Risk evaluation and preimplantation genetic diagnosis in an infertile man with an unbalanced translocation t(10;15) resulting in a healthy baby

De-Hua Cheng · Fei Gong · Chang-Fu Lu · Lu-Yun Li · Guang-Xiu Lu · Yue-Qiu Tan

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Introduction

Balanced chromosome structural rearrangement, including reciprocal translocation, Robertsonian translocation and inversion, is an important cause of male infertility that results in azoospermia, oligozoospermia or asthenozoospermia [1]. Unbalanced translocation between two autosomes, which usually results in mental retardation or multiple congenital anomalies, is rare in infertile patients. To our knowledge, only the t(15;18) unbalanced translocation has been reported, in two healthy infertile brothers. Although fluorescence in situ hybridization (FISH) analysis showed that chromosomal normal sperms existed in their semen, these two brothers and their partners chose donor insemination rather than preimplantation genetic diagnosis (PGD) [2]. Whether patients with this kind of rare karyotype could deliver a healthy baby is unclear.

Here, we report a man with severe oligoasthenoteratozoospermia carrying a derivative chromosome 10 resulting from the rare unbalanced translocation t(10;15). Based on risk evaluation by sperm FISH, we performed PGD for the couple and successfully established a normal pregnancy. Our results show that patients with such an abnormal karyotype can

produce their own offspring, and emphasize the importance of risk evaluation using sperm FISH for infertile male patients.

Patient data

A couple presented to our reproduction center due to primary infertility that had persisted for 5 years. The woman showed no obvious cause of infertility on examinations, which included: (1) clinical findings: physical examination and medical history evaluation, such as menstrual history, obstetrical history, sexual history, family history, personal and lifestyle history; (2) biochemical tests: liver and renal function; blood cholesterol, blood triglycerides and blood glucose; and immunologic function; and (3) hormonal assay: estradiol, testosterone, progesterone, follicle stimulating hormone (FSH), luteinizing hormone, prolactin, 17-hydroxypregnenolone and thyroid stimulating hormone (TSH); (4) other diagnosis test such as hysterosalpingography. Her karyotype was also normal.

However, the man was found to have severe oligoasthenoteratozoospermia after three routine sperm analyses and one sperm morphology examination following the WHO guidelines (Table 1). Conventional G-banding (G-bands after trypsin and Giemsa, GTG) analysis showed that he had only 45 chromosomes, with one derivative chromosome comprising chromosomes 10 and 15 (Fig. 1).

The couple then accepted a genetic counseling and asked for further fertility evaluation. Oligoasthenoteratozoospermia might have been the cause of the primary infertility in this couple. The husband carried a rare unbalanced translocation comprising chromosome 10 and chromosome 15 that had not been reported previously. The genetic risk was unclear for this kind of abnormal karyotype. His chromosomal structure was similar to that of a Robertsonian translocation; therefore, the meiosis pattern might also be similar

Capsule An individual carrying the rare unbalanced translocation der(10)t(10;15)(p15;q11) can produce gametes with a normal karyotype and can deliver healthy offsprings by PGD.

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D.-H. Cheng · L.-Y. Li · G.-X. Lu · Y.-Q. Tan
Reproductive and Genetic Hospital of Citic-Xiangya,
Changsha, Hunan 410078, People's Republic of China

F. Gong · C.-F. Lu · G.-X. Lu · Y.-Q. Tan (✉)
Institute of Reproduction and Stem Cell Engineering,
Central South University,
Changsha, Hunan 410078, People's Republic of China
e-mail: tanyueqiu@sina.com

Table 1 Semen analysis

Parameter	Value
Volume(ml)	2.5
pH	7.2
Sperm concentration ($\times 10^6/\text{ml}$)	3.1
Molality (%;30 min)	22.5
A+B grade (%;Rapid progressive)	19.4
Round cell (%)	0.2
Total malformation (%)	98.6

to a Robertsonian translocation. Sperm chromosome constitution can be obtained by sperm FISH analysis and might predict the probability of producing an embryo with normal karyotype. If chromosomally normal sperms are present in semen at a high prevalence, PGD can be performed to select embryos with balanced chromosomes for transfer and deliver a healthy baby.

This study was approved by the Institutional Review Board of Central South University and Reproductive and Genetics Hospital of Citic-Xiangya, and the patients' written informed consent was obtained.

Methods

Karyotype determination

FISH and GTG analysis were further used to determine the husband's karyotype. The probe combination comprised a

centromeric probe for chromosome 10 (CEP 10, Spectrum Aqua labeled, Cat.32-131010, Lot.411017), a subtelomere probe for chromosome 10p (Tel 10p, Spectrum Green labeled, Cat.30-252010, Lot.421223), and two loci-specific probes for D15S11/D15Z1 (Spectrum Orange/Spectrum Green labeled, Cat.32-190014, Lot.401014) on chromosome 15. All of these probes were purchased from Abbott-Vysis, Inc. (Downers Grove, IL, USA). The DNA probe solution was denatured at 75 °C for 5 min and immediately added to each denatured slide for hybridization overnight. The slides were then washed in 50 % formamide/2×SSC three times at 45 °C, and Tween-20/4×SSC twice and 4×SSC once at room temperature. After drying, the slides were counterstained with DAPI and covered with glass coverslips. The slides were viewed with an Olympus BX51 fluorescence microscope (Olympus, Tokyo, Japan) and images acquired by a CCD camera (Jenoptik, Germany) with Videotest FISH 2.0 software (C1990-2004, Petersburg, Russia).

Sperm FISH analysis

An ejaculated sperm sample was prepared for FISH analysis as described previously. Briefly, 1 ml of the patient's semen was washed twice in 8 ml 1×phosphate-buffered saline and fixed in fresh fixative (methanol:glacial acetic acid = 3:1) for 30 min, and then dropped onto clean microscopic slides and dried at 60 °C in an oven for 2 h. The sperm chromatin was decondensed in 0.01 M sodium acetate, pH 5.2, containing 25 mM dithiothreitol (DTT) in 2.5×10^{-4} M DTT solution for 8 min, after which the slides were denatured

Fig. 1 **a** Partial karyotype of the patient showing normal chromosome 10,15 and der(10). **b** ideogram of the normal chromosome 10,15 and rearranged chromosome. Red, green and aqua bands show the position and color of the FISH probes used for preimplantation genetic diagnosis

Fig. 2 FISH images of the normal chromosome 10, normal chromosome 15 and der(10) from the peripheral blood cell culture

in 70 % formamide/2×SSC at 75 °C for 5 min. The slides were then dehydrated in a cold ethanol series (70 %, 85 %, 100 %) for 2 min at each concentration, and dried at room temperature.

Meiotic segregation pattern was analyzed by three-color FISH using commercially available DNA probes specific for the centromere of chromosome 10 (CEP10, Spectrum Aqua), D15S11 (Spectrum Orange) and D15Z1 (Spectrum Green). All of these probes were purchased from Abbott-Vysis, Inc. (Downers Grove, IL, USA). The sperm FISH procedure was the same as that for metaphase FISH. Only intact isolated spermatozoa were used for scoring FISH signals.

PGD procedure

PGD of blastomeres based on FISH is performed routinely in our institute. Briefly, controlled ovarian stimulation was conducted on the woman with a gonadotropin-releasing hormone analog using a long protocol depending on the individual's condition; this was followed by the administration of recombinant FSH (Gona-F®; Merck Serono Ltd, Switzerland) beginning on the third day of her cycle. Oocytes were retrieved by vaginal ultrasound-guided aspiration; 4 h later, intracytoplasmic sperm injection was performed by microinjecting the husband's sperm into the oocytes (day 0). The pronuclei were observed after 16 h to check for normal fertilization.

Embryos were cultured in G-2.5 medium (Vitrolife, Göteborg, Sweden) in droplet culture and incubated at 37 °C in a humidified atmosphere containing 5 % CO₂. Embryo growth and development were observed after 3 days. Embryos at the 6- to 8-cell stage were biopsied for PGD on day 3. A hole was drilled by laser in the zona pellucida of one blastomere from each embryo and the contents aspirated gently with a biopsy pipette.

Blastomeres used for PGD were checked for the presence of a nucleus.

The biopsied blastomeres were exposed for 5 min to hypotonic solution (1 % sodium citrate in 6 mg/ml bovine serum albumin) and transferred into a small drop of Tween 20 fixative (0.01 N HCl/0.1 % Tween 20) on a slide. The spreading solution was added continuously until the blastomere cell membrane ruptured and the nucleus separated from the bulk of the cytoplasm. Once the slides had dried, they were further fixed through methanol/glacial acid. After the biopsy procedure, the embryos were continued to be grown in IVF-20 medium until the results of the genetic analysis had been fully evaluated.

The procedure for blastomere FISH was the same as that for sperm FISH. Image capture and analysis of the results were also the same. Blastomere FISH results were interpreted

Fig. 3 C banding chromosome for the patient showed the der(10) has only one centromere (White head arrow)

Table 2 Sperm FISH analysis for chromosome 10,15

Normal	der(10)t(10;15)	Disomy 10	Nullisomy 10	Disomy 15	Nullisomy 15	Disomy 10,15	Others
26.80 %	27.84 %	2.06 %	5.15 %	16.49 %	13.06 %	4.12 %	4.47 %

2910 sperms of the patient were scored by triple-colour FISH analysis

according to the scoring criteria previously published by Delhanty et al. [3]. Blastocyst transfer was conducted on day 5 using only chromosome completely normal and high quality embryos.

Results

Normal hybridization signals were observed on the normal chromosome 10, chromosome 15 and the derivative chromosome 10 with the probes Tel 10p, CEP 10 and D15S11. However, there was no hybridization signal with the D15Z1 locus-specific probe on the derivative chromosome 10, and CBG showed only one centromere on the derivative chromosome 10, which indicated that the short arm and partial long arm of chromosome 15 had been deleted (Figs. 2 and 3). Therefore, the husband carried an unbalanced chromosome rearrangement and his karyotype could be described as 45, XY, der(10)t(10;15)(p15;q11), -15.

Centromere probes for chromosome 10 (Spectrum Aqua) and loci-specific probes for chromosome 15 including D15S11 (Spectrum Orange) and D15Z1 (Spectrum Green) were used for FISH analysis of the patient's sperm. Totally 2910 sperms of the patient were scored by

triple-colour FISH analysis, among them 26.8 % of the sperm had normal hybridization signals, 27.84 % had unbalanced karyotypes carrying der(10)t(10;15)(p15;p11), 16.49 % were nullisomic for chromosome 15, 13.06 % were disomic for chromosome 15, 5.15 % were nullisomic for chromosome 10, and 4.12 % were disomic for chromosomes 10 and 15 (Table 2). Figure 4 showed a representative sperm for each hybridization pattern.

Through single blastomere FISH, it was found that 16 of 17 embryos produced accurate hybridization signals. One embryo had no FISH result for the biopsied blastomere without nucleus after fixation. Of the 16 embryos with accurate hybridization signals, four had normal signals (25 %, 4/16), seven had carrier hybridization signals (43.75 %, 7/16) and five had abnormal FISH signals (31.25 %, 5/16). Two fresh embryos with normal FISH signals were selected for transfer, but pregnancy was not established. Frozen embryos with normal FISH signals were selected for a second transfer, which resulted in a successful singleton pregnancy. Prenatal diagnosis on week 16 via amniotic fluid sampling (AFS) was performed and the fetus karyotype was normal. On the 38th week a healthy girl was born. Now the baby is 1 year and 7 months old and she is very healthy.

Fig. 4 Sperm triple-colour FISH analysis for chromosome 10 and 15. Centromere probe of chromosome 10 (*Pseudosematic color white*) and loci-specific probes of D15S11 (*Spectrum Orange*) and D15Z1

(*Spectrum Green*) were used. Figure a and b represented a normal sperm and a carrier sperm (der(10) bearing sperm), respectively. Figure from c to i represented abnormal sperms

Discussion

The incidence of chromosome structural abnormalities is higher in infertile men than in the general population [4, 5]. In infertile couples seeking help by assisted reproductive technology, the incidence of chromosomal abnormalities is as high as 3.95 % [6]. Increasing numbers of genetic factors associated with male infertility are being identified, including chromosomal aneuploidy, structural and numerical karyotype abnormalities and Y chromosomal microdeletions [7]. Semen analysis of a large cohort of infertile men showed a highly correlation between the rate of chromosomal abnormalities and the severity of oligoasthenoteratozoospermia [8, 9].

The infertile couple in the present study had been married for 5 years without using contraception. Routine semen examination of the husband showed severe oligoasthenospermia with the rare karyotype 45, XY, der(10)t(10;15), -15. The derivative chromosome 10 comprised almost a complete chromosome 10 and the long arm of chromosome 15; FISH showed that only 15p and little segment of the long arm of chromosome 15 was missing, while the subtelomeric region of 10p remained intact. The abnormal derivative chromosome could affect the pairing and separation of the homologous chromosomes 10 and 15 during meiosis, leading to disruption of spermatogenesis or formation of abnormal sperm, and ultimately resulting in severe oligoasthenospermia. The patient had no developmental abnormality. The possible reason is that there had been no deletion or duplication in the euchromatin areas of chromosomes 10 and 15.

Only a few cases of unbalanced translocations formed from telocentric and non-telocentric autosomes have been reported [9–11], and the genetic risks for such chromosomal abnormalities have not been reported.

Such derivative chromosomes contain only one centromere; in our case, segments had been deleted from chromosomes 10 and 15.

Our study presents a model to predict the theoretical risk that unbalanced gametes will result from derivative chromosome meiosis (Fig. 5; a total of six possible gamete types). The model was validated by triple FISH of the patient's semen. Based on our experimental data, the types of abnormality of chromosomes 10 and 15 present in the patient's sperm were consistent with the model's predictions. We found that 26.8 % of the sperm had normal hybridization signals, while 27.84 % carried an unbalanced derivative chromosome 10. The likely cause is that segregation in this patient was similar to that which occurs with a Robertsonian translocation. The proportions of chromosome 15 disomic and nullisomic sperm were 16.49 % and 13.06 %, respectively; the proportions of chromosome 10 disomic and nullisomic sperm were 2.06 % and 5.15 %. Sperm disomic for both chromosome 10 and 15 constituted 4.12 %, and the proportion of other abnormal sperm was about 4.47. These findings mean that there would be a high risk of producing abnormal offspring if assisted reproductive technology was used without PGD. Based on the sperm FISH results, we performed PGD for the couple. Blastomere FISH showed that 25 % of the embryos had normal hybridization signals for both chromosome 10 and 15, which was consistent with the 26.8 % normal rate observed on sperm FISH.

In summary, men carrying the unbalanced chromosome translocation der(10)t(10;15)(p15;q11) can produce gametes with a normal karyotype. This is the first report of the clinical use of PGD with multicolor FISH technology for this rare chromosome structural rearrangement, which produced a healthy offspring with a normal karyotype.

Fig. 5 Theoretical balanced and unbalanced meiotic segregations of the carrier and resulting offspring. At least 6 gamete types might exist in the sperms

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