Mosaicism for an unbalanced Y;21 translocation in an infertile man: a case report

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Introduction

Approximately 30 % of male infertility cases are considered ‘idiopathic’ and an additional 40 % have only insufficient/uncertain causes (e.g. varicocele, infections) [1]. It is also estimated that about 30 % of azoo-/oligozoospermia are caused by chromosomal abnormalities (such as 47,XXY or balanced translocations), Y-chromosomal deletions or mutations in genes involved in germ cell production and function [2]. Compared to newborns, infertile males have a higher prevalence of autosomal reciprocal translocations [3], but translocations involving the sex chromosomes are rare [4]. The frequency of Y;autosome translocations in the general population was estimated to be 1 in 2000 [5]. In the more common forms, the heterochromatic region of the long arm of the Y chromosome is translocated onto the short arm of acrocentric chromosomes and fertility is usually not affected in carriers [6]. In contrast, the rare translocations of the euchromatic part of the Y chromosome are frequently associated with azoospermia [7]. Whenever one of the Azoospermia Factor (AZF) regions on Yq is affected by the translocation,

this can be considered the cause for azoo- or severe oligozoospermia [8]. Incorrect or incomplete X/Y pairing during meiosis leading to defective sex vesical formation and consequently to spermatogenetic arrest may be another explanation for infertility of Y;autosome translocation carriers [9, 10].

Translocation carriers have an increased risk for offspring with unbalanced chromosomal aberrations and patients with unbalanced Y;autosome translocations are frequently mentally retarded, depending on the affected autosomal region [11]. The frequency of unbalanced spermatozoa may vary considerably depending on the translocation and utilising fluorescence in situ hybridisation (FISH) may help in assessing the individual risk.

Case report

An otherwise healthy patient presented at the Centre of Reproductive Medicine and Andrology, University of Münster, at the age of 33 years because of couple infertility. The general medical history and specific andrological anamnesis were unremarkable. Clinical examination including genital exam was normal. Sonography of the scrotal contents revealed reduced echogenicity of the testes but otherwise normal findings. Testicular volumes were calculated according to the ellipsoid method as 18 and 14 ml for the right and left testis, respectively. Semen analyses showed severe oligozoospermia with sperm concentrations of 1.6 and 4.6 Mill./ml, 52 % and 28 % progressively motile (a+b) spermatozoa and 2 % and 3 % spermatozoa with normal forms in two samples 3 months apart (analysis according to World Health Organization guidelines [12]). Hormone analyses showed normal luteinising hormone (LH, 2.8 U/l, lab normal range 2–10 U/l) and testosterone (18.6 nmol/l, normal range >12 nmol/l) levels and slightly

Capsule This is an extraordinary case of mosaicism for an unbalanced Y;autosome translocation in an infertile but otherwise healthy man. FISH on spermatozoa may have clinical implications concerning pregnancy chances and preimplantation genetic diagnostics.

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increased follicle stimulating hormone (FSH, 7.6 U/l, lab normal range 2–7 U/l). During our follow-up, the couple did not proceed to any IVF-treatment. All analyses were performed after written informed consent.

In our centre, all patients with sperm concentrations below 10 Mill./ml are routinely offered karyotyping. If the sperm concentration is below 5 Mill./ml, also screening for Y-chromosomal microdeletions is recommended.

Chromosomal analysis and FISH

Metaphase chromosomes were prepared from peripheral blood cells using standard techniques and GTG-banded. All FISH analyses were carried out using standard techniques as described elsewhere [13]. Specific centromeric probes for the X and Y chromosome (DXZ1, DYZ3; Kreatech, Amsterdam, The Netherlands), whole chromosome probes for chromosome 21 and Y (Whole Chromosome 21 and Y; Kreatech), specific subtelomeric probes for 21qter (D21S1446; Kreatech), XYpter (DXYS130; Kreatech), XYqter (DXYS224; Kreatech), and specific probes for chromosomal region 21q21.3 (BAC clone RP11-31B6; BACPAC Resources Oakland, California, U.S.A.) and 21q22.3 (BAC clone RP11-446L19) were used. BAC DNA was isolated using the Nucleobond-AX kit (Machery & Nagel, Düren, Germany). BAC clone RP11-31B6 was labelled with SpectrumOrange dUTP (Abbott, Wiesbaden, Germany) and RP11-446L19 with SpectrumGreen dUTP (Abbott) using the Nick Translation Kit (Abbott) according to the manufacture's

instruction. The labelled products were purified by co-precipitation with 1 µg Cot DNA (ArrayGrade KREAcot DNA, Kreatech). The pellets were then resuspended in FISH Hybridization Buffer (FHB; Kreatech). For each probe 50 metaphases were evaluated. For epifluorescence microscopy an Axioskop 2 Plus photomicroscope (Carl Zeiss, Germany) was used. Multicolor digital images were recorded using the Isis Fluorescence Imaging System (MetaSystems).

Array-CGH

Array-CGH was performed on a research basis to further characterise the translocation. DNA for array-CGH analysis was extracted from peripheral blood cells using standard methods. Whole-genome array-CGH analysis was performed on the 400 k Array Set (Agilent Technologies, Santa Clara, California, USA) as described elsewhere [14]. In short, 1.0 µg of the patient's DNA and 1.0 µg of a pooled DNA probe from 10 male controls were labelled and processed according to the Agilent protocol "Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis" (Version 4.0; June 2006). The array was scanned using a Microarray Scanner (G2565BA, Agilent Technologies). The spot intensities were measured using "Feature Extraction Software" (Version 9.5.31, Agilent Technologies) and further characterised using "CGH Analytics" (Version 4.0.76; Agilent Technologies). All genomic positions are described according to NCBI build 36.1 March 2006 (hg18).

Fig. 1 Cytogenetic results of the aberrant blood cells of the patient. **a** GTG banding. **b** FISH analysis using BAC clone RP11-31B6 (21q21.3; in orange), RP11-446L19 (21q22.3; in green) and a centromeric probe

for the Y chromosome (DYZ3; in blue). **c** FISH analysis using a probe for the subtelomeric region Xpter/Ypter (in green) and for Xqter/Yqter (in orange)

Fig. 2 Array-CGH analysis of the patient's DNA. **a** Chromosome view showing loss of chromosomal region 21q11.2-21q21.2 encompassing the region 14,125,179 to 23,298,289 bp. **b** Gene view demonstrating loss of 13 genes

AZF microdeletion screening

Routine screening for microdeletions of the azoospermia factor regions (AZF) on the long arm of the Y chromosome was performed by multiplex-amplification according to current guidelines [15]. In short, 6 Y-specific chromosomal loci were amplified in two PCR-reactions with markers in the *ZFX/Y*- and *SRY*-gene as internal controls. Format A: *ZFX/Y*, *SRY*, sY254, sY86, sY127; format B: *ZFX/Y*, *SRY*, sY84, sY134, sY255.

FISH analysis in spermatozoa

A smear of 3–10 µl of the semen sample was prepared on a clean glass slide and the sperm concentration was evaluated under a microscope. The spermatozoa were fixed for 10 min and dehydrated in 70, 80, and 100 % Ethanol at –20 °C for 5 min each. The spermatozoa were then denatured for 8 min in 0.5 M NaOH and again in 70, 80, and 100 % EtOH at room

Table 1 Results of the FISH analyses on 200 native spermatozoa

21q21.1 (red)	21q22.3 (green)	Xcen (yellow)	Ycen (blue)	chromosomal content	Native spermatozoa [percentage (N)]
1	1	0	1	Y/21	45 % (90)
1	1	1	0	X/21	28 % (56)
1	1	0	0	-/21	9.5 % (19)
0	0	1	0	X/-	2.5 % (5)
1	2	0	1	derY/21	1 % (2)
2	1	0	1	?	2 % (4)
2	0	0	0	?	2 % (4)
2	2	0	1	?	1.5 % (3)
2	1	0	0	?	1.5 % (3)
2	1	1	0	?	1 % (2)
1	0	1	0	?	1 % (2)
1	0	0	2	?	1 % (2)
1	0	0	1	?	1 % (2)
other constellations found once					3 % (6)

temperature for 2 min. The four probes used (RP11-31B6, RP11-446L19, DXZ1, and DYZ3) were denatured at 90 °C for 10 min and hybridised simultaneously overnight. After hybridisation, the slides were washed in 0.4x SSC and 0.3 % NP40 at 72 °C for 2 min and in 2x SSC, 0.1 % NP40 for 2 min at room temperature. After drying, the spermatozoa were counterstained using DAPI. Spermatozoa were evaluated according to accepted criteria [16]. Only intact, non-overlapping spermatozoa were selected for scoring.

Results

Cytogenetic analysis of 30 metaphase spreads at a 550 band level revealed a mosaicism of 10 metaphases with an unbalanced Y;21 translocation $\text{der}(Y)t(Y;21)(q12;q21)$ and loss of one chromosome 21 and 20 metaphases with an apparently normal male karyotype (46,XY) (Fig. 1a). The karyotype was determined with 45,X,der(Y)t(Y;21)(q12;q21),-21[10]/46,XY[17]. FISH analysis of 50 metaphases each confirmed the mosaicism for the unbalanced translocation. The aberrant metaphases demonstrated only one signal for BAC clone RP11-31B6 (located in chromosomal region 21q21.1) on the normal chromosome 21. One signal of BAC clone RP11-446L19 (located in chromosomal region 21q22.3) was found on the normal chromosome 21, whereas a second signal was seen on the derivative Y chromosome (Fig. 1b). The signal of the subtelomeric Yq-probe was also present on the derivative Y chromosome (Fig. 1c).

Array-CGH demonstrated a loss of approximately 9.2 Mb in the chromosomal region 21q11.2-q21.2 (Fig. 2). The start positions of the oligonucleotides in the breakpoint regions are

as follow (from pter to qter): last normal oligonucleotide 14,092,291, first oligonucleotide in the deleted region 14,125,179, last oligonucleotide in the deleted region 23,298,289, first normal oligonucleotide 23,320,327. Furthermore, array-CGH confirmed the mosaicism found in the conventional and molecular cytogenetic analysis. 13 genes are located in the deleted region: *LIPI*, *RBM11*, *STCH*, *SAMSN1*, *NRIP1*, *USP25*, *C21orf34*, *CXADR*, *BTG3*, *C21orf91*, *CHODL*, *PRSS7*, *NCAM2*. All other copy number variations detected by array-CGH were located in published polymorphic regions as described in the Database of Genomic Variants (<http://projects.tcag.ca/variation/> Accessed May 30, 2013).

Amplicons for all analysed AZF markers could be detected through gel electrophoresis excluding complete AZF deletions.

Altogether 200 spermatozoa were analysed by FISH. The results are summarised in Table 1 and representative FISH images are shown in Fig. 3. Our internal cut-off level for false-positive or negative hybridisation was 5 %. Therefore, only the constellations Y/21 (45 % of spermatozoa), X/21 (27.5 %) and -/21 (9.5 %) were considered informative.

Discussion

We report on an infertile man with oligozoospermia who demonstrated a mosaicism of cells with a normal male karyotype and cells with an unbalanced Y;21 translocation in approximately one third of his peripheral blood cells. This unbalanced translocation led to loss of approximately 9.2 Mb of the proximal part of chromosome 21 containing 13 genes. In contrast, no loss of Y-chromosomal sequences could be detected by array-CGH. Fittingly, FISH analyses showed presence of the

Fig. 3 Four colour-FISH on sperm nuclei using BAC clone RP11-31B6 (21q21.3; in orange), RP11-446L19 (21q22.3; in green), centromeric probe for the X chromosome (DXZ1; in yellow), and a centromeric probe for the Y chromosome (DYZ3; in blue). **a/b** The same sperm nucleus (**a** the raw data; **b** processed figure) showing a normal chromosome 21 and an X chromosome. **c** Sperm nucleus with a normal chromosome 21 and a Y chromosome. **d/e** The same sperm (**d** raw data; **e** processed figure) demonstrating only a normal chromosome 21. **f** Sperm nucleus with only one X chromosome

specific subtelomeric probe on Yq and, therefore, the breakpoint was located close to the telomere of Yq. Because blood lymphocytes were analysed, cells of other germ layers may exhibit other ratios of normal vs. unbalanced cells. The parents of the patients were not available for analysis, but as in any case of mosaicism it was most likely a *de novo* translocation.

Y;autosome translocations are rarely found [5]. To our knowledge, there are no reports describing infertile men with unbalanced Y;autosomal translocations leading to loss of autosomal regions without loss of Y-chromosomal genes. Most published cases demonstrated loss of partial regions of the Y chromosome [6]. On the other hand, unbalanced Y;autosome translocations may lead to genital anomalies in combination with features of an autosomal deletion syndrome depending on the deleted region [7]. Sex chromosome;autosome translocations have been reported to have stronger effects on fertility than autosome;autosome translocations [18]. Hsu (1994) described 25 infertile men with Y;autosome translocations of which 80 % had azoospermia, which seems to be the most frequent phenotype in carriers [6, 7, 19] in contrast to oligoasthenoteratozoospermia in other cases [17, 20, 21]. In our case, the mosaicism may explain the relatively mild phenotype with oligozoospermia. For most of the 13 genes deleted on 21q, no functional information is available and none have been associated with spermatogenesis or infertility.

The FISH analyses showed a normal signal pattern with one chromosome 21 and either X or Y in the majority of spermatozoa (73 %). In contrast, only one normal chromosome 21 was found in 9.5 % lacking a signal for either sex chromosomal probes. Fertilisation with these spermatozoa will lead to a 45,X embryo, i.e. Turner syndrome, with a very high chance of spontaneous termination during the first and second trimester. A derivative Y chromosome and a normal chromosome 21 were seen in two spermatozoa, which would lead to an unbalanced karyotype 46,X,der(Y)t(Y;21)(q12;q21) with a partial trisomy of the distal part of the long arm of chromosome 21 (21q21 to 21qter). Children with this karyotype will present features of Down syndrome [7]. Five spermatozoa with only an X chromosome and without a chromosome 21 were also found. These spermatozoa would lead to fertilized eggs with monosomy 21 and, hence, to an early miscarriage. Spermatozoa with other signal patterns were observed in only a few cases each, for which aberrant chromosomal constellations cannot be distinguished from technical artefacts.

The high level of spermatozoa without sex chromosomes may be caused by the loss of the telomere of the long arm of the Y-chromosome, because the telomeres are required for each chromosome to find its homologous partner and undergo meiotic recombination. In males, sex chromosome pairing and recombination are restricted to the pseudoautosomal regions (PAR) [9, 22]. The unbalanced Y;21-translocation described herein leads to loss of the telomeres as part of the PAR on Yq as well as of the derivative chromosome 21. Thus, the sex

chromosomes and chromosome 21 of the aberrant clone may lead to an incorrect meiotic segregation and are missing in a significant fraction of spermatozoa.

In conclusion, the results of the chromosomal analysis, array-CGH and FISH indicate that the oligozoospermia in this case is caused by mosaicism for an unbalanced Y;21-translocation, most likely through a disturbance in meiotic pairing and segregation. The observation of a high percentage of apparently normal spermatozoa can be considered as good prognostic marker for the couple if undergoing in vitro fertilization with ICSI. However, pre-implantation genetic screening (PGS) should be offered to the couple to increase pregnancy rate and avoid later termination due to an aberrant karyotype (most likely 45,X).

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