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Short Report

Cytogenetic and molecular characterization of the derivative Y chromosome: a case study of an azoospermic patient

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The success of infertility treatment depends on the underlying cause and severity of the infertility problem. The current report addresses the complex genotype–phenotype interactions in an azoospermic man. Cytogenetic, molecular cytogenetic and molecular genetic studies indicated the derivative monocentric Y chromosome with duplication of Yp11 (including *SRY* gene) and partial deletion of Yq11 (including azoospermia factor – AZFb-c regions) as the most probable cause of the severe testicular failure. Our study emphasizes the importance of detailed genetic analysis in male infertility evaluation and helps to estimate the outcome of infertility treatment.

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Cytogenetic aberrations occur in 10–15% of non-obstructive azoospermic patients (1). The most frequent aberrations found in azoospermic men are numerical or structural sex chromosome abnormalities and robertsonian/reciprocal translocations. Structural anomalies of the Y chromosome are particularly hazardous for male reproductive function, as this chromosome is essential for normal testicular differentiation and spermatogenesis (2, 3). Among the structural abnormalities of the Y chromosome, ring chromosomes and isochromosomes/isodicentric chromosomes of both short (p) and long (q) arms are the most prevalent causes of spermatogenic failure and azoospermia (4, 5).

In addition to microscopically visible Y chromosomal aberrations, microdeletions in the Yq euchromatin region (Yq11) encompassing three distinct azoospermia factor (AZF) regions, AZFa-c, are found in 10% of non-obstructive azoospermia cases (6). AZF candidate gene mapping studies focusing on approximately 10 Mbp of Yq11 have revealed the complex and repetitive nature of this genomic region containing several putative single genes (*USP9Y* and

DBY from AZFa) and gene families (*RBMY* and *DAZ* from AZFb and c, respectively) that participate in a variety of spermatogenesis supporting functions (6). However, AZF microdeletions are difficult to correlate with different spermatogenic phenotypes because of genomic plasticity in the AZF region and the diverse polymorphic deletions that occur in fertile men (7).

In the present case report, we unraveled the complex genotype–phenotype relationships in an azoospermic man using: (i) cytogenetic (G-banding technique) and molecular cytogenetic (fluorescence *in situ* hybridization – FISH) analysis of chromosomes from peripheral blood lymphocytes, and (ii) Y chromosome deletion mapping of genomic DNA extracted from peripheral blood and testicular tissue by polymerase chain reaction (PCR) and Y chromosome specific sequence tagged site (STS) markers.

Materials and methods

Clinical characteristics

The study was approved by the Tallinn Medical Research Ethics Committee and was performed on a 38-year-old infertile male patient. Upon physical examination, the patient was found to be appropriately virilized and exhibited normal physical measurements (height: 184 cm; weight: 103 kg and body mass index: 30.4 kg/m^2). External genital examination did not reveal any abnormalities. Reduced testicular volumes (12 ml right and 10 ml left) were measured using a Prader orchidometer and left side grade III and right side grade II varicoceles were diagnosed by palpation. Serum testosterone (T) level was 20.8 nmol/l, which is within the normal range (9.9–52.4 nmol/l). However, follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were slightly elevated (FSH 12.1 mIU/ml, normal value 0.7–11.1 mIU/ml and LH 8.3 mIU/ml, normal value 0.8–7.6 mIU/ml). Semen analyses showed complete azoospermia. The histological examination of the testicular tissue revealed spermatogenic arrest in meiosis, confirming the diagnosis of non-obstructive azoospermia.

Cytogenetic and molecular cytogenetic studies

Cytogenetic analysis was performed on peripheral blood lymphocytes using the GTG-banding (G-banding by trypsin-giemsa technique) method (8). Chromosome spreads were prepared, and 20 metaphases were analyzed at the resolution level of 400–500 G-bands *per* haploid chromosome set. FISH probes specific for *SRY*/DXZ1 loci (LSI SRY, SpectrumOrange/CEP X, SpectrumGreen), Y centromeric DYZ3 locus (CEP Y alpha satellite, SpectrumOrange) and Y heterochromatin specific DYZ1 locus (Satellite III, SpectrumAqua) were used to study X and Y chromosomes (Vysis, Downers, IL). FISH images from mitotic chromosomes and interphase nuclei were visualized using Axioplan 2 Imaging fluorescent microscope (Carl Zeiss, Jena, Germany), were captured by digital camera CV-M4+/M4+CL (JAI, Copenhagen, Denmark) and were analyzed using ISIS 5.0 imaging software (MetaSystems, Altlussheim, Germany).

Molecular genetic studies

Genomic DNA extracted from peripheral blood lymphocytes and testicular tissue was used for PCR with primers specific for 22 STS markers (SRY, M130, M12, sY86, sY84, M168, M46, sY87, M201, M214, sY88, M438, M436, M3, M1, M147, M73, M94, sY127, sY134, sY254, and sY255) covering the whole euchromatic region of Y chromosome p and q arms (9, 10). One STS marker was specific for *SRY* gene, while six STS markers were chosen for AZF regions – AZFa: sY86 and sY84; AZFb: sY127 and sY134; and AZFc: sY254 and sY255. The blood DNA sample of a fertile man was used as a positive control [Fig. 1(**a**)]. PCR products were visualized under the UV light using ethidium bromide staining after the electrophoresis.

Results

Karyotype analysis of G-banded lymphocyte chromosomes demonstrated a normal number of chromosomes, with structurally intact X chromosome and autosomes. However, the Y chromosome was remarkably smaller than would normally be expected. FISH using CEP X/*SRY* probe mixture revealed one X chromosome centromeric signal and two *SRY* specific signals in the terminal segments of both Y chromosome arms (Fig. 2). When Y chromosome centromeric alpha satellite probes were used, one fluorescent signal was displayed (data not shown), confirming the monocentric nature of the derivative Y chromosome – der(Y). In addition, FISH using Y chromosome satellite III probes did not show specific labeling (data not shown), indicating that the whole Y chromosome heterochromatic Yq12 region was missing.

Deletion mapping of the Y chromosome using amplification of 22 STS markers disclosed an 8-Mbp deletion of the Yq11 region [Fig. 1(**b**)]. The

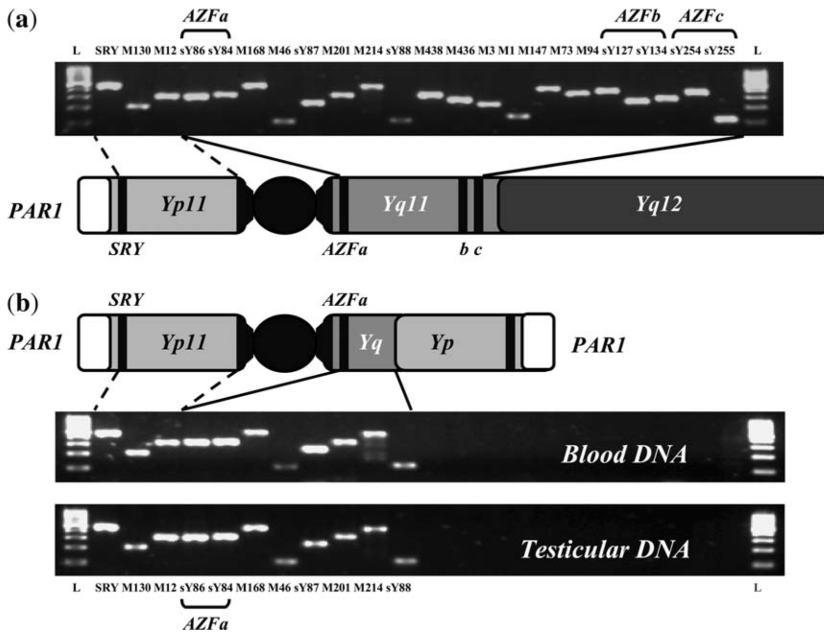

Fig. 1. (**a**) Schematic presentation of an intact Y chromosome with the sequence tagged site (STS) markers analyzed. (**b**) Derivative Y chromosome with partial duplication of Yp and partial deletion of Yq, encompassing AZFb-c region. The following STS markers were analyzed: SRY, M130, M12, sY86, sY84, M168, M46, sY87, M201, M214, sY88, M438, M436, M3, M1, M147, M73, M94, sY127, sY134, sY254, and sY255. AZF denotes azoospermia factor, PAR1 marks pseudoautosomal region 1 and L indicates DNA molecular weight ladder (GeneRuler™ 100 bp DNA Ladder; MBI Fermentas, Vilnius, Lithuania).

deletion breakpoint was localized distally to STS sY88 (present) and proximally to STS M438 (deleted). Considering the location of these STS markers on the Y chromosome map (11), the predicted breakpoint for our case is situated distally to interval 5D and proximally to interval 5J in Yq11.21. Additionally, no PCR-amplification was observed for the AZFb (sY127 and sY134) and AZFc (sY254 and sY255) regions neither with DNA from blood nor testicular tissue, while PCR products from the AZFa (sY86 and sY84) region were present in both reactions [Fig. 1(**b**)].

Cytogenetic, molecular cytogenetic and molecular genetic studies allowed the patient's karyotype to be interpreted as: 46,X,der(Y)(pter→q11.2::p11.3→pter).ish der(Y)(DYZ3+,DYZ1-,SRY++). In general, we identified a monocentric der(Y) with: (i) segmental or complete duplication of Yp (including *SRY* gene), locating in both chromosome arms; (ii) partial deletion of the euchromatic Yq11, encompassing AZFb-c; and (iii) deletion of the heterochromatic Yq12 region [Figs 1(**b**) and 2].

Discussion

In the current report, der(Y) was identified in a non-obstructive azoospermia patient with histologically verified spermatogenic arrest in meiosis. Y chromosome deletion mapping revealed an extensive 8-Mbp Yq11 deletion, encompassing AZFb-c spermatogenic loci, as a very likely causative factor for the testicular failure. Indispensable spermatogenic *RBMY*-AZFb and *DAZ*-AZFc genes share two common properties: both of them form families and code for testis-specific proteins with RNA-binding activity (6). Large AZFb-c microdeletions occur in 8% of AZF-microdeletion infertility patients and almost

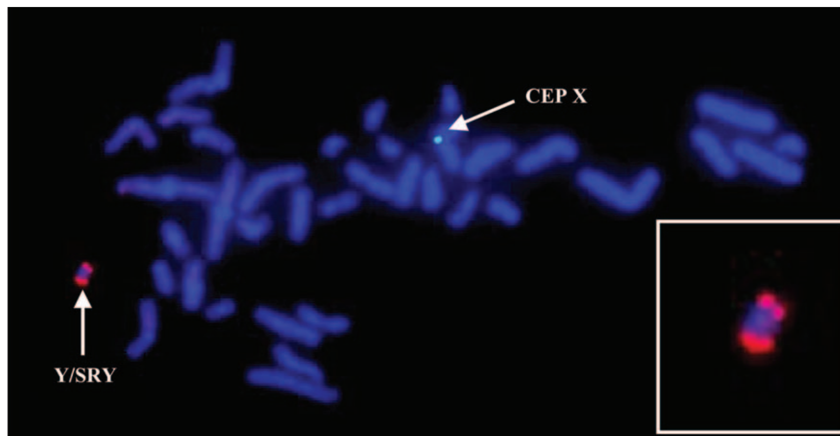

Fig. 2. Fluorescence *in situ* hybridization on metaphase chromosomes using CEP X/*SRY* specific probes. Arrows indicate single X chromosome centromeric signal (green) and two *SRY* specific signals (red) on both ends of the Y chromosome.

certainly result in the complete absence of spermatozoa (6). In accordance, our patient revealed complete azoospermia with spermatogenic arrest in meiosis.

Additionally, the disturbed X and Y chromosome pairing in meiosis can led to severely impaired spermatogenesis. Even though, Xp/Yp pseudoautosomal regions (PAR1) play a key role in the X-Y sex chromosome bivalent formation, this process may be temporarily facilitated by the entire euchromatic Yq (12). Consequently, major cytogenetic aberrations in der(Y) may substantially interfere with the meiotic stages of spermatogenesis, leading to the developmental arrest of male germ cells.

The der(Y) of our patient has a monocentric structure, as evidenced by the single signal observed in FISH analysis with Y chromosome centromere specific alpha satellite probes. This finding is unusual as dicentrics of both p and q arms are the most prevalent Y chromosome abnormalities (13) being often present as part of a mosaic karyotype including a 45,X cell line. Two functional centromeres can lead to profound defects in mitotic chromosomal segregation by bipolar attachment of a single chromatid, anaphase lag and subsequent loss of the entire Y chromosome. Because of the variable distributions of 45,X cells, these mosaic individuals exhibit a wide spectrum of phenotypes from normal men through mixed gonadal dysgenesis to women with Turner syndrome (13). Infertile men with AZF microdeletions have also been associated with the occurrence of 45,X cell line, indicating the general instability of aberrant Y chromosomes in cellular divisions. In extreme cases, the presence of 45,X cell line in a 46,XY AZF-microdeleted infertile patient may lead to an ambiguous genitalia phenotype (7). In this study, we found der(Y) chromosome in all blood cells examined from an infertile male exhibiting appropriate virilization and normally developed external sexual organs.

Our case has a uniform karyotype of 46,X,der(Y), which supports the idea that the abnormal Y chromosome arose during meiosis in the patient's father or in very early stages of embryogenesis. Although not entirely understood, the der(Y) chromosome formation could have involved breaks in Yp11.3 in one chromatid, and Yq11.2 in another chromatid with subsequent joining of segments originally found on different chromatids. These rearrangements resulted in the der(Y) chromosome with a duplication of Yp-*SRY* region and a partial deletion of Yq. *SRY*, the sex-determining region on Yp, codes for a transcription factor that acts as a master regulator in testis development (3). We found *SRY* specific sequences in both terminal regions of der(Y), resembling the pattern in Yp isochromosomes (4). These patients commonly exhibit normal male sexual differentiation but disturbed spermatogenesis because of the lack of AZF genes.

In conclusion, our report addresses the unique interactions between genotype and fertility-phenotype in an azoospermic patient with a derivative Y chromosome. The current report emphasizes the importance of detailed genetic studies of infertile male patients and helps to estimate the outcome of infertility treatment.

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