An infertile azoospermic male with 45,X karyotype and a unique complex (Y;14); (Y;22) translocation: cytogenetic and molecular characterization

Mona K. Mekkawy¹ · Ahmed M. El Guindi² · Inas M. Mazen³ · Alaaeldin G. Fayez⁴ · Amal M. Mohamed¹ · Alaa K. Kamel¹

Received: 23 December 2017 / Accepted: 10 May 2018 / Published online: 2 June 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Introduction

45,X testicular disorder of sex development (DSD) is a very rare disorder. It usually results from Y/ autosomal translocations or insertion [1–3]. The frequency of Y/ autosome translocations in the general population is generally low occurring in about 1 in 2000 [4].

Y/ autosome (Y;A) translocations are usually balanced and segregate in families with minimal effects on the phenotype [4–6]. On the other hand, unbalanced Y;A reciprocal translocations commonly present with infertility and azoospermia [7–9]. Some patients may present with other presentations related to abnormal sex chromosome dosage as ambiguous genitalia, short stature, and some of the Turner syndrome features. Others may have congenital malformations, and mental subnormality depending on the genes involved in the autosome [2, 3, 10, 11]. Almost all autosomes had been reported, but acrocentrics are the most commonly involved [2, 12–16].

The causative factor of male infertility and spermatogenic failure in Y;A translocation is the disruption of AZF loci by the translocation breakpoint or by a positional effect [17–19]. Another explanation is the occurrence of spermatogenic arrest due to abnormal meiotic pairing with defective X/Y body (sex

vesicle) formation and inactivation of autosomal segment and consequently spermatocyte degeneration [13, 20–23].

Case report

Clinical report

A 32-year-old male patient was referred to the endocrinology clinic, Division of Human Genetics and Genome Research, National Research Centre, due to primary infertility and azoospermia.

General examination showed normal male phenotype with a height of 169 cm. The patient had chest hair and normal pubertal growth (P5/A3) and he shaves twice a week. The patient reported normal sexual activity, including desire, frequency of intercourse, erection, orgasm, and ejaculation.

Genital examination revealed normal male external genitalia. Right testicular size was 12 ml and left testis measured 10 ml. The stretched penile length was 9 cm.

Serum hormones revealed values of 12.4 mU/ml for FSH, 6.1 mU/ml for LH, and 4.1 ng/ml for total testosterone. Semen analysis showed repeated azoospermia with normal seminal volume. Trans-rectal ultrasonography revealed bilateral atrophic changes of seminal vesicles. The patient had performed a previous karyotype which showed 45,X pattern. The patient underwent atesticular biopsy, which revealed, upon revision of the histopathology, Sertoli cell-only syndrome, with complete absence of spermatogenic cells.

Conventional cytogenetic analysis

Chromosomal analysis was done for metaphase spreads obtained from cultured blood lymphocytes using GTG-banding technique [24].

✉ Mona K. Mekkawy
monamekkawy2005@yahoo.com; mekkawy.mona@gmail.com

¹ Human Cytogenetics Department, National Research Centre, 33 El-Buhouth st., Dokki, Cairo 12622, Egypt

² Department of Andrology, Kasr Al Ainy Faculty of Medicine, Cairo University, Kasr Al Ainy St., Cairo 11562, Egypt

³ Clinical Genetics Department, National Research Centre, 33 El-Buhouth st., Dokki, Cairo 12622, Egypt

⁴ Molecular Genetics and Enzymology Department, National Research Centre, 33 El-Buhouth st., Dokki, Cairo 12622, Egypt

Karyotype description followed the International System for Human Cytogenetic Nomenclature recommendations, ISCN, 2016 [25].

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) analysis was carried out with commercial probes according to the manufacturer's instructions. These probes included the following: locus-specific sex-determining region Y (SRY) probe (specific for the SRY gene at Yp11.3); CEP X (DXZ1)/CEP Y (DYZ3) probe set which hybridizes to the alpha satellite DNA at the centromeric region of both X and Y chromosomes; TelVysion Xp/Yp probe (specific for the subtelomeric loci: DXYS 129 and DXYS 153 at Xp and Yp, respectively) (Vysis FISH probes, Abbott Molecular Inc., Des Plaines IL); and short stature homeobox (SHOX) region probe (specific for the short stature homeobox gene) which hybridizes to the SHOX gene region on both Xp22.3 and Yp11.3 and WCP-Y probe (whole chromosome paint Y) (Cytocell Inc., Cambridge, UK). DAPI II was applied as a counterstain for the chromosomes and lymphocyte nuclei. FISH procedure was done following Pinkel et al. [26].

Genomic DNA extraction

DNA was isolated from peripheral blood leukocytes collected on K₂EDTA inside a vacutainer sterile tube. The procedure was done using QIAamp DNAMini Kit (50 preps), Germany <https://www.qiagen.com/eg>.

Mapping of Yq classical microdeletions

Investigation of AZFa, AZFb, and AZFc microdeletions was done using six sequence-tagged site (STS) loci ± multiplex PCR analysis according to EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal

microdeletions [27]. These STS primers (Table 1) have been shown to give reproducible results in multiplex PCR reactions by several laboratories and in external quality control trials. The used STSs are the most clinically relevant STSs to azoospermia condition.

The testis-determining factor on the short arm of the Y chromosome (*SRY* gene) was included in the analysis as an internal control for the presence of Y-specific AZF sequences and for absent Y chromosome. A DNA sample from a fertile male, blank (water), and a female was run in parallel with each multiplex PCR as external controls.

Fine Yq microdeletion breakpoint detection

To determine breakpoints of the deleted Y chromosome segments, additional four STSs were used: sY1240, 1251, 121, and 1201, with primer sequence shown in Table 1.

Results

Chromosome analysis of 50 metaphases showed the presence of a 45,X karyotype with some increase in the satellite size on one of chromosomes 14 and an additional material on one of chromosomes 22: 45,X,14ps+, add (22)(p11) (see Fig. 1).

FISH analysis using SRY gene-specific probe showed a positive SRY signal on one of the D group chromosomes. Combined use of SRY and 14q subtelomere probes detected the SRY gene on the short arm of chromosome 14 (Fig. 2a). Signals for the SHOX gene region (Fig. 2b) and for the telomeric region of Yp and for the Y centromere were detected on 14p.

The Y painting probe revealed a red painting signal on 14p; in addition, a fluorescent DAPI II signal for the Y heterochromatic region was evident on chromosome 22 (Fig. 3).

Molecular studies confirmed the intactness of the *SRY* gene and mapping of AZF regions on Y chromosome

Table 1 STS primer sequences

STSs	Position, gene, or region name	Amplicon size (bps)	Forward 5'—3'	Reverse 5'—3'
sY14	Yp, SRY	214	GAATATTCCTCCGCTCTCCGGA	GCTGGTGCTCCATTCTTGAG
sY84	Yq, AZFa	326	AGAAGGGTCTGAAAGCAGGT	GCCTACTACCTGGAGGCTTC
sY86	Yq, AZFa	320	GTGACACACAGACTATGCTTC	ACACACAGAGGGACAACCT
sY127	Yq, AZFb	274	GGCTCACAACGAAAAGAAA	CTGCAGGCAGTAATAAGGGA
sY134	Yq, AZFb	301	GTCTGCCTCACCATAAAACG	ACCACTGCCAAAACCTTTCAA
sY254	Yq, AZFc	400	GGGTGTTACCAGAAGGCAAA	GAACCGTATCTACCAAAGCAGC
sY255	Yq, AZFc	126	GTTACAGGATTCGGCGTGAT	CTCGTCATGTGCAGCCAC
sY1240	Yp, PCDH11Y	385	GGGTCTAGATAGGCTCCAAG	TTCATGTTGGCAGTGATTGG
sY1251	Yq	422	GACTGGAGTGGAACGGTCTC	TCACTTCCCTCCGATTTTCT
sY121	Yq	190	AGTTCACAGAATGGAGCCTG	CCTGTGACTCCAGTTTGGTC
sY1201	Yq, AZFc	677	CCGACTTCCACAATGGCT	GGGAGAAAAGTTCTGCAACG

STSs sequence-tagged sites

Fig. 1 GTG-banded metaphase and karyotype showing 14ps+ and add (22p)

revealed that sY1251, 84, 86, 127, 134, 254, and 255 were all deleted, with preservation of sY14, 1240 (on Yp), and 1201 (on Yq). This indicates a complete AZFa and AZFb deletion and partial AZFc deletion (Fig. 4); Table 1 shows STS primer sequences.

Discussion

Azoospermia is a severe pathology that affects 1% of the male population and accounts for 10–15% male infertility cases. The rate of chromosomal abnormalities ranges from 10 to

Fig. 2 FISH analysis on a blood metaphase using **a** subtelomeric probe for chromosome 14 and SRY gene probe, showing translocation of the SRY signal onto chromosomes 14. **b** SHOX and CEP X probes showing one hybridization signal on the X chromosome and another on a group D chromosome

Fig. 3 FISH analysis on a blood metaphase using whole chromosome paint Y probe showing a painting hybridization signal on a group D chromosome with a fluorescent DAPI signal on a G chromosome

15% in azoospermic males, with the majority involving X and Y chromosomes [28–30]. Males with 45,X karyotype due to Y-autosome (Y;A) translocations are extremely rare. This unbalanced chromosomal abnormality usually involves the translocation of the Y chromosome containing the *SRY* gene onto the short arm of an acrocentric chromosome.

The involvement of acrocentric chromosomes in Y/autosome and X/autosome translocations is explained by the association observed between the sex vesicle chromatin and acrocentric short arm region during male meiosis [30].

We report on a male with azoospermia and a 45,X karyotype due to Y/autosome complex abnormality involving the translocation of the Y short arm containing the Yp subtelomere, the stature gene *SHOX* and *SRY* gene, in addition to the Y centromere onto the short arm of chromosome 14 with translocation of the Y heterochromatic region onto

chromosome 22 short arm. This resulted in the deletion of a large part of the long arm containing AZFa, AZFb, and part of AZFc, documented by molecular analysis.

To our knowledge, only four 45,X males with unbalanced Y;14 translocation were previously reported in literature.

The first two patients were described by Turleau et al. [31] and de la Chapelle et al. [32]; both had the Yp and the Y centromere translocated onto the short arm of chromosome 14. They presented as a male with azoospermia and a boy with penoscrotal hypospadias and slightly retarded psychomotor development, respectively [33].

The third case was reported by Dati et al. [2] as a man presenting with infertility and severe short stature (−4.05 SDS). He had an unbalanced translocation of Yp, including *SRY* and *SHOX* genes, onto the short arm of chromosome 14, with complete deletion of the AZF regions (AZFa, AZFb, AZFc). Short stature was explained by deletion of a specific growth gene in the pericentromeric region of Yq.

SRY gene is normally located on Yp11.2, just proximal to the pseudoautosomal region 1 (PAR1), and is the main gene essential for testis formation and male sexual development [34]. This explains the testicular development and the male phenotype of our patient and all reported male patients. *SHOX* gene is essential for the development of the skeleton and the growth and maturation of bones in the arms and legs. It is located on each of the sex chromosomes (X and Y) in PAR1 at Xp22.33 and Yp11.2, respectively [35]. The preservation of this gene explains the normal height of our patient. It is probable that the region hypothesized by Dati et al. [2] to harbor another gene concerned with growth and stature is not deleted in our patient.

On the other hand, the fourth male with azoospermia and unbalanced Y;14 translocation was reported by Buonadonna

Fig. 4 The investigated STSs on whole Y chromosome that shows deletion on Yq arm between sY1251 and 255; the plus (+) sign indicates the presence of STS and the minus (−) sign indicates the absence of STS (modified from MSY Breakpoint Mapper, <http://breakpointmapper.wi.mit.edu/>)

et al. [21]. The whole Y was translocated onto 14p with only loss of the genetically inert part, Yq heterochromatin, while all the AZF loci were preserved. Their report supported the hypothesis that infertility in Y/ autosome translocation may be due to meiotic disturbance and spermatogenic arrest.

Another report of a familial translocation of the heterochromatic part of Y chromosome to the short arm of chromosome 14 was described by Buys et al. [36] without phenotypic consequences.

Several molecular studies have shown that three non-overlapping azoospermia regions on Yq11 euchromatin, designated AZFa, AZFb, and AZFc, are required for normal spermatogenesis and deletion of which leads to spermatogenesis disruption at different phases of the process [18, 37, 38].

Normal spermatogenesis also requires appropriate X/Y pairing during meiosis, which is hindered by Y/ autosome translocation. In addition, the autosomal part could be included in the sex vesicle with a resulting hypercondensation and inactivation. A subsequent spermatogenic arrest and apoptotic response will result leading to azoospermia or severe oligozoospermia [10, 20, 21, 23, 39].

Familial Y heterochromatin/22p unbalanced translocation was reported by Reitalu [40] in a 14-year-old boy presenting with small genitalia and no pubertal signs, while nearly the same 22 abnormality was detected in his normal father and grandmother. Leschot et al. [41] also reported a normal girl with nearly similar abnormality, t(Y;22) (q12;p12/13), inherited from her mother.

On the other hand, a mosaic 45,X/46,X,+mar karyotype due to a de novo unbalanced translocation, t(Y;22) (q11.2;q11.1), was reported in a male with infertility and azoospermia. The abnormality involved loss of Yq euchromatin containing a large part of AZFb, AZFc, and Yq heterochromatin [42].

Our translocation is very unique as it involved multiple breakpoints and more than two chromosomes, with deletion of the whole AZFa and AZFb and part of AZFc regions. This explains the complete azoospermia and the total absence of spermatogenic cells in testicular histopathology. Furthermore, the transfer of the PAR1 to chromosome 14 could lead to aberrant sex chromosomal recombination with spermatocyte degeneration.

In patients with AZFa deletion, testicular biopsies show a Sertoli cell-only (SCO) phenotype characterized by the absence of germ cells or by the presence of germ cells in a very small number of seminiferous tubules, which is consistent to our patient's findings. While deletions in the AZFb region lead to pre-meiotic spermatogenic arrest or SCO syndrome and, eventually, azoospermia, it is thus virtually impossible to retrieve mature sperms during testicular sperm extraction (TESE) [18, 43], which makes our patient not a candidate for TESE and assisted reproduction.

In conclusion, it is very important to characterize the type and extent of Y and autosome rearrangements in patients with

Y/ autosome translocation in order to understand the underlying molecular mechanisms and to correlate the genomic alterations with the phenotypic consequences and possible surgical intervention. Our patient report adds a complex Yp/ 14p and Yq/ 22p translocation abnormality to the rare cases previously reported.

Funding information This work was funded by the in-house projects program, National Research Centre, grant no. 10010604.

References

- Chernykh VB, Vyatkina SV, Antonenko VG, Shilova NV, Zolotukhina TV, Kurilo LF, et al. Unique mosaic X/Y translocation/insertion in infant 45,X male. *Am J Med Genet A*. 2008;146A: 3195–7.
- Dati E, Valetto A, Bertini V, Chiocia E, Baroncelli GI, Battini R, et al. 45,X maleness: clinical and cytogenetic features in two patients. *Sex Dev*. 2011;5(6):281–6.
- Bilen S, Okten A, Karaguzel G, Ikbali M, Aslan Y. A 45 X male patient with 7q distal deletion and rearrangement with SRY gene translocation: a case report. *Genet Couns*. 2013;24(3):299–305.
- Hsu LYF. Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. *Am J Med Genet*. 1994;53:108–40.
- Cohen MM, Frederick RW, Balkin NE, Simpson SJ. The identification of Y chromosome translocations following distamycin A treatment. *Clin Genet*. 1981;19:335–42.
- Morales C, Soler A, Bruguera J, Madrigal I, Alsius M, Obon M, et al. Pseudodicentric 22;Y translocation transmitted through four generations of a large family without phenotypic repercussion. *Cytogenet Genome Res*. 2007;116:319–23.
- Guichaoua MR, Quack B, Speed RM, Noel B, Chandley AC, Luciani JM. Infertility in human males with autosomal translocations: meiotic study of a 14;22 Robertsonian translocation. *Hum Genet*. 1990;86:162–6.
- Pinho MJ, Neves R, Costa P, Ferrás C, Sousa M, Alves C, et al. Unique t(Y;1)(q12;q12) reciprocal translocation with loss of the heterochromatic region of chromosome 1 in a male with azoospermia due to meiotic arrest: a case report. *Hum Reprod*. 2005;20:689–96.
- Braun-Falco M, Schempp W, Nevinny-Stickel-Hinzpeter C, Köhn FM. Azoospermia due to a unique de novo balanced reciprocal translocation (Y;1) (q12;q25). *J Androl*. 2007;28(5):647–51.
- Gardner RJM, Sutherland GR. Chromosome abnormalities and genetic counseling. Sex chromosome translocations. New York, Oxford: Oxford University Press; 1996. p. 95–114.
- Mareri A, Iezzi M, Salvatore A, Ligas C, D'Alessandro E. A unique case of growth hormone and human chorionic gonadotropin treatment in a 45,X male with Y: autosome translocation and literature review. *J Pediatr Endocrinol Metab*. 2016;29:857–62.
- Boutouil M, Fetni R, Qu J, Dallaire L, Richer C-L, Lemieux L. Fragile site and interstitial telomere repeat sequences at the fusion point of a de novo (Y;13) translocation. *Hum Genet*. 1996;98:323–7.
- Shanske A, Ellison J, Vuguin P, Dowling P, Wasserman E, Heinrich J, Saenger P. Deletion of the pseudoautosomal region in a male with a unique Y;13 translocation and short stature. *Am J Med Genet* 1999; 82: 34–39.
- Alves C, Carvalho F, Cremades N, Sousa M, Barros A. Unique (Y;13) translocation in a male with oligozoospermia: cytogenetic and molecular studies. *Eur J Hum Genet*. 2002;10:467–74.

15. Borie C, Léger J, Dupuy O, Hassan M, Ledu N, Lebbar A, et al. Translocation (Y;22) resulting in the loss of SHOX and isolated short stature. *Am J Med Genet A*. 2004;125A(2):186–90.
16. Orrico A, Marsegli G, Pescucci C, Cortesi A, Piomboni P, Giansanti A, et al. Molecular dissection using array comparative genomic hybridization and clinical evaluation of an infertile male carrier of an unbalanced Y;21 translocation: a case report and review of the literature. *Int J Fertil Steril*. 2016;9:581–5.
17. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet*. 1996;5:933–43.
18. Vogt PH, Bender U. Human Y chromosome microdeletion analysis by PCR multiplex protocols identifying only clinically relevant AZF microdeletions. *Methods Mol Biol*. 2013;927:187–204.
19. Liow SL, Yong EL, Ng SC. Prognostic value of Y deletion analysis: how reliable is the outcome of Y deletion analysis in providing a sound prognosis? *Hum Reprod*. 2001;16:9–12.
20. Kauppi L, Barchi M, Baudat F, Romanienko PJ, Keeney S, Jasin M. Distinct properties of the XY pseudoautosomal region crucial for male meiosis. *Science*. 2011;331:916–20.
21. Buonadonna AL, Cariola F, Caroppo E, Di Carlo A, Fiorente P, Valenzano MC, et al. Molecular and cytogenetic characterization of an azoospermic male with a de-novo Y;14 translocation and alternate centromere inactivation. *Hum Reprod*. 2002;17:564–9.
22. Röpke A, Stratis Y, Dosow-Scheele D, Wieacker P, Kliesch S, Tüttelmann F. Mosaicism for an unbalanced Y;21 translocation in an infertile man: a case report. *J Assist Reprod Genet*. 2013;30:1553–8.
23. Delobel B, Djlelati R, Gabriel-Robez O, Croquette MF, Rousseaux-Prevost R, Rousseaux J, et al. Y-autosome translocation and infertility: usefulness of molecular, cytogenetic and meiotic studies. *Hum Genet*. 1998;102:98–102.
24. Verma RS, Babu A. Human chromosomes—principles and techniques. McGraw-Hill. 1995:1–183.
25. ISCN 2016: An International System for Human Cytogenomic Nomenclature (2016) Reprint of: Cytogenetic and Genome Research 2016, Vol. 149, No. 1–2 1st Edition. McGowan-Jordan J, Simons A, Schmid M (Editors). Karger, Basel.
26. Pinkel D, Gray J, Trask B, Van den Engh G, Fuscoe J, Van Dekken H. Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. In Cold Spring Harbor symposia on quantitative biology (Vol. 51, pp. 151–157). Cold Spring Harbor Laboratory Press, 1986.
27. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions. State of the art 2004. *Int J Androl*. 2004;27:240–9.
28. Krausz C. Male infertility: pathogenesis and clinical diagnosis. *Best Pract Res Clin Endocrinol Metab*. 2011;25:271–85.
29. Lee JY, Dada R, Sabanegh E, Carpi A, Agarwal A. Role of genetics in azoospermia. *Urology*. 2011;77:598–601.
30. Stahl A, Hartung M, Devictor M, Bergé-LeFranc JL. The association of the nucleolus and the short arm of acrocentric chromosomes with the XY pair in human spermatocytes: its possible role in facilitating sex-chromosome acrocentric translocations. *Hum Genet*. 1984;68:173–80.
31. Turleau C, Chavin-Colin F, de Grouchy J. A 45,X male with translocation of euchromatic Y chromosome material. *Hum Genet*. 1980;53:299–302.
32. de la Chapelle A, Page DC, Brown L, Kaski U, Parvinen T, Tippet PA. The origin of 45,X males. *Am J Hum Genet*. 1986;38:330–40.
33. Andersson M, Page DC, Pettay D, Subrt I, Turleau C, De Grouchy J, et al. Y; autosome translocations and mosaicism in the aetiology of 45,X maleness: assignment of fertility factor to distal Yq11. *Hum Genet*. 1988;79:2–7.
34. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*. 1990;346:240–5.
35. Binder G, Renz A, Martinez A, Keselman A, Hesse V, Riedl SW, et al. SHOX haploinsufficiency and Leri-Weill dyschondrosteosis: prevalence and growth failure in relation to mutation, sex, and degree of wrist deformity. *J Clin Endocrinol Metab*. 2004;89:4403–8.
36. Buys CH, Anders GJ, Borkent-Ypma JM, Blenkers-Platter JA, van der Hoek-van der Veen AY. Familial transmission of a translocation Y/14. *Hum Genet*. 1979;53:125–127.
37. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet*. 1995;10:383–93.
38. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev*. 2001;22:226–239.
39. Helena Mangs A, Morris BJ. The human pseudoautosomal region (PAR): origin, function and future. *Curr Genomics*. 2007;8:129–36.
40. Reitalu J. A familial Y-22 translocation in man. *Hereditas*. 1973;74:155–60.
41. Leschot NJ, von den Velden J, Marinkovic-Ilsen A, Darling SM, Nijenhuis LE. Homozygosity for a Y/22 chromosome translocation: t(Y;22) (q12;p12/13). *Clin Genet*. 1986;29:251–7.
42. Brisset S, Izard V, Misrahi M, Aboura A, Madoux S, Ferlicot S, et al. Cytogenetic, molecular and testicular tissue studies in an infertile 45,X male carrying an unbalanced (Y;22) translocation: case report. *Hum Reprod*. 2005;20:2168–72.
43. Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. *Hum Reprod*. 2003;18:1660–5.