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Infertility caused by male partners with genetic defects in Sichuan Province of China

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ABSTRACT. The purpose of this study was to detect chromosomal aberrations and azoospermia factor (AZF) microdeletions in male patients with reproductive problems and to summarize related clinical features to provide reliable information for evaluating prenatal and preimplantation diagnoses. A large cohort of 5083 men with various phenotypes of male infertility was analyzed via G-banding karyotyping, and Origin 8.0 was used to analyze the prevalence of abnormalities. Additionally, patients with azoospermia, oligozoospermia, and oligoasthenozoospermia were analyzed using multiplex polymerase chain reaction to detect microdeletion in the AZF. We identified 387 patients with abnormal karyotypes, and the ratio was 7.61%. Among them were 175 patients with Klinefelter's syndrome, which was the most common numerical chromosomal abnormality and accounted for 45.22% of all chromosomal aberrations. The frequencies of increased satellites, balanced translocations, and Robertsonian translocations were 6.47, 7.00, and 3.62%, respectively. Multiplex polymerase chain reaction performed in 810 cases with azoospermia, oligozoospermia, and oligoasthenozoospermia found a ratio of AZF microdeletions of 4.94%. The finding suggests that chromosomal

abnormalities and AZF deletion are main factors that result in male infertility. Detecting these common genetic variations is necessary in infertile men seeking assisted reproductive technology.

Key words: Male infertility; Klinefelter's syndrome; Chromosome; Prenatal diagnosis; Preimplantation genetic diagnosis

INTRODUCTION

According to the World Health Organization, infertility affects approximately 1 in 6 couples worldwide, and male factor infertility accounts for an estimated half of all infertility cases. Male infertility occurs because of various factors, including those of environmental and genetic origin. The most common genetic causes of male infertility are chromosomal abnormalities and Y chromosome microdeletions, which often result in azoospermia (AS) or oligozoospermia (OS). Among patients with chromosomal aberrations, Klinefelter's syndrome is the most frequent cause of infertility, affecting nearly 4% of infertile men and resulting in significant financial and emotional costs.

Assisted reproductive technology (ART) is widely used to help infertile men produce healthy offspring. Therefore, determining the exact genetic defects in infertile patients is critical to evaluating the risks of ART. The aim of this study was to investigate the frequencies of various chromosomal abnormalities and Y chromosome microdeletions in patients with AS, OS, and oligoasthenozoospermia (OAT) in Sichuan Province, China. The results of this study provide valuable information for improving the diagnosis of male infertility and developing appropriate therapies for infertile men in that region.

MATERIAL AND METHODS

Subjects

A total of 5083 male patients with reproductive problems diagnosed at the Affiliate Hospital of Sichuan Genitalia Hygiene Research Center (Chengdu, China) between September 2010 and May 2012 were recruited for this study. All patients lived in Sichuan Province at the time of the study. The study was approved by the ethics committee of Sichuan University.

Karyotype analysis

Karyotyping was performed using standard G-banding. Briefly, peripheral blood lymphocytes were cultured for 72 h in RPMI-1640 with phytohemagglutinin and fetal bovine serum. Two hours before the completion of culturing, colcemid was added to the medium. G-banding of metaphase chromosomes was performed using Giemsa staining. At least 20 metaphase spreads were analyzed for each patient, and at least 50 metaphase spreads were analyzed to confirm abnormalities.

Semen analysis

Conventional semen analysis was performed in patients with Klinefelter's syndrome according to World Health Organization guidelines.

Detection of azoospermia factor (AZF) microdeletions

Multiplex polymerase chain reaction was performed to detect microdeletions in the AZF region for patients with AS, OS, and OAT. Genomic DNA was extracted from peripheral blood lymphocytes using an H.Q. & Q. Blood DNA Kit. Using previous studies in the Chinese population and the diagnostic criteria of European Academy of Andrology, we chose 6 sequence-tagged site (STS) markers in the AZF region to detect microdeletions: sY86 and USP9Y in AZFa, sY127 and sY134 in AZFb, and sY254 and sY255 in AZFc. Additionally, the SRY (sY14; sex-determining region on Y) was used as an internal control, and deionized water and female samples were used as positive and negative controls, respectively. Two multiplex polymerase chain reaction experiments were designed as follows: multiplex1 contained STSs markers for USP9Y, sY134, and sY255, and multiplex2 contained STS markers for SRY, sY86, sY127, and sY254.

Statistical analysis

Data management and statistical analysis were carried out using Origin 8.0.

RESULTS

The results of the study revealed that 387 (7.61%) of all patients (N = 5083) had chromosomal abnormalities, as shown in Table 1. Available karyotypes and clinical characteristics are summarized in Figure 1. Among patients with abnormalities, sex chromosome and autosomal chromosome aberrations accounted for 76.75 and 21.96%, respectively. Additionally, we identified 4 (1.03%) patients with complicated translocations between sex and autosomal chromosomes and 8 (2.07%) patients with sex reversal. Klinefelter's syndrome was the most common chromosomal aneuploidy, with 175 cases accounting for 45.22% of all chromosomal aberrations. The number of subjects with increased satellites, balanced translocations, and Robertsonian translocations was 25 (6.46%), 27 (7.00%), and 14 (3.62%), respectively. Chromosomal variants were found in all chromosomes except 4 and 20, whereas chromosomes 1, 13-15, and Y displayed higher frequencies of abnormalities (Figure 2).

DISCUSSION

According to our data, the main causes of male infertility are chromosomal abnormalities, including structural and numerical aberrations. We identified 175 patients with Klinefelter's syndrome, which accounted for the majority of the abnormalities. The incidence of Klinefelter's syndrome in infertile men was 3.42%, which agrees with previously published data (Guichaoua et al., 1993). Increased satellites, balanced translocations, and Robertsonian translocations were the most common autosomal chromosomal abnormalities leading to male infertility.

Klinefelter's syndrome is the most common chromosomal aneuploidy leading to male infertility (Lanfranco et al., 2004), and our study reconfirmed this conclusion. The prevalence of Klinefelter's syndrome is 0.1-0.2% in the general population (Lanfranco et al., 2004), 3.1-4% in infertile male, and 11% in azoospermic men (Van Assche et al., 1996). Only an estimated 25% of individuals with Klinefelter's syndrome in the general population are diagnosed

Table 1. Clinical features of 387 patients with chromosomal aberration.

	Karyotpye	Total	Clinical feature
Sex chromosome			
Numerical abnormalities			
	45, X0/46, XY	2	AS
	47, XXY	166	Small testis, gynecomastia, hypogonadism; AS, OS, OAT
	47, XXY, 21ps+	1	Small testis, gynecomastia, hypogonadism, gonadal dysgenesis; AS, OS,
	47, XXY/46, XY	4	OAT
	48, XXY, +der	1	AS
	48, XXYY	2	Gynecomastia, hypogonadism, gonadal dysgenesis; OS
	46, XX (43)/48, XXYY(7)	1	
Structural abnormalities			
	46, XY, dup (Yp)	1	AS
	46, XY, i (Yq)	2	AS
	46, XY, Y≥18	55	Genital development defect, poor maternal history; AS, OS, OAT
	46, XY, Y≤21	49	Miscarriage; AS, OS, OAT
	46, XY, del (Yq)	4	AS
	46, XY, t (X; Y)	1	AS
	46, XY, t (Xq; Y)	1	AS
Autosomal chromosome			
Numerical abnormalities			
	45, XY, rob (13; 14)	11	Miscarriage, poor materal history
Structural abnormalities			
	46, XY, rob (14; 21)	1	Miscarriage
	45, XY, rob (14; 14)	1	Miscarriage
	45, XY, rob (14; 15)	1	
	46, XY, 1ph+	1	OAT
	46, XY, 13ps+	3	
	46, XY, 14ps+	4	Poor materal history
	46, XY, 15ps+	9	Fetal anomaly, AS
	46, XY, 16ps+	1	
	46, XY, 21ps+	3	AS
	46, XY, 22ps+	4	AS
	46, XY, 1qh+	10	Miscarriage
	46, XY, 16qh+	1	Fetal anomaly
	46, XY, dup (1q12)	5	Poor maternal history
	46, XY, dup (9p13)	1	
	46, XY, dup (9p21)	2	Poor maternal history
	46, XY, dup (19) (pter→p13:p13→p12::p13→qter)	1	Miscarriage
	46, XY, inv (1)	1	Miscarriage
	46, XY, inv (1p)	1	Miscarriage
	46, XY, inv (9)	2	Poor maternal history
	46, XY, inv (21q)	1	Miscarriage
	46, XY, t (1; 11)	2	OAT
	46, XY, t (1; 12)	1	
	46, XY, t (1; 17)	1	
	46, XY, t (1; 5)	2	Miscarriage
	46, XY, t (1; 7)	2	
	46, XY, t (1; 8)	1	Miscarriage
	46, XY, t (1; 9)	1	Miscarriage
	46, XY, t (2; 13)	1	
	46, XY, t (3; 15)	1	
	46, XY, t (5; 17)	1	Poor materal history
	46, XY, t (6; 14)	1	AS
	46, XY/46, XY, t (7; 13) (20/1)	1	AS
	46, XY/46, XY, t (8p; q) (20/1)	1	OAT
	46, XY/46, XY, t (7; 14) (10/2)	1	OAT
	46, XY, t (15; 18)	1	AS
	46, XY, t (1q; 17p)	1	Miscarriage
	46, XY, t (2; 9; 18)	1	Miscarriage

Continued on next page

Table 1. Continued.

	Karyotpye	Total	Clinical feature
	46, XY, t (6; 19) (6pter→6q22::19q14→19qter; 19pter→19q14::6q22→6qter)	1	
Translocation between sex chromosome and autosome			
	46, XY, t (5; X)	1	AS
	46, XY, t (1; Y)	1	OAT
	46, XY, t (13; Y)	1	AS
	46, XY, t (16q; Xp)	1	AS
Sex reversal			
	46, XX	8	Male phenotype, infertile, small testis
		387	

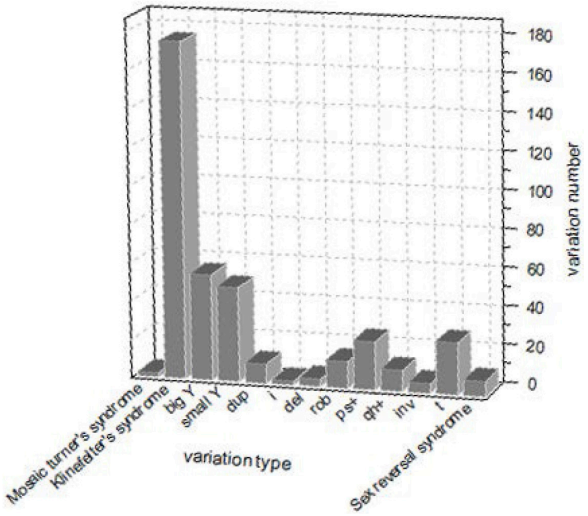

Figure 1. Types of chromosomal abnormality and prevalence, big Y meaning Y ≥ 18 Chromosome, small Ymeaning Y ≤ 21 Chromosome.

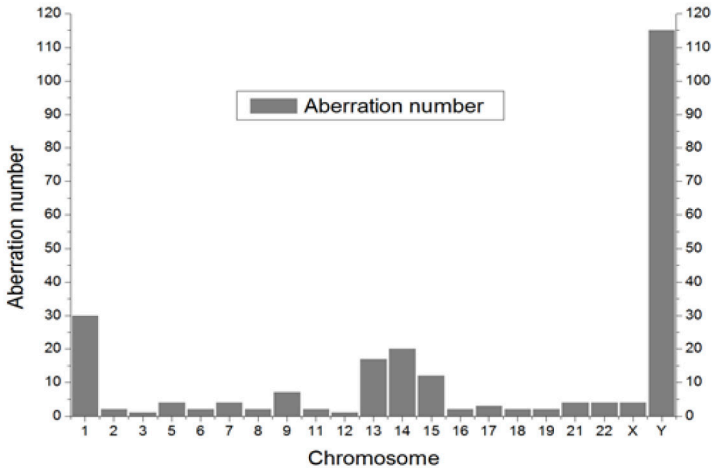

Figure 2. Distribution of abnormality in chromosomes (Klinefelter's syndrome was excluded).

as such, and the syndrome is detected in only a limited number of individuals before puberty because physical anomalies are difficult to notice in children. The most common reported karyotype of Klinefelter's syndrome is non-mosaicism (47, XXY); mosaicism accounts for only 15% of all cases (Mitra et al., 2006). We identified 5 patients with mosaicism (2.86%), which is a notable departure from published reports of 10% (Staessen et al., 2003) and 15% (Mitra et al., 2006). This difference may be due to sample size and study population variations. Patients may have also had inconsistencies in peripheral blood and gonadal karyotypes.

Our results showed that 27.43% of patients with Klinefelter's syndrome have sperm counts higher than 1 x 10^7/mL, as show in Table 2. This percentage is inconsistent with the conclusions of Reubinoff et al. (1998), who consider that non-mosaic Klinefelter's syndrome patients are generally azoospermic owing to primary testicular failure. We hope to launch more thorough research on this kind of patient to explore the reason. However, the sperm of these patients displayed abnormalities in both morphology and mobility, which may result from abnormal concentrations of follicle-stimulating hormone and luteinizing hormone and lower testosterone levels in the microenvironment for sperm genesis (Figure 3). Differences in physical qualities of patients and life habits may also contribute to variations in semen parameters.

Table 2. Semen analysis of 175 patient with Klinefelter's syndrome.

Sperm concentration (10^6/mL)[a]	N	Ratio (%)
0	119	68
0-10	8	4.57
10-50	32	18.29
50-100	16	9.14

[a]Moderate OS 2 x 10^7-1 x 10^7/mL; mild OS 1 x 10^7-0.5 x 10^7/mL; severe OS < 0.5 x 10^7/mL.

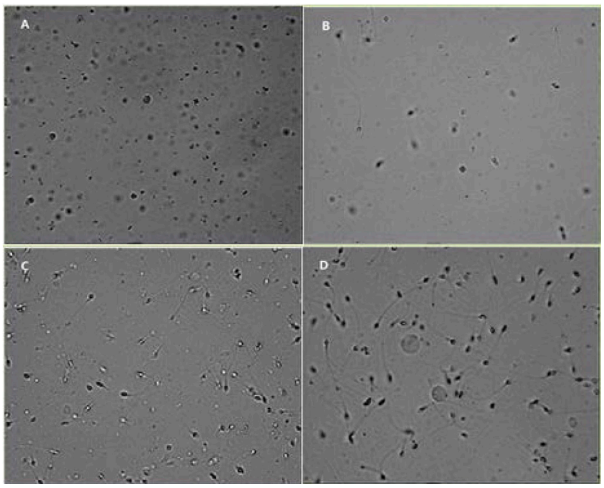

Figure 3. A. Klinefelter's Syndrome with no spermatozoon; **B.** Klinefelter's Syndrome with minimum spermatozoon density = 9.091 x 10^6/mL; **C.** Klinefelter's Syndrome with maximum spermatozoon density = 57.046 x 10^6/mL; **D.** Normal control part spermatozoon density = 69.389 x 10^6/mL.

A syndrome known as 48, XXYY is a rare karyotype of sex chromosome aneuploidy occurring at a rate of approximately 1 in 18,000 males (Tartaglia et al., 2009). Two

subjects with this syndrome were identified in our study. The frequency of 48, XXYY increases to 1 in 2500 in infertile men, so the higher incidence in our study compared to that of Tartaglia et al. (2009) is not surprising given that our study focused on infertile males. The pathogenesis of this rare karyotype is complicated and may result from the nondisjunction of primary spermatocyte in meiosis I followed by the nondisjunction of sister chromatids in meiosis II or from the combination of an aneuploidy gamete derived from nondisjunction of secondary spermatocytes and maternal secondary oocytes (Kleiman et al., 1999).

Sex reversal syndrome (SRS) is a disease of gonadal sex and chromosomal sex inconsistency (Rajender et al., 2006). SRS can be divided into 46, XX male SRS and 46, XY female SRS. In humans, XX maleness occurs with an incidence of approximately 1 in every 20,000-30,000 male births (Ergun-Longmire et al., 2005; Rajender et al., 2006; Vorona et al., 2007). The incidence in the infertile men in our study was 1.57% above average. We identified 8 patients with SRS who were phenotypically male with infertility and small testes. This phenotype may result from the expression of the SRY gene or upregulated expression of genes in the *SOX* family (Alves et al., 2010).

The analysis of 810 patients with OA, OS, and OAT revealed that the prevalence of chromosomal abnormality and AZF deletion was 13.58% (cf.; 14.5%, Retief et al., 1984; 15.4%, Bourrouillou et al., 1985) and 4.94% (c.f.; 5-10%, Zhu et al., 2008), respectively, as shown in Table 3. The frequency of abnormal karyotype with AZF deletion was 1.85% lower than that in a study by Devroey et al. (2009), who reported 3.68% (see Table 3). The analysis for microdeletions revealed that sY254 and sY255 in AZFc were the most common deletion sites (Table 4). On the contrary, a study by Li et al. (2012) in populations of northern Chinese has shown that AZFa and AZFb display deletion rates higher than that in AZFc. More samples should be studied to confirm this difference.

Table 3. Six STS analysis for microdeletion in AZF region and chromosomal abnormalities in 810 patients with AS, OS.

	Chromosomal abnormality	AZF deletion	Chromosomal abnormality + AZF deletion
N	110	40	15
Ratio (%)	13.58	4.94	1.85

Six STSs include sY86 and USP9Y in AZFa; sY127 and sY134 in AZFb; sY254 and sY255 in AZFc.

Table 4. Forty cases AZF region deletion sites.

Case No.	AZFa		AZFb		AZFc		SRY
	sY86	USP9Y	sY127	sY134	sY254	sY255	
1	▲						+
6	▲	▲					+
2			▲	▲			+
22					▲	▲	+
2			▲	▲	▲	▲	+
5	▲	▲	▲	▲	▲	▲	+
2	▲	▲	▲	▲	▲	▲	-
Total number	14	13	11	11	31	31	2

▲ and blank cells mean absence or presence of AZFs region, respectively; (+) and (-) mean positive and negative of SRY, respectively.

We identified 55 patients with Y ≥ 18 Chromosome and 49 patients with Y ≤ 21 Chromosome, which accounted for 2.05% of all patients in our study population. These variations in the Y chromosome may result in chromosomal instability and increase the risk of deletions in AZF regions in these patients.

The main autosomal variations in the study population included balanced translocations, Robertsonian translocation, increased satellites, increased secondary constriction, deletion, duplication, and inversion. These variations were found in 98 patients. Generally, these changes greatly alter chromosome structure, which may affect a large number of genes; therefore, they often result in AS, OS, OAT, infertility, and habitual abortion (Huang et al., 2012) or, more severely, lead to life-threatening disorders in development and metabolism.

In conclusion, the causation of male infertility is complicated. Chromosomal aberration, microdeletion in the AZF region, and deletion of sex-determined genes may play essential roles. Currently, the primary ART for male infertility is intracytoplasmic sperm injection/*in vitro* fertilization. However, these techniques remain risky if the genetic backgrounds of infertile patients are unclear. Therefore, combining ART and other techniques such as fluorescence *in situ* hybridization and primed *in situ* labeling to detect chromosomal aberration and gene deletion is essential for those who have family members with serious genetic disorder and are seeking reproductive assistance from ART (Tournaye et al., 1996; Van Assche et al., 1999).

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