9 and another on derivative 16 visible on inverted DAPI (Figure 1D). This confirmed the partially cryptic translocation t(9;16;17)(q34;p13.3;q25.3) which was not detected on karyotyping. The chromosome 22 showed 2 normal green signals. The karyotype after metaphase FISH was therefore 46,XY,t (9;16;17)(q34;p13.3;q25.3).

According to the detailed ISCN nomenclature, the karyotype was designated 46,XY,t(9;16;17) (9pterà9q34,,17q25.3à17qter;16qterà16p13.3,,9q34à9qter; 7pterà17q25.3,16p13.3à16pter).

PGT therefore required the use of FISH probes for 9q34 besides 16pter, centromere 16, 17qter, and common aneuploidies. This was carried out in three rounds on the same trophectoderm cells. Signal size polymorphism was also detected on pre-PGT workup of the wife, where one of the aqua signals for CEP 16 was much smaller in size in interphase cells (Figure 1E).

PGT

Of the trophectoderm cells biopsied from three embryos, cells from one embryo were normal with no unbalanced translocation and no aneuploidy for the chromosomes tested, as they showed two signals for each probe used. The corresponding normal embryo was transferred in the subsequent cycle. Pregnancy was confirmed on beta-HCG test after fifteen days.

Discussion

Detection of a balanced translocation in one of the partners is an acceptable indication for PGT. A cryptic translocation is one which cannot be observed by conventional cytogenetic analysis, either

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because the size and banding pattern of the chromosome regions involved are too similar for the exchange to be detected, or because the size of the exchanged segments is close to the limit of resolution of the cytogenetic technique.[3] Small translocations involving telomeric regions of chromosomes are especially difficult to detect since most telomeres have a similar banding pattern. To date, detection of cryptic telomeric translocations has mostly relied upon the presence of clinical or cytogenetic clues that point to a specific chromosome deletion or duplication syndrome. The availability of DNA probes that permit informative DNA or molecular cytogenetic studies is also crucial.[4]
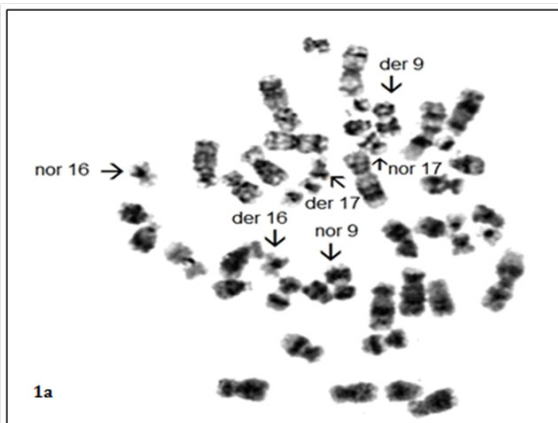

Figure 1A G-banded metaphase obtained from husband's lymphocyte culture.
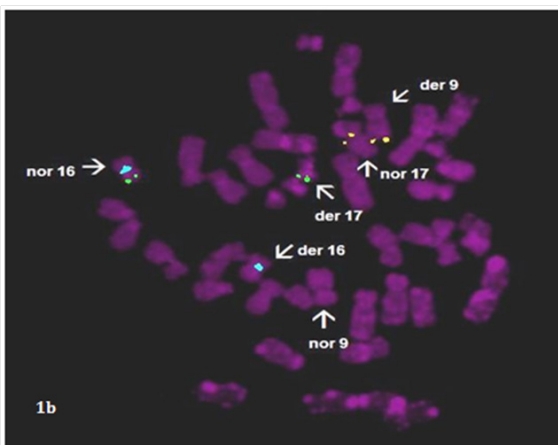

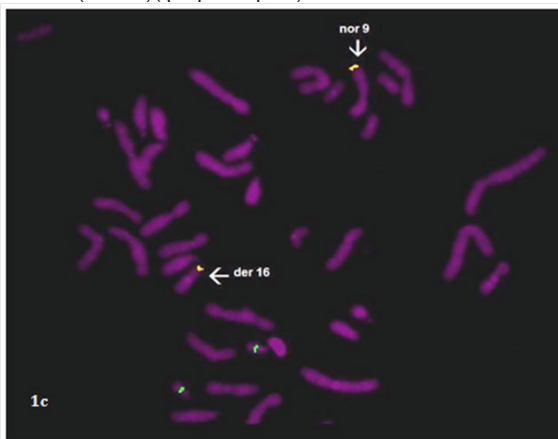**Figure 1B** Reflex FISH on metaphase of Figure 1a showing three way translocation t(9;16;17)(q34;p13.3;q25.3).

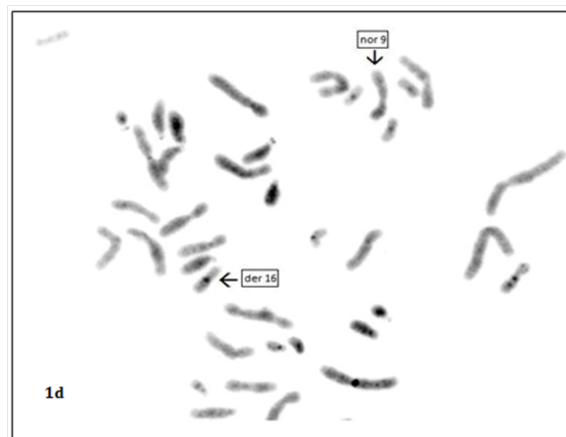**Figure 1C** FISH on metaphase showing 9q (Orange) signal on 16p.

Figure 1D Inverted DAPI image of Fig. 1c showing normal 9 and derivative 16.
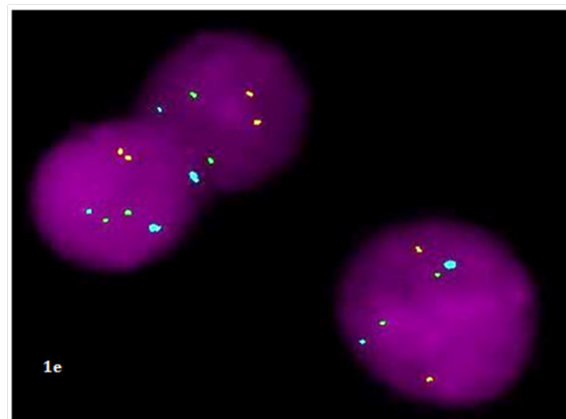

Figure 1E Signal size polymorphism of CEP16 (Aqua) signal in wife.

With the advent of technologies such as FISH, aCGH and NGS, it is possible to detect unbalanced chromosomal translocations in preimplantation embryos. FISH was the initial technique used to detect aneuploidies and translocations in PGT embryos. However, as FISH could only detect aneuploidy of about 9 chromosomes besides translocations, in multiple rounds, its use was limited. New techniques like CMA and NGS have been developed where all 24 chromosomes can be tested.[5] Depending on the technology; CMA is preferred for reciprocal translocation cases over NGS. Telomeric regions were excluded from CGH analysis because the absolute green and red fluorescence intensities gradually decrease at the telomeres and unreliable ratio changes may appear as the fluorescence intensities approach background fluorescence.[6,7]

In 2010, the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium best practice guidelines were published for FISH based PGT. As per the guidelines, for chromosome rearrangement cases, preliminary work on peripheral blood lymphocytes from both reproductive partners is recommended for each different probe and combined probe set, and should include both metaphase spread and interphase nuclei analysis. For probe mixes containing subtelomeric probes and/or locus-specific probes with known polymorphism and cross-hybridization, preliminary work should be carried out using (diploid) cells from both reproductive partners.[8] In the present case, signal size polymorphism was observed for the centromere of chromosome 16 in the wife, where one of the 2

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aqua signals was very small. This information helped in interpretation of FISH results during PGT. Also, a 3-way cryptic translocation involving 9q34 was picked up on reflex FISH in the husband, substantiating the importance of detailed pre-PGT workup.

Due to limitations of technology, newer techniques such as CMA, NGS and multiplex ligation-dependent probe amplification (MLPA) cannot detect balanced rearrangements in carriers. Another limitation of certain CMA platforms used for PGD, is the inability to reliably detect unbalanced derivatives from rearrangements that have breakpoints in the telomere or subtelomere, while FISH probes can cover all subtelomeric regions.[1] FISH has the advantage of providing an instant location of certain rearrangements, such as insertions, inversions, and balanced translocations, which cannot be achieved by the microarray-based method. Subtelomeric FISH is a very robust technique, as it can detect and locate rearrangements that involve small and specific regions of the chromosomes, uncover low level mosaicism, and identify balanced chromosomal rearrangements.[9] A comparative study of three molecular cytogenetic methods in 100 children with idiopathic mental retardation, concluded that telomere (T) FISH and multiplex ligation-dependent probe amplification (MLPA) are both very useful and interchangeable methods for the detection of unbalanced chromosome rearrangements, but T-FISH also detects balanced rearrangements. The resolution power of comparative genomic hybridization was too low for subtelomeric screening compared with T-FISH and MLPA.[10] A pre-PGD work up would help to determine selected cases where FISH is preferable.[11] Recent oligoarray-CGH platforms are now accurately able to detect unbalanced rearrangements at the telomeres and do not require a workup prior to PGD.[12] A prospective blinded cohort study of miscarriage specimens compared traditional cytogenetic analysis with single nucleotide polymorphism microarrays (SNP) and array CGH and concluded that given their similar overall performance, providers may chose a method based on individual availability and consideration of limitations in each clinical scenario.[13]

Informed consent was obtained from the couple. All procedures performed were in accordance with ethical standards and approved by the Institutional Review Board.

Acknowledgements

Conflicts of interest

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