Normal sperm in a 2;2 homologous male translocation carrier

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Abstract

Purpose Carriers of balanced structural chromosomal abnormalities are phenotypically normal but are at high risk of infertility. Translocations usually occur between two non-homologous chromosomes. When occur between homologous chromosomes, an extremely rare event, generally involve acrocentric chromosomes. We present an infertile male referred for genetic analysis with a pure balanced homologous 2;2 translocation and normal sperm in the ejaculate.

Methods Conventional cytogenetic and fluorescence in situ hybridization (FISH) were used in karyotype and sperm analysis, respectively.

Results Male's karyotype revealed a pure balanced translocation involving homologous chromosomes 2: 46,XY,t(2;2)(p23;q21.2). Sperm analysis by FISH revealed the presence of 15.8 % of normal and 84.2 % of abnormal spermatozoa for chromosome 2.

Conclusions This is the first report of confined gonadal mosaicism in a pure homologous non-acrocentric chromosome translocation carrier. Preimplantation genetic diagnosis for chromosome 2 should be offered as a reproductive option.

Keywords Confined gonadal mosaicism · Homologous chromosome translocation · Male infertility · Preimplantation genetic diagnosis · Sperm analysis

Introduction

Infertility affects about 15% of couples in reproductive age, and male factor infertility accounts for approximately 50% of cases. There are numerous known causes of male infertility, associated with genetic and non-genetic conditions [11]. Among genetic ones, cytogenetic abnormalities, both somatic and meiotic, are a major cause of male infertility [7].

Somatic chromosomal abnormalities can be numerical or structural, and one of the most common are rearrangements of acrocentric chromosomes [7]. Carriers of balanced rearrangements are phenotypically normal, but they are at high risk of infertility, recurrent spontaneous abortions and phenotypically abnormal offspring [1,13]. Translocations usually occur between two non-homologous chromosomes. Translocations between homologous chromosomes are extremely rare, the majority involving acrocentric chromosomes [6]. Unlike translocations between two non-homologous acrocentric chromosomes, these have no chance of having normal offspring as they only produce abnormal gametes [5,6]. However, it was recently described the presence of normal sperm in the ejaculate of a homologous Robertsonian translocation carrier [2]. This report opened a new line of investigation for the fertility potential of men with homologous chromosome translocations.

A survey of the literature revealed only two reports regarding homologous non-acrocentric chromosome translocations, involving chromosomes 2 and 7 [9,10]. We here report the presence of a balanced translocation between both homologous chromosomes 2 and the presence of normal sperm in the ejaculate of an infertile man.

Capsule A homologous 2;2 chromosome translocation male carrier showed 15.8 % of normal spermatozoa for chromosome 2. Preimplantation genetic diagnosis for chromosome 2 should be offered as a reproductive option.

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Materials and methods

A 31-year-old man and a 30-year-old woman with an infertility history of 7 years and one spontaneous abortion were referred to our department for genetic analysis. The man presented normal physical examination but semen analysis revealed severe oligoasthenozoospermia (2.5×10^6 spermatozoa/mL). The woman had normal ovarian function.

Peripheral blood was collected for lymphocytes culture and chromosomes banded with Leishman stain as described elsewhere [4]. Male's karyotype revealed a pure balanced translocation involving homologous chromosomes 2: 46, XY,t(2;2)(p25.1;q23) (Fig. 1). A total of 100 metaphases were analyzed in order to discard possible mosaicism. The partner showed normal karyotype: 46,XX. Additionally, Yq11.2-AZF region and DAZ gene copies were normal.

Fluorescence in situ hybridization on spermatozoa

Semen sample was collected by masturbation after a period of 3 days of abstinence. After liquefaction (30 min, 37 °C), semen sample was centrifuged for 20 min at 437 g, the seminal liquid removed and about 5 ml of warmed phosphate buffered saline solution (PBS, Sigma, Barcelona, Spain) added. The sample was left at 37 °C for 10 min, washed twice in PBS (2× 10 min, 437 g) and then fixed with methanol/acetic acid (fixative solution; 3:1; VWR International, Stockholm, Sweden/Pan-reac, Barcelona, Spain). After two washes with fixative solution (2× 10 min, 437 g) the sample was kept at −20 °C until fluorescence in situ hybridization (FISH) procedure.

FISH was performed using a probe mixture containing TelVysion 2p (VIJyRM2052; Spectrum Green) and TelVysion 2q (D2S447; Spectrum Orange), both from Abbott Molecular (Illinois, USA). Briefly, 10 µl of fixed semen sample was spread on a slide, washed in saline-sodium citrate buffer (SSC; Invitrogen, Scotland, UK; 1:10), dehydrated in ethanol series and sperm heads decondensed with 1,4-dithiothreitol (DTT; Roche Applied Systems, Penzberg, Germany). Spermatozoa and probe mixture were denatured separately and hybridization occurred overnight at 37 °C in a humidified chamber. In order to counterstain the DNA (blue), 5 µl of 4'-diamidino-2-phenylindole (DAPI; Abbott Molecular) were applied. Spermatozoa were observed and analyzed in a fluorescence microscope (Axio Imager Z1, Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA) fitted with a CCD camera (AxioCam MRm, Zeiss) and an automated image software (FISH Imaging System, version 5.1, MetaSystems GmbH, Altlussheim, Germany).

Results

FISH analysis revealed the presence of spermatozoa with different segregation patterns (Fig. 2). The analysis of a total of 481 spermatozoa showed the presence of 15.8 % of normal sperm (monosomy 2) and 84.2 % of abnormal sperm for chromosome 2. From these, 67.2 % showed disomy 2q, 3.1 % disomy 2p and 13.9 % had other segregations (Fig. 2).

Fig. 1 **a** Male's karyotype; **b** Chromosome 2 ideogram and respective derivatives chromosomes resulted from the homologous translocation involving p25.1 and q23 chromosome 2 bands

Fig. 2 Sperm cells after hybridization with TelVysion 2p (Spectrum Green) and TelVysion 2q (Spectrum Red). **a** Normal spermatozoa (1× green, 1× orange); **b** Abnormal spermatozoa with disomy 2q (2× orange); **c** Abnormal spermatozoa with disomy 2p (2× green)

Discussion

Most translocation carriers are potentially fertile but produce high numbers of unbalanced gametes that frequently result in spontaneous abortions. Preimplantation genetic diagnosis is usually offered to these patients in order to transfer only chromosomally normal or balanced embryos, increasing the probability of a successful pregnancy. In male translocation carriers, fluorescence in situ hybridization on sperm is useful on determining the meiotic segregation patterns most present and usually helpful on predicting the chances of conceiving [3].

Balanced homologous translocations are rare and usually occur between acrocentric chromosomes. The main mechanism proposed for homologous acrocentric translocations

origin is mitotic and when exceptionally present in a chromosomally normal conceptus, mosaicism is expected to be seen [12]. In theory, carriers of these rearrangements have no chance of having normal offspring as they only produce abnormal gametes, resulting in monosomic or trisomic zygotes for the translocated chromosome [5]. Genetic counseling for these patients should include assisted reproductive technologies with donor sperm or ovum.

Balanced homologous translocations between non-acrocentric chromosomes are extremely rare and only two reports are found in the literature [9,10]. We report a homologous 2;2 translocation in a infertile man. Although parents karyotype was not possible to be performed, it is conceivable that it is a *de novo* rearrangement, originated during the first

Fig. 3 Mechanism suggested for gonadal mosaicism origin. In the fetal testis, primordial germ cells (PGCs) enclosed in the tubules form a population of gonocytes with proliferative activity. After birth and starting the reproductive life, gonocytes give rise to a population of spermatogonia. We suggest that at some point of this proliferative activity (*double arrow*), an error has occurred originating different

pools of spermatogonia: **a** spermatogonia with the same genetic material as lymphocytes, i.e., with a balanced translocation involving homologous chromosomes 2; **b** spermatogonia normal for chromosome 2. These second pool of normal spermatogonia would be in the origin of the 15.8 % normal sperm for chromosome 2 analyzed

divisions after fertilization. As previously described [6], the presence of mosaicism for a normal cell line should be expected unless the rearrangement occur in the first zygotic cleavage where the chromatin is more unstable and more prone to rearrangements. In the present case, no normal cell line was found on lymphocytes and so, theoretically, no normal gametes and a successful pregnancy are expected. Even so, cryptic mosaicism is not possible to discard when analyzing only lymphocytes [6]. Mosaicism in other tissues is a hypothesis to be considered.

In order to search for possible confined gonadal mosaicism, fluorescence in situ hybridization for chromosome 2 was performed in sperm. Normal sperm for chromosome 2 were found confirming the gonadal mosaicism and increasing the fertility potential of this patient. The possible mechanism involved in this mosaicism is represented in Fig. 3.

In the presence of 15.8 % of normal sperm, preimplantation genetic diagnosis can be offered in order to select and transfer normal embryos and, hopefully, achieve a viable pregnancy [8]. These results underline the need for an extremely good investigation in cases of very poor reproductive prognosis, where donor sperm or adoption would be, otherwise, the only option.

This is the first report of a man with a balanced non-acrocentric homologous chromosome translocation with a normal sperm cell line.

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