Case Report

An Infertile Azoospermic Male With 45, X T(Yp;15) Karyotype

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Abstract

Objective: 45, X is a very rare condition that usually results from Y/autosomal translocations or insertions. Here we present an infertile azoospermic man who had 45, X t(Yp;15) karyotype and deletion of AZF (azoospermia factor) gene region.

Case report: A 35-year-old infertile azoospermic man with a typical male appearance came for infertility genetic counseling. He was infertile for more than ten years and had short height. High-resolution of metaphase chromosomes of 50 peripheral white blood cells were analyzed for karyotyping. Fluorescence in situ hybridization (FISH) analysis and Polymerase chain reaction (PCR) were done for SRY and AZF gene localization. Karyotyping and FISH analysis revealed 45, X t(Yp;15) karyotype and no mosaicism. More investigation on the Y chromosome revealed no deletion in the SRY region, but AZF a/b/c were deleted. It was revealed that Yp's subtelomeric region but not Yq was translocated to chromosome 15.

Conclusion: This study shows that despite the lack of a complete Y chromosome in this person, the occurrence of secondary male traits is a result of the short arm translocation of the Y chromosome, which contains the (ex-determining region Y) SRY gene. Infertility is also due to the Y chromosomes long arm's deletion containing the AZF gene region.

Keywords: Aneuploidy; Genetic Translocation; Azoospermia; Sex-Determining Region Y Protein; Fluorescence In Situ Hybridization

Introduction

45, X is a very rare condition that usually results from Y/autosomal translocations or insertions (1, 2). The frequency of Y/autosome translocations is generally about 1 in 2000 (3). Balanced Y/autosome (Y; A) translocations have minimal effects on the phenotype (3) but unbalanced Y; A reciprocal translocations commonly are associated with infertility and azoospermia (4, 5). Almost all autosome chromosomes had been involved in translocation, but acrocentric chromosomes are more common (6-10). The SRY locus, an essential gene in the determination of male phenotype, is located in Yp11.2. This gene encodes the sex-determining region Y protein witch involved in

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male sexual development (11). Another critical genetic loci in male fertility are azoospermia factor (AZF) in Yq11. Disruption of spermatogenesis locus AZF results in spermatogenic failure and male infertility in Y; A translocation (12).

Case report

A 35-year-old azospermic male was referred to the urology clinic, Shahid Akbarabadi Clinical Research Development Unit, due to primary infertility for more than ten years and azoospermia. The general analysis revealed a normal phenotypic male with a height of 160 cm. The patient had normal chest hair. He shaves twice a week. According to urological studies, the size of the penis was standard and sexual activity was regular. Right and left testicular size were 11 ml and 10 ml, respectively. However, testicles were atrophic. Serum hormones analysis revealed 25.1 mU/ml for Follicle-stimulating hormone (FSH), 5.26 mU/ml for *luteinizing hormone* (Alves, #8), and 1.1 nmol/l for total testosterone values. Semen analysis showed normal seminal volume with repeated azoospermia.

Conventional cytogenetic analysis: 50 metaphase spreads obtained from peripheral blood lymphocytes using the Giemsa Banding (GTG-banding) procedures (13). The International System for Human Cytogenetic Nomenclature recommendations, International System for Human Cytogenomic Nomenclature (ISCN), were utilized for Karyotype description (14).

Fluorescence in situ hybridization (FISH): The following probes were used for FISH analysis according to the manufacturer's instructions: Xp/Yp probe (specific for the subtelomeric loci at Xp and Yp, respectively), Xq/Yq probe (specific for the subtelomeric loci at Xq and Yq, respectively) (Vysis FISH probes, Abbott Molecular Inc., Des Plaines IL); The FISH procedure was done according to Pinkel et al. (15).

Genomic DNA extraction: Peripheral blood leukocytes DNA extraction was done using QIAamp DNAMini Kit (Qiagen, Germany).

Mapping of Yq classical microdeletions: Using six sequence-tagged sites (STS) loci primers of AZFa, AZFb, and AZFc and multiplex PCR, microdeletions were analyzed according to the guideline of EAA/EMQN best practice for molecular diagnosis of Y-chromosomal microdeletions (16). The testis-determining factor (SRY gene) on the Yp chromosome was used as an internal control. A DNA sample from a fertile male, blank (water), and a normal female was used as external controls.

Chromosome analysis of 50 metaphases showed the presence of a 45, X karyotype (Figure 1a and b). Although the Y chromosome was not fully present, further karyotype studies showed that the Y chromosome's derivative was translocation on the short arm of chromosome 15.

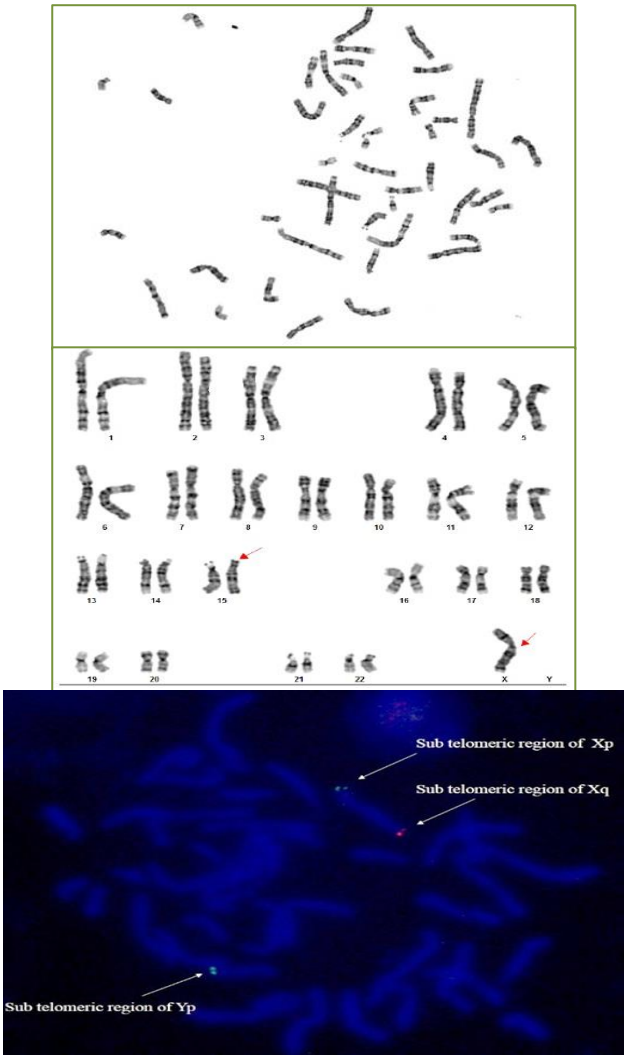

Figure 1a, b and c: The karyotype of the patient involves 45, X,t(Y;15). Red arrows (a and b)) indicate the absence of Y chromosome integrity and the translocation of Yp to chromosome 15. FISH analysis (c) on a metaphase cell spread of the peripheral blood using the subtelomeric probes for chromosome Yp and Yq and Xp and Xq (white arrow) showed the subtelomeric region of Yq was deleted.

FISH analysis using subtelomeric probes of X p and q arms and Y p and q arms revealed that Yq was deleted (Figure 1c). Molecular analysis confirmed the intactness and presence of the SRY gene and deletion of AZFa, b and c regions (data not shown); Table 1 shows STS primer sequences used in multiplex PCR analysis.

Table 1: STS primer sequences used in multiplex PCR analysis

Sequence-Tagged Site	Position, gene, or region name	Amplicon size (bps)	Forward (F) and Reverse (R) primers
sY14	Yp, SRY	214	F: 5'- GAATATTCCCGCTCTCCGGA- 3' R: 5'- GCTGGTGCTCCATTCTTGAG- 3'
sY84	Yq, AZFa	326	F: 5'- AGAAGGGTCTGAAAGCAGGT- 3' R: 5'- GCCTACTACCTGGAGGCTTC- 3'
sY86	Yq, AZFa	320	F: 5'- GTGACACACAGACTATGCTTC- 3' R: 5'- ACACACAGAGGGACAACCCT- 3'
sY127	Yq, AZFb	274	F: 5'- GGCTCACAAACGAAAAGAAA- 3' R: 5'- CTGCAGGCAGTAATAAGGGA- 3'
sY134	Yq, AZFb	301	F: 5'- GTCTGCCTCACCATAAAACG- 3' R: 5'- ACCACTGCCAAAACTTTCAA- 3'
sY254	Yq, AZFc	400	F: 5'- GGGTGTTACCAGAAGGCAAA- 3' R: 5'- GAACCGTATCTACCAAAGCAGC- 3'
sY255	Yq, AZFc	126	F: 5'- GTTACAGGATTCGGCGTGAT- 3' R: 5'- CTCGTCATGTGCAGCCAC- 3'

Discussion

An azoospermic male phenotype with the karyotype of a 45, X-chromosome represents a rare chromosomal abnormality condition. Some of the patients diagnosed as 45, X male are, in fact, the mosaics of 45, X/46,XY (17). But in others, Y-autosome (Y; A) translocations have occurred. In azoospermic males which affects 1% of the male population, chromosomal abnormalities involving X and Y chromosomes range from 10 to 15% (18).

Males with 45, X karyotype due to Y-autosome (Y; A) translocations with unbalanced chromosomal abnormality usually involves the translocation of the Y chromosome containing the SRY gene onto the short arm of an acrocentric chromosome (chromosome 13, 14, 15, 21 and 22) (19, 20). However, non-acrocentric chromosome translocation has also been observed (21).

SRY gene and azoospermia regions are the most critical regions in the Y chromosome for maleness phenotype and fertility, respectively. SRY gene is the primary gene essential for formation of testis and development of the male sex and is located on Yp11.2 (22). Three azoospermia regions on Yq11 euchromatin, designated AZFa, AZFb, and AZFc, are important for normal spermatogenesis and deletion of them results in azoospermia (12, 23).

Here, a male with azoospermia and a 45, X karyotype due to Y/ autosome translocation was reported, which short arm of the Y chromosome containing the Yp subtelomere and SRY gene, translocated onto chromosome 15. We hypothesized that the breakage event resulting in this translocation might have occurred in his father's mitotic division. The acentric segment of 15p must also have been eliminated. The translocation resulted in the deletion of long arm containing AZFa, AZFb, and AZFc, confirmed by molecular analysis.

Conclusion

The 45, X t(Yp;15) karyotype and presence of SRY gene region (located in Yp) and deletion of AZF gene region (located in Yq) resulted in male appearence and infertility, respectively.

Conflict of Interests

Authors have no conflict of interests.

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