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Sperm meiotic segregation of a balanced interchromosomal reciprocal insertion resulting in recurrent spontaneous miscarriage

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KEY MESSAGE

In carriers of interchromosomal reciprocal insertions, sperm FISH analysis is useful to estimate the amount of unbalanced products, providing information for genetic reproductive advice.

A B S T R A C T

Research question: Is sperm fluorescence in-situ hybridization (FISH) useful to evaluate the risk of chromosomally unbalanced gametes in interchromosomal reciprocal insertion (IRI) carriers? How do these imbalances lead to recurrent miscarriages?

Design: This study reports a clinical and molecular study of a rare familial balanced IRI resulting in recurrent spontaneous miscarriage. Sperm FISH was performed to estimate the number of unbalanced gametes.

Results: A 31-year-old healthy male (proband) and his 28-year-old female partner were referred to the Genetics Department for three spontaneous miscarriages occurring during the first trimester of pregnancy. FISH analysis of the proband with the LSI TRA/D (14q11.2) and DiGeorge N25 (22q11.2) break-apart probes showed the presence of a balanced IRI between 14q11.2 and 22q11.2 chromosomal regions. This IRI was also identified in the proband's father. Sperm FISH with the same probes showed that more than 40% of gametes of the proband were unbalanced for either 14q11.2 or 22q11.2, despite normal sperm parameters. FISH analysis of a product of conception indicated that unbalanced gametes result in a non-viable fetus.

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Conclusions: This study shows the value of sperm FISH analysis in improving genetic reproductive advice for IRI carriers. Disruption of critical genes through this rearrangement and their consequent functional impairment could result in recurrent miscarriages. In this case, several genes located in the 14q11.2 region, particularly *RNase 3*, would be good candidates to explain the lethality of the imbalances.

Introduction

Chromosomal abnormalities are one of the major causes of spontaneous miscarriage before the fourth month of pregnancy. Reciprocal and Robertsonian translocations were the most common type of aberration observed among couples who experienced recurrent spontaneous miscarriages (Tunç et al., 2016). Classic interchromosomal insertions with three break-points are rare events. Their frequency has been estimated at about one in 80,000 (Van Hemel and Eussen, 2000). However, the detection rate has increased with the use of array comparative genomic hybridization (CGH) and recent reports suggest a frequency of about one in 500 (Kang et al., 2010). Interchromosomal reciprocal insertions (IRI) that require four break-points are even rarer, and only 12 cases have been reported (Bernardini et al., 2008; Harbuz et al., 2013; Kang et al., 2010; Manolakos et al., 2011; Neill et al., 2011; Ou et al., 2008; Van Esch et al., 1999; Van Hemel and Eussen, 2000; Wallis et al., 2016; Wang et al., 1994). This paper reports a familial balanced IRI between 14q11.2 and 22q11.2 resulting in recurrent spontaneous miscarriage. Sperm fluorescence in-situ hybridization (FISH) was performed to estimate the number of unbalanced gametes and to investigate the possible occurrence of interchromosomal effects (ICE) affecting the normal disjunction of other chromosome pairs not involved in the IRI. To the best of our knowledge, this is the first reported case of sperm FISH analysis in a carrier of balanced IRI. Altered expression of critical genes caused by this rearrangement could explain the pregnancy outcomes.

Clinical report

A 31-year-old healthy male (proband) and his 28-year-old female partner were referred to the Genetics Department for three spontaneous miscarriages occurring during the first trimester of pregnancy. The mother of the proband had had six miscarriages. On physical examination, the patient was phenotypically normal with no developmental abnormalities, and his medical history revealed neither early neonatal problems nor motor impairment. His wife had a normal 46,XX karyotype.

Chromosome analysis of the proband was performed with GTG and RGH banding techniques on peripheral blood lymphocytes. The 400–550 band level resolution karyotype revealed the presence of an additional band at the q11 region of one of the chromosome 14 homologues, which stained darkly on RGH banding and faintly on GTG banding (**Figure 1**).

Multiplex fluorescence in-situ hybridization (M-FISH) failed to identify the nature of the additional band. FISH using whole chromosome painting xcp14 probes (Metasystem) showed the absence of signal on the unusual chromosome 14q11 region and two signals on the two chromosome 22 homologues by cross-hybridization of alpha satellite DNA sequences (**Figure 2a**). Both chromosomes 22 were integrally painted by the xcp22 probes (Metasystem) and two signals were present on the two chromosome 14 homologues (cross-hybridization) but with an enlarged signal on the derivative chromosome 14 (**Figure 2b**). FISH using the LSI TRA/D break-apart probe mapping at 14q11.2 (Vysis) showed that the 5' TRA/D signal hybridized on chromosome 22 whereas the 3' TRA/D signal remained on chromosome 14. The LSI DiGeorge N25 signal (Vysis) was delocalized at 14q11 (**Figure 3**).

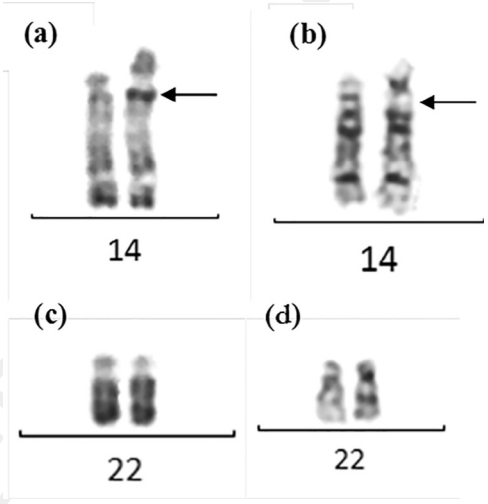

Figure 1 – Partial karyotype for normal and derivative chromosomes 14 and 22 of the patient. RGH banding (a) shows the insertion of the 22q11.2 dark segment at the 14q11.2 band and GTG banding; (b) shows a corresponding fair band (arrows). The insertion is less visible on chromosome 22 (c and d).

Finally, the karyotype of the proband was 46,XY,ins(14;?)(q11;?).ish der(14)ins(14;22)(q11.2;q11.2q11.2)(xcp14+,xcp22+,N25+,3'TRA/D+), der(22)ins(22;14)(q11.2;q11.2q11.2)(xcp22+,xcp14+,N25-,5'TRA/D+)

Semen analysis performed according to World Health Organization recommendations (2010) showed nearly normal parameters: volume 3.05 ml; pH 8.3; concentration 84.75 × 10^6 spermatozoa/ml; vitality 83.5%; total motility after 1 h: 71%, after 3 h: 63%; progressive motility after 1 h: 58%, after 3 h: 55.5%; normal forms 3.5%.

Sperm FISH was performed with the same LSI N25 and TRA/D break-apart probes. A centromeric probe from chromosome 18 was used as a control. Five hundred cells were analysed for each probe. The percentage of normal or balanced spermatozoa ranged from 56 to 58%. About 40% of spermatozoa showed chromosomal imbalance resulting from the presence of the derivative chromosome 14 in 18–24% of cells, depending on the FISH probe used, or of the derivative chromosome 22 in 20–24% of cells (**Figure 4**).

The occurrence of ICE for chromosomes 13, 18, 21 and for the sex chromosomes was also evaluated by FISH using the AneuVysion kit

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