

Multicenter study of genetic abnormalities associated with severe oligospermia and non-obstructive azoospermia

Chong Xie^{1,*}, Xiangfeng Chen^{2,*}, Yulin Liu^{3,*},
Zhengmu Wu¹ and Ping Ping¹

Abstract

Objective: Genetic defects are identified in nearly 20% of infertile males. Determining the frequency and types of major genetic abnormalities in severe male infertility helps inform appropriate genetic counseling before assisted reproductive techniques.

Methods: Cytogenetic results of 912 patients with non-obstructive azoospermia (NOA) and severe oligozoospermia (SOS) in Eastern China were reviewed in this multicenter study from January 2011 to December 2015. Controls were 215 normozoospermic men with offspring.

Results: Among all patients, 22.6% (206/912) had genetic abnormalities, including 27.3% (146/534) of NOA patients and 15.9% (60/378) of SOS patients. Chromosomal abnormalities (all autosomal) were detected in only 1.9% (4/215) of controls. In NOA patients, sex chromosomal abnormalities were identified in 25.8% (138/534), of which 8% (43/534) had a 47,XXY karyotype or its mosaic; higher than the SOS group prevalence (1.1%; 4/378). The incidence of Y chromosome microdeletions was lower in the SOS group (13.2%; 50/378) than in the NOA group (17.8%; 95/534).

Conclusions: The high prevalence of genetic abnormalities in our study indicates the importance of routine genetic testing in severe male infertility diagnosis. This may help determine the choice of assisted reproductive technique and allow specific pre-implantation genetic testing to minimize the risk of transmitting genetic defects.

*Chong Xie, Xiangfeng Chen, and Yulin Liu contributed equally to this work.

Corresponding author:

Dr Ping Ping. Institutional address: Assisted Reproductive Center, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. Mailing address: Rm 204, Reproductive Medicine Building, No 1961, Hua Shan Road, Shanghai, China. 200030.

Email: deer16@126.com

¹Assisted Reproductive Center, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

²Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai Human Sperm Bank, Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai, China

³Shanghai Ji Ai Genetic and IVF Institute, Shanghai, China



Keywords

Male infertility, genetic abnormalities, oligospermia, azoospermia, assisted reproductive techniques, autosome, sex chromosome, Klinefelter syndrome, microdeletions

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Introduction

Infertility is an important health problem with a multifactorial etiology that affects approximately 15% of couples who attempt pregnancy.¹ Its cause in approximately 50% of infertile couples is male factors, which may exist either alone or in combination with female factors.² Spermatogenesis is an extremely complex cell differentiation process involving 2,300 genes that regulate germ cell development and maturation.³ The prevalence of numerical and structural chromosomal abnormalities in the infertile population ranges from 2%–10%,^{4,5} with 8% reported in severe oligospermia (SOS) cases⁶ and up to 20% in non-obstructive azoospermia (NOA);⁷ many other genetic abnormalities causing infertility remain unknown.

Molecular defects and genetic alterations associated with infertility have negative effects on hormonal homeostasis, spermatogenesis, and sperm quality.^{8–10} Among the various chromosomal defects detected in severely infertile males, structural aberrations of the autosomes, such as the Robertsonian translocation, balanced translocations, and inversions, are found in some SOS cases, while microdeletions on AZF regions of the Y chromosome and aneuploidies of sex chromosomes such as 47,XXY (i.e., Klinefelter syndrome, KS) account for 1%–3% in NOA cases.^{11–13}

Infertile males with SOS and NOA can have offspring with the help of assisted reproduction techniques (ART), particularly intracytoplasmic sperm injection (ICSI). However, the risks of passing genetic aberrations to their descendants are increased.

The main purpose of this multicenter study was to evaluate the frequency and types of major chromosomal abnormalities of SOS and NOA, and to highlight the urgent need for genetic counseling and testing prior to ART treatments.

Materials and methods

Patients

From January 2011 to December 2015, 912 infertile men, including 378 with SOS and 534 with NOA, from reproductive medical centers in the International Peace Maternity and Child Health Hospital, the Shanghai Ji Ai Genetic and IVF Institute and Center for Reproductive Medicine, and the Ren Ji Hospital were prospectively enrolled in this study. A total of 215 male volunteers (aged 22–45 years) with normozoospermia from the Shanghai Human Sperm Bank who already have offspring were recruited as controls. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research and ethic committee of Shanghai Jiaotong University.

SOS was defined as a sperm cell count $\leq 5 \times 10^6$ /ml. NOA was first established on the basis of at least three semen evaluations performed on separate occasions, when the seminal specimen after centrifugation showed no sperm under the microscope and was then diagnosed according to several clinical parameters. The investigation included a medical history, a physical examination including a thorough evaluation of the scrotum and testes, and laboratory tests, and imaging in some cases. The history was

based on general health, sexual health, past fertility, libido, and sexual activity. Past exposure to a number of agents, including medical agents such as hormone/steroid therapy, antibiotics, 5-ASA inhibitors (sulfasalazine), alpha-blockers, 5 alpha-reductase inhibitors, chemotherapeutic agents, pesticides, and recreational drugs (marijuana, excessive alcohol), as well as heat exposure of the testes, was queried. A history of surgical procedures of the genital system was also elicited. The family history was assessed to identify genetic abnormalities. Additionally, elevated serum follicle-stimulating hormone, testicular volume, and histopathology of testicular biopsy or testicular sperm aspiration, if available, were taken into consideration. All patients underwent an andrological work-up, including physical examination, two semen analyses, hormonal analysis, testicular ultrasonography, karyotyping, and Y chromosome microdeletion screening. Other possible causes of spermatogenic failure, such as abnormal endocrinology, varicocele, cryptorchidism, and seminal duct obstruction, were excluded. Semen samples were obtained after a 3- to 7-day period of ejaculatory abstinence, and semen analysis was performed according to 2010 World Health Organization (WHO) guidelines,¹⁴ based on the parameters of semen volume, sperm count, sperm motility, and sperm morphology. WHO regards 1.5 ml as the lower semen volume reference limit and $>15 \times 10^6/\text{ml}$ sperm as a normal sperm count. Sperm with progressive motility and non-linear motility should be over 32%, and $\geq 4\%$ of observed sperm should have a normal morphology.

Cytogenetic analysis and fluorescence in situ hybridization

Karyotyping was performed using standard G-banding. A total of 20 GTG-banded metaphases with a minimum resolution of

550 bands per haploid set were analyzed in each case. Chromosomal abnormalities were reported according to the current international standard nomenclature.¹⁵

PCR analysis of microdeletions in the AZF region of the Y chromosome

Genomic DNA was extracted from whole blood using a Blood Genomic DNA Mini Kit (CoWin Biosciences, Co., Ltd, Beijing, China) according to the manufacturer's instructions. The DNA concentration and purity were measured using the BioPhotometer Plus (Eppendorf, Hamburg, Germany). The following six sequence tagged sites were analyzed using a Y chromosome deletion detection kit (Shanghai Tellgen Corporation Co., Ltd., Shanghai, China): sY84 and sY86 (AZFa), sY127 and sY134 (AZFb), and sY254 and sY255 (AZFc). The SRY (sY14) gene and ZFX/Y gene (located on both X and Y chromosomes) were used as internal controls. Two multiplex PCRs were performed for each sample using the ABI 7500 Real-Time PCR System (Life Technologies, Gaithersburg, MD, USA), with the following program: 2 min at 50°C, 5 min at 95°C, and 38 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. Fertile male samples, female samples, and deionized water were used as positive, negative, and blank controls, respectively. A positive amplification result on the real-time instrument FAM(Control)/VIC(AZFa)/ROX(AZFb)/Cy5(AZFc) channel generated a Ct value ($Ct < 32$) and a typical S-shaped amplification curve. A negative amplification result generated no Ct value or amplification curve.

Statistical analyses

Statistical analyses were performed using SPSS 16.0 software (Chicago, IL, USA). Differences in group frequencies were

assessed by the χ^2 -test, and significance was declared at $P \leq 0.05$.

Results

Cytogenetic evaluation

The mean ages of each group (\pm SD) were 33.1 ± 12.9 years (NOA group), 35.2 ± 14.7 years (SOS group), and 30.8 ± 9.6 years (control group); these values did not differ statistically. The mean sperm count was $2.1 \pm 1.7 \times 10^6$ /ml (range, $0.1-5 \times 10^6$ /ml) in the SOS group, and the mean motility was also reduced to $10.3 \pm 6.5\%$ (range, 2%–32%). In the control group, the mean sperm count was $41 \pm 23 \times 10^6$ /ml (range, $15-105 \times 10^6$ /ml), and the mean motility was $45 \pm 13\%$ (range, 32%–62%).

Karyotyping showed that a total of 22.6% (206/912) of severely infertile patients had genetic abnormalities, including 27.3% (146/534) of NOA patients and 15.9% (60/378) of SOS patients, while 1.9% (4/215) of chromosomal abnormalities were detected in the control group. Characteristics of the patient's chromosomal abnormalities are summarized in Table 1. In the NOA group, 94.5% (138/146) of genetic abnormalities were

sex chromosomal abnormalities and Y chromosome deletions. Sex chromosomal abnormalities accounted for 25.8% (138/534) of all abnormal karyotypes, of which approximately 6.7% (36/534) were represented by a 47,XXY karyotype, and 1.3% (7/534) bore 47, XXY/46,XY or other types of mosaic. Approximately 17.8% (95/534) of NOA cases showed various categories of Y chromosome deletions. The remaining 1.5% (8/534) of NOA men had autosomal abnormalities, including six chromosomal translocations and two chromosomal inversions.

In the SOS group, 1.1% (4/378) showed 47, XXY/46, XY or mosaic, which is significantly lower than that of NOA men (8%, 43/534, $P = 0.002$), and 13.2% (50/378) had Y chromosome deletions. The other 1.6% (6/378) had autosomal abnormalities, including four cases of chromosomal translocations and two cases of chromosomal polymorphisms or inversions. No sex chromosomal abnormalities were detected in the control group. The eight abnormal control cases were all autosomal abnormalities, including six chromosomal inversions, and two chromosomal translocations.

Table 1. Genetic abnormalities in non-obstructive azoospermia, severe oligozoospermia, and control groups.

Genetic abnormalities	Non-obstructive azoospermia, n (%)	Severe oligozoospermia, n (%)	Controls, n (%)
Abnormal sex chromosome	43/534 (8)*	4/378 (1)*	0 (0)*
47,XXY	36/534 (6.7)	1/378 (0.26)	0 (0)
46,XY/ 47,XXY	7/534 (1.3)	3/378 (0.79)	0 (0)
YqAZF microdeletion	95/534 (17.8)**	50/378 (13.2)**	0 (0)**
Abnormal autosome	8/534 (1.5)***	6/378 (1.5)***	4/215 (1.9)***
Balanced translocation	6/534 (1.1)	4/378 (1)	1/215 (0.46)
Pericentric inversion	2/534 (0.4)	2/378 (0.5)	3/215 (1.4)
Total	146/534 (27.3)***	60/378 (15.9)***	4/215 (1.9)***

*There is significant difference between any two groups. $P < 0.01$.

**There is significant difference between any two groups. $P < 0.01$.

***There is no significant difference between any two groups. $P > 0.05$.

****There is significant difference between any two groups. $P < 0.01$.

Table 2. Frequency of different YqAZF microdeletions in non-obstructive azoospermia, severe oligozoospermia, and control groups.

AZF region	Non-obstructive azoospermia, n (%)	Severe oligozoospermia, n (%)	Controls, n (%)
AZFa	8 (8.4)	2 (4)	0 (0)
AZFb	6 (6.3)	0 (0)	0 (0)
AZFc	49 (51.6)	48 (96)	0 (0)
AZFab	1 (1)	0 (0)	0 (0)
AZFbc	21 (22.1)	0 (0)	0 (0)
AZFac	0 (0)	0 (0)	0 (0)
AZFabc	10 (10.5)	0 (0)	0 (0)
Total	95 (100)	50 (100)	0 (0)

Prevalence and type of Yq microdeletions

NOA and SOS patients were analyzed for the incidence of Y chromosome deletions, which included AZFa, AZFb, AZFc, AZFab, AZFac, AZFbc, and AZFabc in this study. As shown in Table 2, 15.9% (145/912) of severely infertile males presented with Y chromosome microdeletions.

In the NOA group, the prevalence was 17.8% (95/534), which is slightly higher than that of SOS males (13.2%, 50/378) although the difference was not significant. Deletion of AZFc was the most frequent Y chromosome deletion type in both groups; 9.2% (49/534) of NOA males and 12.3% (48/378) of patients with SOS had deletions in the AZFc region. Other deletion types, such as the deletion of AZFabc and AZFbc, were found in the NOA group but were not detected in the SOS group (Table 2).

Discussion

Chromosomal anomalies are closely associated with male infertility. Several previous studies have reported their high prevalence in infertile males; for example, 11%–24% of patients with NOA and 2%–16% of patients with oligospermia^{16–19} have chromosomal anomalies. The reasons for discrepancies between studies might reflect the criteria

used for patient selection or dissimilar compositions of the studied populations, such as the extent of spermatogenesis dysfunction. In the present study, 1.3% (8/603) of volunteers with normozoospermia had chromosomal abnormalities, while 15.9% (60/378) of patients with SOS and 27.3% (146/534) with NOA had abnormalities. All eight abnormal cases with normozoospermia were autosomal aberrations: six were balanced translocations, and two were pericentric inversions. Carriers of structural chromosomal abnormalities with normal phenotypes may nevertheless experience fertility problems. Therefore, patients with unexplained infertility with normal semen parameters should be referred for cytogenetic analysis because of their high chance of miscarriage and live birth of children with unbalanced karyotypes.

Of the 146 patients in the NOA group with chromosomal abnormalities, 5.5% (8/146) had pericentric inversions, and 94.5% (138/146) had gonosomal abnormalities, which is in line with other studies.²⁰ The combined incidence of structural aberrations in sex and autosomal chromosomes was higher for NOA than for SOS (27.3% vs 15.9%). In contrast to other published data, Y chromosomal terminal deletions accounted for most karyotypic abnormalities identified in our work. The

frequency of AZF microdeletions was 17.7% (95/534) in NOA men, which is higher than the 13.2% frequency (50/378) observed in patients with SOS. These results are similar to the published frequencies of 0%–15% in NOA patients and 5%–10% in oligozoospermic patients.²¹ Deletions of the different AZF regions occurred with different frequencies. We found that AZFc deletions were the most frequent in severely infertile males, representing 66.9% of all deletion categories, followed by deletions in ACFbc (14.5%), AZFabc (6.9%), ACFb (4.1%), AZFa (6.9%), and AZFab (0.7%) regions.

Molecular research into the Y chromosome has demonstrated that each AZF subregion acts during a different phase of spermatogenesis.²² Therefore, complete deletion of the AZFa region results in Sertoli cell-only syndrome and azoospermia, while deletion of the AZFb region causes Sertoli cell-only syndrome or arrested spermatogenesis at the early spermatocyte stage, with phenotypic azoospermia. Deletions of the AZFc locus manifest in cases of moderate oligozoospermia (0.7%), SOS (4%–14%), or azoospermia (11%–18%).²³ Deletion of the AZFc region results in various phenotypes, from unaffected to oligozoospermia to azoospermia. Because ART techniques such as ICSI, testicular sperm extraction, and *in vitro* fertilization can assist infertile men with Y chromosome microdeletions to achieve pregnancies, Y microdeletion screening is strongly recommended for men with severe sperm defects ranging from azoospermia to oligozoospermia.²⁴ Additionally, although pre-implantation genetic diagnosis (PGD) technology is not routinely used in the evaluation of male infertility, it could potentially prevent the transmission of Y microdeletions to male offspring.

KS was the next most common condition observed in our study, in keeping with the fact that KS (47,XXY) is the most common

sex chromosomal defect detected in newborn males (0.1%–0.2%).²⁵ Ferlin et al.¹⁶ previously reported a KS prevalence of 10% in NOA and 5% in SOS patients. In our cohort, the incidence of KS was 6.7% (36/534) in NOA and 1.1% (4/378) in SOS patients. Recent studies have suggested that the impact of KS on pregnancy outcomes is limited.²⁶ Sperm can be retrieved in approximately 50% of azoospermic KS cases from focal areas of spermatogenesis in the testis using the microsurgery testicular sperm retrieval technique. Moreover, although the abnormal embryo rate of ICSI–PGD of patients with KS was reported to be 12.6%, retrospective reviews of 200 normal babies born with ICSI without PGD demonstrate that most of these children are chromosomally normal.

The loss of one of the X chromosomes from spermatogonia may represent a particular characteristic of the X chromosome; for example, X inactivation as suggested by Sciarano et al.^{27,28} This phenomenon is seen in mammals with more than one X chromosome, in which the genes on all but one X chromosome are not expressed. It occurs in XXY males as well as in normal XX females.²⁹ Levron et al.³⁰ speculated that during multiplication of the primordial germ cells in the prenatal testis, ‘correcting mitotic errors’ might give rise to isolated testicular mosaicism, with normal germ lines surviving to produce sperm later in adulthood. Mroz et al.³¹ demonstrated in a mouse model that surviving germ cells are all XY and restricted to single continuous segments, indicating that they arose from the clonal proliferation of single germ cells that had lost an X chromosome.

In conclusion, our results on the frequencies of AZF microdeletions and chromosomal abnormalities in infertile men from eastern China are largely in agreement with previous reports performed in different societies and ethnic groups. However, they show a relatively higher incidence of

chromosomal anomalies and Y chromosome deletions in NOA in our centers. Because of the small sample size in the SOS group, further studies with larger patient groups should be conducted to clarify the chromosomal abnormality incidence in our region. Screening for chromosomal abnormalities and Yq microdeletions is strongly recommended for infertile patients during diagnosis and before ART. Specific PGD should also be performed to minimize the risk of transmitting genetic defects to offspring.

Author contributions

PP and XFC designed the study. CX drafted the manuscript, CX, XFC, YLL and ZMW collected and interpreted the data. XFC critically revised the paper for intellectual content, and PP reviewed the paper. All authors read and approved the final manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. der Kretser DM. Male infertility. *Lancet* 1997; 349: 787–790.
2. Dada R, Thilagavathi J, Venkatesh S, et al. Genetic testing in male infertility. *Open Reprod Sci J* 2011; 3: 42–56.
3. Schultz N, Hamra FK and Garbers DL. A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. *Proc Natl Acad Sci U S A* 2003; 100: 12201–12206.
4. Elghezal H, Hidar S, Braham R, et al. Chromosome abnormalities in one thousand infertile males with nonobstructive sperm disorders. *Fertil Steril* 2006; 86: 1792–1795.
5. Gekas J, Thepot F, Turleau C, et al. Chromosomal factors of infertility in candidate couples for ICSI: an equal risk of constitutional aberrations in women and men. *Hum Reprod* 2001; 16: 82–90.
6. Pylyp LY, Spinenko LO, Verhoglyad NV, et al. Chromosomal abnormalities in patients with oligozoospermia and non-obstructive azoospermia. *J Assist Reprod Genet* 2013; 30: 729–732.
7. Koşar PA, Özçelik N and Koşar A. Cytogenetic abnormalities detected in patients with non-obstructive azoospermia and severe oligozoospermia. *J Assist Reprod Genet* 2010; 27: 17–21.
8. O'Flynn O'Brien KL, Varghese AC and Agarwal A. The genetic causes of male factor infertility: a review. *Fertil Steril* 2010; 93: 1–12.
9. Asero P, La Vignera S and Lanzafame F. Genetic aspects of male infertility. *J Androl Sci* 2010; 17: 1–16.
10. Brugh VM 3rd and Lipshultz LI. Male factor infertility: evaluation and management. *Med Clin North Am* 2004; 88: 367.
11. Bache I, Assche EV, Cingoz S, et al. An excess of chromosome 1 breakpoints in male infertility. *Eur J Hum Genet* 2004; 12: 993–1000.
12. Lipshultz LI and Lamb DJ. Risk of transmission of genetic diseases by assisted reproduction. *Nat Clin Pract Urol* 2007; 4: 460–461.
13. Bojesen A and Gravholt CH. Klinefelter syndrome in clinical practice. *Nat Clin Pract Urol* 2007; 4: 192–204.
14. Esteves SC. Clinical management of infertile men with nonobstructive azoospermia. *Asina J Androl* 2015; 17: 459–470.
15. Mitelman F, ISCN (eds). *An international system for human cytogenetic nomenclature*. Basel: Karger; 1995.
16. Ferlin A, Raicu F, Gatta V, et al. Male infertility: role of genetic background. *Reprod Biomed Online* 2007; 14: 734–745.
17. Hofherr SE, Wiktor AE, Kipp BR, et al. Clinical diagnostic testing for the cytogenetic

- and molecular causes of male infertility: the Mayo Clinic experience. *J Assist Reprod Genet* 2011; 28: 1091–1098.
18. Elghezal H, Hidar S, Braham R, et al. Chromosome abnormalities in one thousand infertile males with nonobstructive sperm disorders. *Fertil Steril* 2006; 86: 1792–1795.
 19. Yatsenko AN, Yatsenko SA, Weedon JW, et al. Comprehensive 5-year study of cytogenetic aberrations in 668 infertile men. *J Urol* 2010; 183: 1636–1642.
 20. Hotaling J and Carrell DT. Clinical genetic testing for male factor infertility: current applications and future directions. *Andrology* 2014; 2: 339–350.
 21. Wang RX, Fu C, Yang YP, et al. Male infertility in China: laboratory finding for AZF microdeletions and chromosomal abnormalities in infertile men from Northeastern China. *J Assist Reprod Genet* 2010; 27: 391–396.
 22. Kleiman SE, Almog R, Yogev L, et al. Screening for partial AZFa microdeletions in the Y chromosome of infertile men: is it of clinical relevance? *Fertil Steril* 2012; 98: 43–47.
 23. Foresta C, Moro E and Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev* 2001; 22: 226–239.
 24. Silber SJ, Alagappan R, Brown LG, et al. Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. *Hum Reprod* 1998; 13: 3332–3337.
 25. Fröhesser A and Kotzot D. Chromosomal variants in Klinefelter syndrome. *Sex Dev* 2011; 5: 109–123.
 26. Ni TX, Yan JH, Wang B, et al. Outcomes of 13 ICSI-PGD cycles with ejaculated spermatozoa in patients with Klinefelter syndrome. *Asian J Androl* 2016; 18: 498–499.
 27. Sciarano RB, Luna Hisano CV, Rahn MI, et al. Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. *Hum Reprod* 2009; 24: 2353–2360.
 28. Maiburg M, Repping S and Giltay J. The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. *Fertil Steril* 2012; 98: 253–260.
 29. Chow JC, Yen Z, Ziesche SM, et al. Silencing of the mammalian X chromosome. *Annu Rev Genomics Hum Genet* 2005; 6: 69–92.
 30. Levron J, Aviram-Goldring A, Madgar I, et al. Sperm chromosome analysis and outcome of IVF in patients with non-mosaic Klinefelter's syndrome. *Fertil Steril* 2000; 74: 925–929.
 31. Mroz K, Carrel L and Hunt PA. Germ cell development in the XXY mouse: evidence that X chromosome reactivation is independent of sexual differentiation. *Dev Biol* 1999; 207: 229–238.