African Journal of Biotechnology Vol. 10(69), pp. 15509-15513, 7 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2059
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Analysis of sex chromosomal constitution in sperm from a 47, XYY/46, XY male by using fluorescence *in situ* hybridization (FISH)

Wei Le[1], Qiuming Chen[1], Junhai Qian[1], Chunyan Li[2], Shengdao Dan[1], Denglong Wu[1], LiHe Guo[3] and JinFu Zhang[1]*

[1]Department of Urology, Tongji Hospital of Tongji University School of Medicine, 389 Xincun Road, Shanghai, 200065, P. R. China.
[2] Tongji University School of Medicine, 1239 Siping Road, Shanghai, 200092, P.R.China.
[3]Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, P. R. China.

Accepted 9 September, 2011

This study was carried out to analyze sex chromosomal constitution in sperm from a 46, XY [60%] / 47, XYY [40] karyotype male and to evaluate the risks of his reproductive genetics. Sperm samples harvested from the mosaic karyotype patient and four healthy male (control group) were assessed using duel-color (specific centremeric probes for X and Y chromosome) fluorescence *in situ* hybridization (FISH) studies. Sex chromosome numerical abnormalities were observed. The ratio of the X-bearing sperm between Y-bearing sperm in both patient and controls was closel to 1:1. The incidence of sex chromosome abnormal combinations (YY, XY and XX) were significantly increased in the patient's semen sample compared with normal control (0.466 versus 0.10%, p<0.0001; 0.39 versus 0.21%, p<0.001; 1.16 versus 0.09%, p<0.0001, respectively). High risks of chromosome numerical abnormalities of the patient's offspring and miscarriage rate were suggested from the study. FISH analysis has the potential merits to evaluate the rate of sex chromosome numerical abnormalities on spermatozoa for these karyotyped abnormal patients. Prenatal and genetic diagnoses (PGD) are recommended to increase the likelihood of a successful pregnancy.

Key words: 47, XYY, mosaic, sperm, chromosome numerical abnormalities.

INTRODUCTION

47, XYY / 46, XY mosaic karyotype is a common chromosomal abnormality (Jacobs et al., 1974). The majorities of 47, XYY / 46, XY mosaic patients are fertile and have chromosomally normal offspring. However, high chromosomal numerical abnormalities, abortion and prenatal death rates of these karyotyped abnormal males were revealed in recent years (Jones et al., 1997). In this study, fluorescence *in situ* hybridization (FISH) was carried out to assess the sex chromosomal constitution on sperm from a 47, XYY / 46, XY mosaic patient, and theoretical risks of reproductive genetic disease was provided by this study as well.

*Corresponding author. E-mail: jinfuzhang@yahoo.com.cn. Tel: +86-021-66111292.

Case report

Study subject was a 27 years old man married four years with infertility. His wife, had normal karyotype, and aborted three times during four years. No history of exposure to high temperature and toxic environment was provided by the couple. All of the patient's parents and sister were healthy and no genetic disease history was provided. The patient's sperm conventional analysis procedure was followed by fifth edition of examination and processing of human semen (Cooper et al., 2010). He had normal sperm parameters: 65% progressive motility (PR); 15%non-progressive motility (NP); 70×10^6

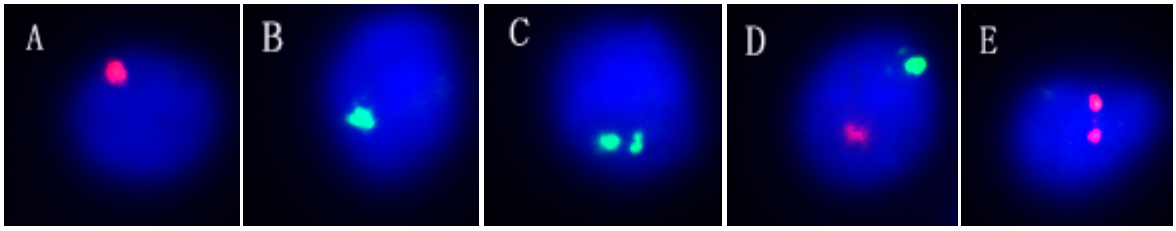

Figure 1. Dual-color fluorescence *in situ* hybridization analysis of sperm. A, X-bearing sperm from normal control group (23.X); B, Y-bearing sperm from normal control group (23,Y); C, XX-typed sperm from 47,XYY/46,XY male (24,XX); D, XY-typed sperm from 47,XYY/46,XY male (24,XY); E, YY-typed sperm from 47,XYY/46,XY male (24,YY).

/ml sperm concentration; 50% normal morphology. Result of chromosome analysis via peripheral blood was a mosaic karyotype: 46, XY [60%] / 47, XYY [40%].

MATERIALS AND METHODS

Semen samples acquired from control group

Semen samples were kindly provided by four healthy fertile male donors from Renji Hospital, Shanghai Jiaotong University (Shanghai sperm bank). Donors had the following characteristics: aged from 22 to 30 years; normal karyotype (46, XY); without alcohol and tobacco habits, genetic diseases, infectious diseases and radiation, pesticides, chemical medicines and other exposure history. More also, three to five days abstinence was required by donors before ejaculation. Semen samples were collected by masturbation and analyzed according to fifth edition of examination and processing of human semen. The analyzed results of the semen samples were normal.

Sperm sample preparation

Semen samples were washed with phosphate buffer saline (PBS) and centrifuged twice. Sperm heads were decondensed by dithiothreitol (DTT) -Tris solution (DTT 10 mmol/L, Tris 100 mmol/L, pH = 8.0) for 40 min approximately. Observation on the expansive morphology of sperm head by microscope was required in the aforementioned procedure. Then, sperm heads were fixed and dripped to slides and slides were treated by 2 × saline-sodium citrate (SSC) solution (pH = 7.0, 37°C) for 10 min and dehydrated by gradient ethanol.

Fluorescence *in situ* hybridization

Slides were denatured for 4 min in 70% formamide / 2 × SSC solution (74°C) and quickly placed into 70, 90 and 100% ethanol for 1 min, respectively. 10 µl probe mixture (SE X, SE Y, Kreatech, Netherlands) was added to the hybridization region after denaturing at 90°C for 10 min. Rubber cement was used to mount the hybridization region after covering with coverslips and slides were hybridized in humid chamber overnight at 3°C. After mounted off the rubber cement, slides were then washed in solution (0.4 × SSC/0.3% NonidetP-40, 73°C) for 2 min without agitation, placed into the solution (2 × SSC/0.1% NP-40, room temperature) for 1 min, dehydrated by gradient ethanol, and air dried. Each hybridization region was counterstained by 10 µl 4',6-diamidino-2-phenylindol (DAPI) (including Antifade) before covering with coverslip.

Signal observation

Slides were observed by fluorescence microscope (Olympus BX51) after counterstaining with DAPI. Strict scoring criteria were followed in order to minimize the inter-observer variability and subjective deviation (Blanco et al., 1996; Tomas, 2010) such that only non-overlapping, well-delineated and intact spermatozoa can be evaluated. The scoring signals were described as follows: normal sperm: expressed as a green signal (X chromosome) or a red signal (Y chromosome); sperm chromosome numerical abnormalities: one green and one red spots were recognized as the XY type, two spots for the green fluorescent were evaluated as XX type, and two spots for the red fluorescent were scored as YY type; null signal sperm: neither red nor green fluorescent signal was observed in the sperm cells (Figure 1). More than 1×10^4 sperms were counted per slide.

Statistical analysis

Chi-Square test was used to analyze sex chromosome numerical constitution between sperm samples from the subject and control group by SPSS 16.0 software (Table 1 and Figure 2).

RESULTS AND DISCUSSION

In total, 10449 sperm cells from the subject and 40537 sperm cells from the control group were counted. Hybridization rate was above 99%. The types of sex chromosome numerical abnormalities are illustrated in Figure 2. Results of the comparison of sex chromosome numerical abnormalities rate are also shown in Table 1. The incidences of sex chromosome abnormal combinations (YY, XY and XX) were significantly increased in the patient's semen sample compared with the normal control (0.466 versus 0.10%, $p<0.0001$; 0.39 versus 0.21%, $p<0.001$; 1.16 versus 0.09%, $p<0.0001$, respectively).

There is an extremely close relationship between male infertility, genetic disease and chromosome numerical abnormalities. The majority of 47, XYY males are fertile, however, an increased rate of miscarriage and prenatal death has been suggested (Jones, 1997). In recent

Table 1. Dual-color fluorescence in situ hybridization analysis of sperm.

Parameter	Number of sperm counted	X-Bearing sperm	Y-Bearing sperm	X:Y ratio	XX-Typed sperm (%)	XY-Typed sperm (%)	YY-Typed sperm (%)	Sex nullisomy (%)	Overall abnormality rate (%)
Patient (47,XYY/46,XY)	10449	5157	4977	1.04	48 (0.46%)**	41(0.39%)*	121 (1.16%)**	106 (1.01%)$^{n.s}$	2.01**
Normal control (46,XY)	40537	20025	19951	1.00	41 (0.10%)	84 (0.21%)	36 (0.09%)	403 (0.99%)	0.40

**P-value<0.0001 compared with normal control; *p-value<0.001 compared with normal control; ns, not significant observed compared with normal control (Statistical analyses were performed using Chi-square test).

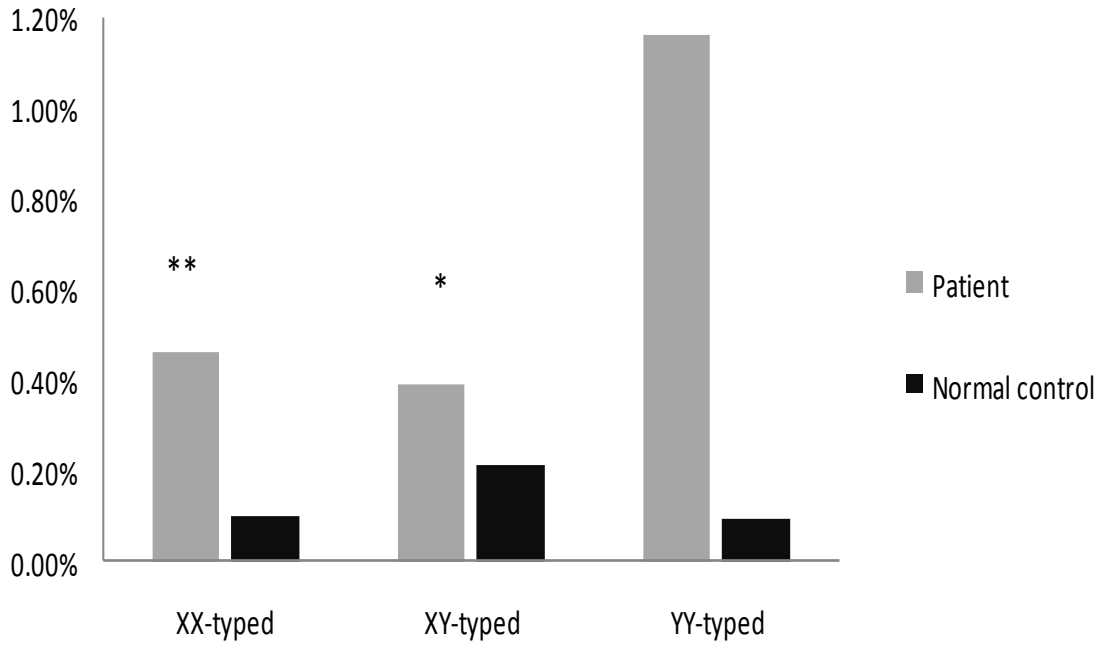

Figure 2. Sex chromosome numerical abnormalities on sperm. **P-value<0.0001 compared with normal control; *p-value<0.001 compared with normal control.

years, sex chromosome constitutions of sperm from males with 46, XY/47, XYY karyotype have been studied and higher incidences of chromosome numerical abnormalities were revealed. Sperm numerical abnormalities have been found as an important reason for chromosomal disease and pregnancy abortion (Blanco et al., 1997; Chevret et al., 1997). The sperm density and motility can be quite varied among the males with 47, XYY mosaic patient, as some were showed as oligoasthenospermia, while others were present as normal density and motility (Lim et al., 1999; Wang et al., 2000).

The patient had normal sperm parameters in this study. It has been reported that the rate of sperm chromosome numerical abnormalities increased in patients with oligoasthenospermia (Milazzo et al., 2006). 40% peripheral lymphocytes of the patient belonged to abnormal cell line (47, XYY karyotype), but results of his sex chromosome numerical abnormalities showed a lower rate according to the abnormal karyotype proportion of his lymphocytes. The mechanism of checkpoint on sex chromosome in pachytene of meiosis stage may be the possible explanation to the relatively low sperm chromosome numerical abnormalities. Sex chromosome configurations in pachytene have been studied in spermatogenesis stage of 47, XYY male (Wang et al., 2000). It has been revealed that sex chromosome may have several pairing configurations in pachytene stage, such as X+YY, XY+Y, X+Y+Y types and XYY trivalent structure as well (Codina-Pascual et al., 2006; Gonsalves et al., 2004; Speed et al., 1991; Gabriel-Robez et al., 1996; Solari et al., 1997). X+YY pairing was the most common configuration among the types aforementioned.

It was indicated by a former research that the XYY trivalent structure may evade checkpoint in synaptic

stage, thus leading to the increased incidence on sex chromosome numerical abnormalities in sperm (Rodriguez et al., 2000; Milazzo et al., 2006). Based on immune-fluorescence staining pachytene nuclei for synaptonemal complex protein, it has been revealed that X+YY form occupies the majority pairing configurations in 47,XYY spermatogonial cells during pachytene stage (Wong et al., 2008). Since the evidence that the un-synapsed chromosome (such as X+YY pairing configuration) may activate checkpoint mechanisms to prevent the progression of meiosis, this also gives a reasonable explanation to the relatively lower incidence of sex chromosome numerical abnormalities compared with the high abnormal karyotype proportion in patient's peripheral lymphocyte. It has been confirmed that partially normal XY chromosome are not synapsed in the pachytene stage and the failure of chromosome synapses may prevent the recombination between sex chromosome (Wong et al., 2008; hassold et al., 1991; Martin, 2005; Ma et al., 2006), thus leading to non-disjunction of sex chromosome. The failure of sex chromosome recombination in pachytene may cause sex chromosome numerical abnormalities in spermatozoa (Codina-Pascual et al., 2006; Gonsalves et al., 2004). The increased sex chromosome numerical abnormalities are mostly associated with the abnormal testicular internal environment. Low sperm density of infertile males suggests the disorder of spermatogenesis function in testicular. Based on previous studies, sex chromosome abnormalities rate were often increased in the infertile males (Durakbasi-Dursun et al., 2008; Guttenbach et al., 1997). The changes of spermatogenic environment may result in abnormal separation of sex chromosome or chromosome fragments (Sakkas and Alvarez, 2010). The increased ratio of sex numerical abnormalities in 47, XYY/46, XY males with lower sperm density may also be related to this factor.

However, increased rates of XX, XY and YY typed abnormalities were revealed as different in previous studies. Some results only showed that XX and YY typed abnormalities increased, while others revealed that all abnormal types increased in the patient's sperm (Lim et al., 1999; Rives et al., 2003). Dual-color fluorescence *in situ* hybridization in this study was used to determine the sex chromosome constitution, and the rate of aneuploidy and diploidy were included in the numerical abnormalities results. The incidences of the sex chromosome numerical abnormalities (XX, XY and YY typed) were significantly increased in patient's spermatozoa compared with the control group in this study. Individual variant of patients, proportion of 47, XYY abnormal cells, difference in experimental methods, auto or manual signal counting and conditions as earlier mentioned may contribute the difference of study results.

The rates of sex chromosome abnormalities in 47, XYY mosaic male in this study was relatively low when compared with the high proportion of abnormal cells in peripheral lymphocytes. However, his wife still had three consecutive miscarriages and as such it is still uncertain whether the consecutive miscarriage has a definite causal relationship with the relatively lower incidence of sex chromosome numerical abnormalities. Further studies are needed to reveal the mechanisms of the miscarriage for 47, XYY males. High risks of chromosome numerical abnormalities of the patient's offspring and miscarriage rate are suggested from this study. FISH analysis has potential merits to evaluate the rate of sex chromosome numerical abnormalities on spermatozoa for these karyotyped abnormal patients. Prenatal and genetic diagnoses are therefore advised to increase the likelihood of a successful pregnancy.

REFERENCES

Blanco J, Egozcue J, Vidal F (1996). Incidence of chromosome 21 disomy in human spermatozoa as determined by fluorescent in-situ hybridization. Hum. Reprod. 11(4): 722-726.

Blanco J, Rubio C, Simon C, Egozcue J, Vidal F (1997). Increased incidence of disomic sperm nuclei in a 47, XYY male assessed by fluorescent in situ hybridization (FISH).Hum Genet. 99(3): 413-416.

Chevret E, Rousseaux S, Monteil M, Usson Y, Cozzi J, Pelletier R, Sele B (1997). Meiotic behaviour of sex chromosomes investigated by three-colour FISH on 35,142 sperm nuclei from two 47, XYY males. Hum. Genet. 99(3): 407-412.

Codina-Pascual M, Navarr, J, Oliver-Bonet M, Kraus J, Speicher MR, Arango O, Egozcue J, Benet J (2006). Behaviour of human heterochromatic regions during the synapsis of homologous chromosomes. Hum. Reprod. 21(6): 1490-1497.

Cooper TG (2010). WHO laboratory manual for the examination and processing of human semen - 5th ed, (World Health Organization, Geneva, WHO Press)

Durakbasi-Dursun HG, Zamani AG, Kutlu R, Görkemli H, Bahce M, Acar A (2008). A new approach to chromosomal abnormalities in sperm from patients with oligoasthenoteratozoospermia: detection of double aneuploidy in addition to single aneuploidy and diploidy by five-color fluorescence in situ hybridization using one probe set. Fertil. Steril. 89(6): 1709-1717.

Gabriel-Robez O, Delobel B, Croquette MF, Rigot JM, Djlelati R, Rumpler Y (1996). Synaptic behaviour of sex chromosome in two XYY men. Ann. Genet. 39(3): 129-132.

Gonsalves J, Sun F, Schlegel PN, Turek PJ, Hopps CV, Greene C, Martin RH, Pera RA (2004). Defective recombination in infertile men. Hum. Mol. Genet. 13(22): 2875-2883.

Guttenbach M, Martinez-Expósito MJ, Michelmann HW, Engel W, Schmid M (1997). Incidence of diploid and disomic sperm nuclei in 45 infertile men. Hum. Reprod. 12(3): 468-473.

Jacobs PA, Melville M, Ratcliffe S, Keay AJ, Syme J (1974). A cytogenetic survey of 11,680 newborn infants. Ann. Hum. Genet. 37(4): 359-376.

Jones KL (1997). XYY syndrome. In Jones, K.L.(ed), Smith's Recognizable Patterns of Human Malformation, 5th edn. W.B. Saunders, Philadelphia, pp. 70-71.

Lim AS, Fong Y, Yu SL (1999). Analysis of the sex chromosome constitution of sperm in men with a 47, XYY mosaic karyotype by fluorescence in situ hybridization. Fertil. Steril. 72(1): 121-123.

Martin RH (2005). Mechanisms of nondisjunction in human spermatogenesis. Cytogenet Genome Res. 111(3-4): 245-249.

Ma S, Ferguson KA, Arsovska S, Moens P, Chow V (2006). Reduced recombination associated with the production of aneuploid sperm in an infertile man: a case report. Hum. Reprod. 21(4): 980-985.

Milazzo JP, Rives N, Mousset-Siméon N, Macé B (2006). Chromosome constitution and apoptosis of immature germ cells present in sperm of two 47, XYY infertile males. Hum Reprod. 21(7): 1749-1758.

Rives N, Siméon N, Milazzo JP, Barthélémy C, Macé B (2003). Meiotic

segregation of sex chromosomes in mosaic and non-mosaic XYY males: case reports and review of the literature. Int. J. Androl. 26(4): 242-249.
Rodriguez TA, Burgoyne PS (2000). Evidence that sex chromosome asynapsis, rather than excess Y gene dosage, is responsible for the meiotic impairment of XYY mice. Cytogenet Cell Genet. 89(1-2): 38-43.
Sakkas D, Alvarez JG (2010). Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil. Steril. 93(4): 1027-1036.
Solari AJ, Rey Valzacchi G (1997). The prevalence of a YY synaptonemal complex over XY synapsis in an XYY man with exclusive XYY spermatocytes. Chromosome Res. 5(7): 467-474.
Speed RM, Faed MJ, Batstone PJ, Baxby K, Barnetson W (1991). Persistence of two Y chromosomes through meiotic prophase and metaphase I in an XYY man. Hum. Genet. 87(4): 416-420.
Tomas L (2010). FISH on spermatocytes and oocytes. Fluorescence In Situ Hybridization (FISH)- Application Guide. Part II, Oliver-Bonet, M. (Berlin, Germany: Springer), 16: 157-172.
Wang JY, Samura O, Zhen DK, Cowan JM, Cardone V, Summers M, Bianchi DW (2000). Fluorescence in-situ hybridization analysis of chromosomal constitution in spermatozoa from a mosaic 47, XYY/46,XY male. Mol. Hum. Reprod. 6(7): 665-668.
Wong EC, Ferguson KA, Chow V, Ma S (2008). Sperm aneuploidy and meiotic sex chromosome configurations in an infertile XYY male. Hum. Reprod. 23(2): 374-378.