Asian Journal of Andrology (2020) 22, 642–648
www.asiaandro.com; www.ajandrology.com

Open Access

ORIGINAL ARTICLE

Male Infertility

The association between the two more common genetic causes of spermatogenic failure: a 7-year retrospective study

Hong-Ge Li, Li-Hong Fan, Bei Liu, Ye-Qing Qian, Min Chen, Yi-Xi Sun, Min-Yue Dong

Chromosomal abnormalities and Y chromosome microdeletions are considered to be the two more common genetic causes of spermatogenic failure. However, the relationship between chromosomal aberrations and Y chromosome microdeletions is still unclear. This study was to investigate the incidence and characteristics of chromosomal aberrations and Y chromosome microdeletions in infertile men, and to explore whether there was a correlation between the two genetic defects of spermatogenic failure. A 7-year retrospective study was conducted on 5465 infertile men with nonobstructive azoospermia or oligozoospermia. Karyotype analysis of peripheral blood lymphocytes was performed by standard G-banding techniques. Y chromosome microdeletions were screened by multiplex PCR amplification with six specific sequence-tagged site (STS) markers. Among the 5465 infertile men analyzed, 371 (6.8%) had Y chromosome microdeletions and the prevalence of microdeletions in azoospermia was 10.5% (259/2474) and in severe oligozoospermia was 6.3% (107/1705). A total of 4003 (73.2%) infertile men underwent karyotyping; 370 (9.2%) had chromosomal abnormalities and 222 (5.5%) had chromosomal polymorphisms. Karyotype analysis was performed on 272 (73.3%) patients with Y chromosome microdeletions and 77 (28.3%) had chromosomal aberrations, all of which involved sex chromosomes but not autosomes. There was a significant difference in the frequency of chromosomal abnormalities between men with and without Y chromosome microdeletions ($P < 0.05$).

Asian Journal of Andrology (2020) **22**, 642–648; doi: 10.4103/aja.aja_13_20; published online: 01 May 2020

Keywords: azoospermia factor; chromosomal aberrations; infertile men; nonobstructive azoospermia and oligozoospermia; spermatogenic failure; Y chromosome microdeletions

INTRODUCTION

Infertility affects 10%–15% of married couples around the world, and about half of these cases are due to male factors.[1,2] Genetic abnormalities, including cytogenetic and molecular abnormalities, account for 15%–30% of male infertility by affecting spermatogenesis and sperm transport.[3] 47,XXY (Klinefelter's syndrome) is the most frequent genetic cause of spermatogenic failure and accounts for approximately two-thirds of all the cytogenetic abnormalities observed in infertile men.[4] Y chromosome microdeletions are the second-most common genetic cause of male infertility[5,6] that are associated with severe spermatogenic failure and are the most common molecular genetic cause of nonobstructive azoospermia and severe oligozoospermia.[7] It has been reported that the prevalence of Y chromosome microdeletions in severe oligozoospermia is 5%–10% and in azoospermia is 10%–15%.[8] The "azoospermia factor (AZF)" region is responsible for the Y chromosome microdeletions and is divided into three nonoverlapping regions described as AZFa, AZFb, and AZFc.

Nowadays, it is frequently recommended that men with nonobstructive azoospermia and severe oligozoospermia undergo karyotyping and Y chromosome microdeletion analysis for the detection of cytogenetic and molecular abnormalities before assisted reproductive technology (ART).[9] In previous reports, the important roles of Y chromosome microdeletions and chromosomal aberrations have been extensively studied. However, the relationship between chromosomal aberrations and Y chromosome microdeletions is poorly understood. Some studies on small samples have found that there might be a correlation between the two genetic causes, but the specific relationship is unclear. This study was aimed at determining the incidence and characteristics of chromosomal aberrations and Y chromosome microdeletions in infertile men from southeastern China, and to explore whether there was an association between the two more common genetic causes of spermatogenic failure.

PATIENTS AND METHODS

Patients

5465 outpatients who visited the Women's Hospital, School of Medicine, Zhejiang University (Hangzhou, China) for infertility counseling between May 2012 and December 2018, were included in the retrospective study. The patients' ages ranged from 17 to 62 (mean ± standard deviation [s.d.]: 31.4 ± 5.3) years, and infertility lasted from 2 to 21 years. Primary spermatogenic failure of nonobstructive azoospermia or oligozoospermia was confirmed after obtaining the medical history, genital examination, semen analysis results, ultrasonography, hormonal analysis, karyotyping, as well as the detection of AZF microdeletions.

Women's Hospital, School of Medicine, Zhejiang University, Hangzhou 310006, China.
Correspondence: Dr. MY Dong (dongmy@zju.edu.cn)
Received: 19 August 2019; Accepted: 14 January 2020

Semen parameters were evaluated following the WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction (WHO, 2010, 5th edition).[10] Semen samples were collected in the laboratory after 3–5 days of sexual abstinence and were evaluated within 1 h after ejaculation by SQA-V GOLD (Medical Electronic Systems Ltd., Haifa, Israel) and Makler counting chamber (0.01 mm^2, 10 μm deep; Sefi Medical Instruments, Haifa, Israel). Semen (taken at intervals of 1–3 weeks) from all the patients was analyzed on at least two occasions. All the experimental operations followed the standard protocol of the Women's Hospital, School of Medicine, Zhejiang University of Male Laboratory Guidelines. Patients were defined as having mild oligozoospermia if their last three semen samples had sperm concentrations of $\geq 10 \times 10^6$ ml^{-1} and $<15 \times 10^6$ ml^{-1}, moderate oligozoospermia if their sperm concentrations were $\geq 5 \times 10^6$ ml^{-1} and $<10 \times 10^6$ ml^{-1}, severe oligozoospermia if their sperm concentrations were $<5 \times 10^6$ ml^{-1}, or azoospermia if no spermatozoa were present in the ejaculate after centrifugation (1 ml, 3000g, 15 min; Thermo Fisher Scientific, Waltham, MA, USA). This study was reviewed and approved by the Ethics Committee of the Women's Hospital, School of Medicine, Zhejiang University. Informed consent was obtained from all the participants before enrollment in the project. All the methods used in the study followed the current approved guidelines.

Analysis of Y chromosome microdeletions

Human genomic DNA was extracted from peripheral blood lymphocytes with the QIAampH DNA Blood Mini Kit (QIAGEN, Hilden, Germany) with a phenol-chloroform Proteinase-K standard protocol according to the manufacturer's instructions. Y chromosome microdeletions were performed by multiplex PCR amplification with specific sequence-tagged site (STS) markers following the standard protocol of the Women's Hospital, School of Medicine, Zhejiang University of Human Molecular Genetics Guidelines. Multiplex PCR amplification was performed on the LightCycler 480 II thermocycler (Roche Applied Science GmbH, Mannheim, Germany). The Human Y Chromosome Microdeletion Gene Detection Kit (AmoyDx Biomedicine Technology Co. Ltd., Xiamen, China) was used for the detection of Y chromosome microdeletion. All six STS markers recommended by the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMGQ) were used for the detection of Y chromosome microdeletions, including sY84 and sY86 for AZFa, sY127 and sY134 for AZFb, and sY254 and sY255 for AZFc. In addition, the sY-14 (an STS located within the sex-determining region of Y, *SRY*) and *ZFX/ZFY* were used as internal controls. Primer sequences used for the PCR amplification are summarized in **Table 1**. Specifically, FAM-, HEX-, ROX-, and Cy5- labeled probes and their corresponding primers were designed to detect the target STS markers located on the AZFa, AZFb, and AZFc regions of the Y chromosome; deletion of the AZF region would result in the absence of the amplification curve in the corresponding fluorescent channel. Primers were pooled into reaction A, consisting of primers and probes of sY-14, sY86, sY127, and sY254, and reaction B, consisting of primers and probes of *ZFX/ZFY*, sY84, sY134, and sY255. PCR for each sample was performed in 25 μl reaction mixture consisting of 2 μl genomic DNA, 22.75 μl PCR reaction solution, and 0.25 μl mixed enzyme solution. PCR was performed with the following thermal cycling conditions: 95°C for 3 min, then 10 cycles at 95°C for 15 s, 63°C for 20 s, 72°C for 20 s, followed by 30 cycles at 95°C for 15 s, 63°C for 32 s, 72°C for 20 s, and a final extension at 72°C for 10 min. DNA samples from normozoospermic men and healthy women were used as positive and negative controls, respectively, and water was used as blank control. The amplification curves for all the corresponding fluorescent channels should be present in normozoospermic men and absent from healthy women. An STS marker was considered to be deleted only after at least two failed PCR amplification attempts with single primer pairs, while the *SRY* and *ZFX/ZFY* were successfully amplified to confirm the assay validity.

Table 1: Primer sequences used in polymerase chain reaction amplification for the analysis of Y chromosome microdeletions

STS markers	*Regions*	*Primer sequences (5'–3')*	*PCR products (bp)*
sY84	AZFa	Forward: 5'-AGAAGGGTCTGAAAGCAGGT-3'	326
		Reverse: 5'-GCCTACTACCTGGAGGCTTC-3'	
sY86	AZFa	Forward: 5'-GTGACACACAGACTATGCTTC-3'	320
		Reverse: 5'-ACACACAGAGGGACAACCCT-3'	
sY127	AZFb	Forward: 5'-GGCTCACAAACGAAAAGAAA-3'	274
		Reverse: 5'-CTGCAGGCAGTAATAAGGGA-3'	
sY134	AZFb	Forward: 5'-GTCTGCCTCACCATAAAACG-3'	301
		Reverse: 5'-ACCACTGCCAAAACTTTCAA-3'	
sY254	AZFc	Forward: 5'-GGGTGTTACCAGAAGGCAAA-3'	400
		Reverse: 5'-GAACCGTATCTACCAAAGCAGC-3'	
sY255	AZFc	Forward: 5'-GTTACAGGATTCGGCGTGAT-3'	126
		Reverse: 5'-CTCGTCATGTGCAGCCAC-3'	
SRY	Yp11.3	Forward: 5'-GAATATTCCCGCTCTCCGGA-3'	472
		Reverse: 5'-GCTGGTGCTCCATTCTTGAG-3'	
ZFX/ZFY	Xp21.3	Forward: 5'-CCATTCACACGAAAGACTATCC-3'	585
		Reverse: 5'-AGACCTGACTGTAAAATCTCCC-3'	

STS: specific sequence-tagged site; AZF: azoospermia factor; PCR: polymerase chain reaction; *SRY*: sex-determining region of Y; *ZFX/ZFY:* zinc finger protein X-linked/zinc finger protein Y-linked

Karyotype analysis

Karyotype analysis was performed on peripheral blood lymphocytes following the standard protocol of the Women's Hospital, School of Medicine, Zhejiang University of Human Cytogenetics Guidelines, as previously described.[11] Five milliliter peripheral blood was collected in a heparinized blood collection tube, and about 1 ml was incubated aseptically in lymphocyte culture solution (Yishengjun, BaiDi Biotech Co. Ltd., Guangzhou, China) at 37°C for 72 h. The remaining blood was stored at 4°C in case the experiment needed to be repeated. After 72 h of culture, 20 μg ml^{-1} colchicine (BaiDi Biotech Co. Ltd.) was added to the culture system half an hour before the termination of cell culture to arrest the chromosomes at metaphase. Metaphase chromosomes with targeted 400-band level of resolution were obtained by hypotension, fixation, trypsinization, Giemsa staining, and so forth. Karyotype analysis was performed by GSL-120 high-throughput automatic chromosome scanning platform (CytoVision, Leica, Wetzlar, Germany). After G-banding, at least 30 metaphases were counted and 5 metaphases were analyzed for each patient. If different cell lineages were present in the same patient, the count was increased to 50–100 metaphases to establish the mosaicism. Karyotype results were described by certified physicians following the criteria established by the International System for Human Cytogenetic Nomenclature guidelines (ISCN, 2016, 5th edition).[12] The C-banding technique was used when polymorphisms were difficult to identify.

Statistical analyses

The results were presented as mean ± s.d. and percentages. Statistical analysis was performed with SPSS® version 10.0 (SPSS Inc., Chicago, IL, USA). The Pearson's Chi-squared test was used to determine correlation. All the *P*-values were based on two-sided comparisons with $P < 0.05$ considered as statistically significant.

Genetic counseling

Genetic counseling was provided to patients when chromosomal aberrations or Y chromosome microdeletions were detected. The patients received an explanation about the predicted success rate of testicular sperm retrieval in the absence of seminal spermatozoa and the possibility of their male offspring having the same subfertility problem if intracytoplasmic sperm injection (ICSI) treatment was chosen. Options such as artificial insemination by donor (AID) or gender selection by preimplantation genetic diagnosis (PGD) were provided to the patients during the genetic counseling.

RESULTS

Clinical characteristics of the entire cohort in the study

In total, 2474 patients with nonobstructive azoospermia and 2991 with oligozoospermia (including 1705 with severe, 510 with moderate, and 776 with mild oligozoospermia) were included in the present study. The prevalence of Y chromosome microdeletions in azoospermia was 10.5% (259/2474) and in severe oligozoospermia was 6.3% (107/1705). However, the prevalence of Y chromosome microdeletions in moderate oligozoospermia was 1.0% (5/510), and none of the patients with mild oligozoospermia had AZF microdeletions. Clinical characteristics of the entire study cohort are shown in **Table 2**.

Incidence and characteristics of Y chromosome microdeletions in the study

Of the 5465 infertile males, 371 (6.8%) were diagnosed to have Y chromosome microdeletions. There were nine different microdeletion patterns in these patients. The most common microdeletions were detected in the AZFc region in 238 men (64.2%), followed by AZFb+c in 73 (19.7%), AZFb in 27 (7.3%), AZFa+b+c in 19 (5.1%), AZFa in 8 (2.2%), partial deletions of AZF regions in 5 (1.3%), and AZFb+sY254 in 1 (0.3%). Deletions involving only a part of the region or only a single STS marker were defined as partial AZF deletions. The partial deletions of AZF regions included partial deletion of the AZFa region (sY84) in one patient and partial deletion of the AZFb region (sY127 or sY134) in four patients. The patterns and numbers of AZF microdeletions are shown in **Table 3**.

Semen analysis results of the 371 patients with Y chromosome microdeletions

Semen analysis showed that 259 (69.8%) of 371 infertile men with Y chromosome microdeletions had azoospermia and 112 (30.2%) had oligozoospermia. As shown in **Table 3**, 126 of 238 (52.9%) patients with AZFc microdeletions had azoospermia and 112 of 238 (47.0%) patients had oligozoospermia, whereas all the patients with AZFa, AZFb, AZFb+c, and AZFa+b+c microdeletions had azoospermia. Azoospermia was also observed in all the patients with partial deletion of AZFa (sY84), partial deletion of AZFb (sY127 or sY134), and AZFb+sY254 microdeletions. However, no Y chromosome microdeletion was found in patients with a sperm concentration $>10 \times 10^6$ ml^{-1}.

Incidence and characteristics of chromosomal aberrations and AZF microdeletions

A total of 4003 (73.2%) of 5465 infertile men underwent karyotyping, of which 370 (9.2%) had chromosomal abnormalities and 222 (5.5%) had chromosomal polymorphisms. The incidence and characteristics of chromosomal aberrations and AZF microdeletions in the study are shown in **Supplementary Table 1**. We found that 54 of 370 patients (14.6%) with chromosomal abnormalities had Y chromosome microdeletions. All the patients with karyotypes 46,X,del(Y)(q11-q12), 46,X,+mar, and 46,X,der(X)t(X;Y)(p22.3;p11.2) had Y chromosome microdeletions. We also found that 9 of 15 (60.0%) patients with karyotype 46,XX and 12 of 18 (66.7%) with 45,X/46,XY mosaicism or its variants had Y chromosome microdeletions. However, only 2 of 184 (1.1%) patients with 47,XXY were found to have Y chromosome microdeletions. In addition, 23 of 222 (10.4%) patients with chromosomal polymorphisms coexisted with Y chromosome microdeletions, including 7 of 61 (11.5%) with 46,XY,Y≤21 and 16 of 35 (45.7%) with 46,X,Yqh-. None of the patients with autosomal aberrations in the study had Y chromosome microdeletions. The incidence and characteristics of Y chromosome microdeletions in patients with chromosomal aberrations are shown in **Table 4**. Abnormal karyotypes might also be present in the 1462 men without karyotype results.

Prevalence and characteristics of chromosomal aberrations in the patients with Y chromosome microdeletions

Karyotype analysis was available for 272 (73.3%) of 371 patients with Y chromosome microdeletions, and 77 (28.3%) of these had chromosomal abnormalities or chromosomal polymorphisms. All the 77 patients who had both chromosomal aberrations and Y chromosome microdeletions were azoospermic. As shown in **Table 5**, the abnormal karyotypes were more likely to be related to large deletions such as AZFa+b+c and AZFb+c. All patients with AZFa+b+c deletions had abnormal karyotypes, while 42 of 53 (79.2%) patients with AZFb+c microdeletions and only 16 of 168 (9.5%) patients with AZFc microdeletions involved karyotypic abnormalities. However, all the patients with microdeletions of AZFa, AZFb, and AZFb+sY254 had normal karyotypes. Moreover, all the patients with partial deletion of a single STS marker deletion, including sY84, sY127, and sY134, had normal karyotypes. In addition, 10 of 53 (18.9%) patients with AZFb+c microdeletions and 2 of 168 (1.2%) patients with AZFc microdeletions had the mosaic phenotype 45,X/46,XY. All the patients with Y chromosome microdeletions had only sex chromosomal aberrations and no relationship with autosomal aberrations in the study.

Table 2: Clinical characteristics of the entire study cohort

Descriptions	*Sperm concentration (10^6 ml^{-1})*				*Total*
	0	*0–5*	*5–10*	*10–15*	
Patients (*n*)	2474	1705	510	776	5465
Patients with Yq microdeletion (*n*)	259	107	5	0	371
The frequencies of Yq microdeletion (%)	10.5	6.3	1.0	0	6.8

Table 3: The patterns and number of microdeletions in AZFa, AZFb, and AZFc regions in the study

Deletion patterns	*Patients (n)*	*Percentage (%)*	*Sperm concentration (10^6 ml^{-1})*			
			0	*0–5*	*5–10*	*10–15*
AZFc	238	64.2	126	107	5	0
AZFb+c	73	19.7	73	0	0	0
AZFb	27	7.3	27	0	0	0
AZFa+b+c	19	5.1	19	0	0	0
AZFa	8	2.2	8	0	0	0
sY134	3	0.8	3	0	0	0
sY127	1	0.3	1	0	0	0
sY84	1	0.3	1	0	0	0
AZFb+sY254	1	0.3	1	0	0	0
Total	371	100.0	259	107	5	0

AZF: azoospermia factor

Table 4: The incidence and characteristics of Y chromosome microdeletions in patients with chromosomal aberrations

Chromosomal abnormalities	*Patients (n)*	*Sperm concentration (10^6 ml^{-1})*				*AZF microdeletions (n)*
		0	*0–5*	*5–10*	*10–15*	
47,XXY	184	181	3			AZFc (2)
46,X,del(Y)(q11-q12)	26	26				AZFc (5) AZFb+c (15) AZFa+b+c (6)
46,XX	15	15				AZFa+b+c (9)
46,X,+mar	3	2	1			AZFc (1) AZFa+b+c (2)
46,X,der(X)t(X;Y)(p22.3;p11.2)	2	2				AZFa+b+c (2)
45,X[12]/46,X,del(Y)(q12)[8]	1	1				AZFb+c (1)
45,X[25]/46,X,del(Y)(q12)[5]	1	1				AZFb+c (1)
45,X[26]/46,XY,Y≤21[14]	1	1				AZFb+c (1)
45,X[34]/46,X,Yqh-[16]	1	1				AZFb+c (1)
45,X[6]/46,XY[16]	1	1				AZFc (1)
46,X,del(Y)(q12)[17]/45,X[13]	1	1				AZFb+c (1)
46,X,del(Y)(q12)[8]/45,X[7]	1	1				AZFb+c (1)
46,X,Yqh-[14]/45,X[6]	1	1				AZFc (1)
46,X,Yqh-[21]/45,X[9]	1	1				AZFb+c (1)
46,X,Yqh-[23]/45,X[7]	1	1				AZFb+c (1)
46,X,Yqh-[44]/45,X[6]	1	1				AZFb+c (1)
46,X,Yqh-[45]/45,X[5]	1	1				AZFb+c (1)
46,XY,Y≤21	61	14	2	2	43	AZFc (4) AZFb+c (3)
46,X,Yqh-	35	21	1	1	12	AZFc (2) AZFb+c (14)
Total	338	273	7	3	55	77

AZF: azoospermia factor

Correlation analysis between chromosomal abnormalities and Y chromosome microdeletions

In this study, 54 of 272 (19.9%) patients with Y chromosome microdeletions had chromosomal abnormalities, while 316 of 3731 (8.5%) patients without Y chromosome microdeletions had chromosomal abnormalities. We performed statistical analysis in the frequency of chromosomal abnormalities in men with Y chromosome microdeletions and compared this to the frequency of chromosomal abnormalities in men without Y chromosome microdeletions. The difference was statistically significant between the two groups ($\chi^2 = 39.161$, $P < 0.05$).

DISCUSSION

Chromosomal abnormalities are one of the major causes of human infertility,[13] and some authors believe that the presence of chromatin abnormalities interferes with spermatogenesis and affects sperm production.[14] Chromosomal abnormalities are more frequently observed in azoospermic and oligozoospermic patients than in the general population.[15] Karyotype analysis has become increasingly important for distinguishing multiple cytogenetic causes of human infertility. In the present study, a majority (73.2%) of infertile men underwent karyotyping, but few of them (9.2%) had chromosomal abnormalities and even fewer (5.5%) had chromosomal polymorphisms. We found that 14.6% of patients with chromosomal abnormalities and 10.4% with chromosomal polymorphisms had Y chromosome microdeletions. The data from this study also showed that the chromosomal aberrations in patients with Y chromosome microdeletions only involved the sex chromosomes, not autosomes. The main autosomal variations in the study included balanced translocations, Robertsonian translocations, and inversions. However, none of the patients in the study with autosomal variations had AZF microdeletions; the cause of infertility in these patients might be the chromosomal abnormalities.

The prevalence of chromosomal abnormalities in infertile men here was 9.2%, which is consistent with previous values ranging from 2.2% to 14.3%.[16,17] We identified 4.6% (184/4003) of patients with Klinefelter's syndrome, which accounted for about half (49.7%, 184/370) of the chromosomal abnormalities. However, only 1.1% of patients with Klinefelter's syndrome had AZF microdeletions. Klinefelter's syndrome usually causes the arrest of spermatogenesis at the primary spermatocyte stage, but occasionally, it is observed in the later stages of sperm development.[18] Previous studies have found that 74% of men with Klinefelter's syndrome are azoospermic,[19] and 25% of patients with Klinefelter's syndrome have spermatozoa in their ejaculates.[8] However, in this study, less than 2% (1.6%, 3/184) of patients with Klinefelter's syndrome had seminal spermatozoa, and the remaining 98% of patients were azoospermic. 46,X,del(Y)(q11-q12) ranked the second among the abnormal karyotypes and accounted for a relatively low percentage (7.0%, 26/370) of abnormalities. Patients with the 46,X,del(Y)(q11-q12) karyotype had a structural deletion on the Y chromosome, which is close to the AZF region. All the patients with the 46,X,del(Y)(q11-q12) karyotype had AZF microdeletions (15 was AZFb+c, 5 was AZFc, and 6 was AZFa+b+c) in this study. Karyotyping is a reliable technique for the identification of most chromosomal abnormalities, but it cannot detect subtle variations in chromosomal structure, only detecting unbalanced anomalies of at least 5–20 Mb.[20] Therefore, although all patients with deleted positions of q11-q12 had AZF deletions in the study, it was not accurate to classify them as having AZF microdeletions when the Y chromosome was lacking q11-q12.

Table 5: Prevalence and characteristics of chromosomal aberrations in patients with Y chromosome microdeletions

Deletion patterns	*Patients (n)*	*Karyotype results*				
		ND	*Normal*	*Abnormal*	*Abnormal (%)*	*Abnormal karyotypes*
AZFb	27	9	18	0	0	
AZFa	8	0	8	0	0	
AZFb+c	73	20	11	42	79.2	46,X,Yqh- (*n*=14) 46,X,del(Y)(q11.23) (*n*=9) 46,X,del(Y)(q12) (*n*=6) 46,XY,Y≤21 (*n*=3) 45,X[26]/46,XY,Y≤21[14] (*n*=1) 46,X,Yqh-[21]/45,X[9] (*n*=1) 46,X,Yqh-[23]/45,X[7] (*n*=1) 46,X,Yqh-[44]/45,X[6] (*n*=1) 46,X,Yqh-[45]/45,X[5] (*n*=1) 45,X[34]/46,X,Yqh-[16] (*n*=1) 45,X[12]/46,X,del(Y)(q12)[8] (*n*=1) 45,X[25]/46,X,del(Y)(q12)[5] (*n*=1) 46,X,del(Y)(q12)[8]/45,X[7] (*n*=1) 46,X,del(Y)(q12)[17]/45,X[13] (*n*=1)
AZFc	238	70	152	16	9.5	46,X,del(Y)(q11.23) (*n*=3) 46,X,del(Y)(q11.21) (*n*=2) 46,X,Yqh- (*n*=2) 46,XY,Y≤21 (*n*=4) 47,XXY (*n*=2) 46,X,Yqh-[14]/45,X[6] (*n*=1) 45,X[6]/46,XY[16] (*n*=1) 46,X,+mar (*n*=1)
AZFa+b+c	19	0	0	19	100.0	46,X,del(Y)(q11.22) (*n*=3) 46,X,del(Y)(q11.23) (*n*=3) 46,X,der(X)t(X;Y)(p22.3 p11.2) (*n*=2) 46,XX (*n*=9) 46,X,+mar (*n*=2)
sY84	1	0	1	0	0.0	
sY127	1	0	1	0	0.0	
sY134	3	0	3	0	0.0	
AZFb+sY254	1	0	1	0	0.0	
Total	371	99	195	77	28.3	

AZF: azoospermia factor; ND: no date

Sex reversal syndrome (SRS) is a disease characterized by a variable degree of mismatch between the phenotype and the genotype of the affected individual. SRS can be divided into 46,XX males and 46,XY females. 46,XX males are a rare SRS characterized by a female karyotype in discordance with a male phenotype. About 90% of these individuals have Y chromosomal material, including the *SRY* gene, which is usually translocated to the distal tip of the short arm of the X chromosome or autosomal chromosomes. Once karyotype analysis fails to detect a Y chromosome in a phenotypic male, fluorescence *in situ* hybridization (FISH) or molecular amplification with PCR should be performed to determine the presence or absence of the *SRY* gene. In our study, 60.0% of male patients with 46,XX karyotypes had AZFa+b+c microdeletions, due to a recombination of *SRY* and a sex chromosome or autosome (FISH analysis was performed to confirm the recombination). Because of Y chromosome instability, abnormalities in it were frequently observed with sex chromosome mosaicisms, such as 45,X/46,XY or 47,XXY/46,XY.[21,22] The association between Y chromosome microdeletions and the 45,X/46,XY mosaic karyotype has been previously reported,[22,23] suggesting that microdeletions in the long arm of Y chromosome might be associated with Y chromosomal instability leading to the formation of 45,X cell lines. The frequency of sex chromosome mosaicisms detected in the present study was 5.9% (22/370) in patients with chromosomal abnormalities, and 54.5% (12/22) of them carried *AZF* microdeletions, all of which had a 45,X/46,XY mosaic karyotype or its variant (including six cases of 45,X/46,X,Yqh-, four cases of 45,X/46,X,del(Y)(q12), and two cases of 45,X/46,XY). Therefore, Y chromosome instability might be detected at the molecular level as Y chromosome microdeletions and at the cytogenetic level as a 45,X/46,XY mosaicism; more extensive studies are needed to elucidate these intriguing findings. Moreover, deletion of the AZFc region might also make men more likely to lose their Y chromosomes; several studies have found AZFc deletion to be a premutation for 45,X[22,24] and for the mosaic phenotype 45,X/46,XY.[25] In this study, 15.6% (12/77) of patients with AZF microdeletions had the mosaic 45,X/46,XY phenotype or a variant, including 23.8% (10/42) with AZFb+c microdeletions and 12.5% (2/16) with AZFc microdeletions.

Although chromosomal polymorphisms have been categorized as minor chromosomal rearrangements that do not correlate with abnormal phenotypes, many researchers believe that polymorphic variants on chromosomes play a significant role in infertility.[26–28] Higher frequencies of chromosomal polymorphisms have been reported in infertile couples than in the general population.[29,30] However, the correlation between chromosomal polymorphisms and male infertility remains undefined. Therefore, a profound understanding of the relationship between male infertility and polymorphisms is essential. In this study, about 5.5% of patients were found to have chromosomal polymorphisms, but Y chromosome microdeletions involved 45.7% of the patients with 46,X,Yqh- and 11.5% of the patients with 46,XY,Y≤21; no Y chromosome microdeletions were found in other polymorphisms. In conclusion, Y chromosome microdeletions only involved Y chromosome polymorphic variants

(especially Yqh- and Y≤21 variants) and had no relationship with other chromosome polymorphisms. Moreover, the frequency of chromosomal polymorphisms (especially the Yqh- and 46, XY,Y≤21 variants) in patients with Y chromosome microdeletions was significantly higher than in patients without Y chromosome microdeletions, suggesting that a relationship with infertility should not be ignored, which was consistent with evidence from previous studies.[31,32] The mechanism for the association between male infertility and polymorphisms needs further exploration. The cause of infertility may be attributable to the Y chromosome microdeletion, although the nature of the association between the Y chromosome microdeletions and the Y chromosomal polymorphisms remains to be elucidated.

To date, the frequency of chromosomal abnormalities in infertile men with Y chromosome microdeletions has been poorly evaluated, either because the study samples have been too small or the inclusion criteria excluded abnormal karyotypes. In the present study, 6.8% of infertile men were found to have Y chromosome microdeletions, and the prevalence of microdeletions was 10.5% in azoospermia and 6.3% in severe oligozoospermia, which was also consistent with previous studies.[8] Karyotype analysis was performed on 73.3% of patients with Y chromosome microdeletions, and a high frequency (28.3%) of chromosomal aberrations coexisted with Y chromosome microdeletions. In addition, a large combination of deletions appeared to be associated with chromosomal abnormalities. In this study, all patients with AZFa+b+c microdeletions and 79.2% of patients with AZFb+c microdeletions had karyotype abnormalities, while only 9.5% of patients with AZFc microdeletions, and no patients with microdeletions of AZFa or AZFb or AZFb+sY254 had karyotype abnormalities. These data indicated that patients with AZFa, AZFb, or AZFc had a low risk of chromosomal abnormalities, and patients with combined deletions of AZFb+c had an intermediate risk of chromosomal abnormalities, while all the patients with AZFa+b+c, unsurprisingly, had chromosome abnormalities. This might be used to guide practitioners when to obtain a karyotype.

We further explored the relationship between AZF microdeletions and chromosomal aberrations through simple statistical analysis. Because the vast majority of AZF microdeletions occurred *de novo*, since they had a severe effect on male fertility, we only correlated AZF microdeletions with other chromosomal rearrangements that were also likely to be *de novo*. This meant that common chromosomal polymorphisms should be excluded from the statistical analysis. We performed statistical analysis on the frequency of chromosomal abnormalities in men with and without AZF microdeletions and there was statistically significant between the two groups, indicating a direct relationship between chromosomal abnormalities and Y chromosome microdeletions.

CONCLUSIONS

Chromosomal aberrations and Y chromosome microdeletions coexisted at a high frequency, and there was a relationship between the two more common genetic causes of spermatogenic failure in the infertile men studied here. All the patients with Y chromosome microdeletions had only sex chromosome aberrations, and a large combination of deletions appeared to be associated with chromosomal aberrations. Therefore, karyotyping and analyzing Y chromosome microdeletions should be routine cytogenetic and molecular methods in infertile males, and professional genetic counseling should be provided to affected couples to avoid the birth of fetuses carrying genetic defects. Moreover, the frequency of chromosomal polymorphisms (especially the Yqh- and 46,XY,Y≤21 variants) was high in patients with Y chromosome microdeletions, suggesting that its relationship with infertility should not be ignored.

AUTHOR CONTRIBUTIONS

All the authors participated in the study and manuscript preparation. HGL wrote the manuscript; LHF and BL helped with the manuscript revisions; YQQ, MC, and YXS helped prepare, revise, and review the manuscript critically for accurate intellectual content; MYD contributed to the study design, data interpretation, and article revision. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

We should like to thank our patients for agreeing to donate their personal data and allowing the data to be published. We are grateful to Dr. Jiong Gao (BGI Genomics, BGI-Shenzhen, Shenzhen, China) for improving the article. The study was funded by the National Key Research and Development Program of China (Grant No. 2018YFC1004903, and 2016YFC1000703), Key Research and Development Program of Zhejiang Province (Grant No. 2019C03025), the National Nature Science Foundation of China (Grant No. 81801441), and Zhejiang Provincial Natural Science Foundation of China (Grant No. LQ19H090019).

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

REFERENCES

1 Bushnik T, Cook JL, Yuzpe AA, Tough S, Collins J. Estimating the prevalence of infertility in Canada. *Hum Reprod* 2012; 27: 738–46.
2 Kamali M, Baghestani AR, Kashfi F, Kashani H, Tavajohi S, *et al*. A survey on infertility in Royan Institute. *Int J Fertil Steril* 2007; 1: 23–6.
3 Zhou Y, Ge Y, Xiao L, Guo Q. Rapid and simultaneous screening of 47,XXY and *AZF* microdeletions by quadruplex real-time polymerase chain reaction. *Reprod Biol* 2015; 15: 113–21.
4 De-Braekeleer M, Dao TN. Cytogenetic studies in male infertility: a review. *Hum Reprod* 1991; 6: 245–50.
5 Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev* 2001: 22: 226–39.
6 Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, *et al*. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet* 2002; 71: 906–22.
7 Krausz C, Forti G. Clinical aspects of male infertility. *Results Probl Cell Differ* 2000; 28: 1–21.
8 Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, *et al*. Male infertility: role of genetic background. *Reprod Biomed Online* 2007; 14: 734–45.
9 Practice Committee of American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril* 2012; 98: 294–301.
10 World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: WHO Press; 2010. p. 287.
11 Zhang YS, Dai RL, Wang RX, Zhang HG, Chen S, *et al*. Analysis of Y chromosome microdeletion in 1738 infertile men from northeastern China. *Urology* 2013; 82: 584–8.
12 Mcgowan-Jordan J, Simons A, Schmid M. An International System for Human Cytogenetic Nomenclature. Basel: S. Karger Press; 2016. p. 38.
13 Martin RH. Cytogenetic determinants of male fertility. *Hum Reprod Update* 2008; 14: 379–90.
14 Etem EÖ, Yüce H, Erol D, Deveci ŞD, Ceylan GG, *et al*. Cytogenetic analysis in infertile males with sperm anomalies. *Marmara Med J* 2009; 22: 217–24.
15 Tuerlings JH, de France HF, Hamers A, Hordijk R, Van Hemel JO, *et al*. Chromosome studies in 1792 males prior to intra-cytoplasmic sperm injection: the Dutch experience. *Eur J Hum Genet* 1998; 6: 194–200.
16 Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, *et al*. Cytogenetics of infertile men. *Hum Reprod* 1996; 11: 1–24.
17 Vicdan A, Vicdan K, Gunalp S, Kence A, Akarsu C, *et al*. Genetic aspects of human male infertility: the frequency of chromosomal abnormalities and Y chromosome microdeletions in severe male factor infertility. *Eur J Obstet Gynecol Reprod Biol* 2004; 117: 49–54.
18 Georgiou I, Syrrou M, Pardalidis N, Karakitsios K, Mantzavinos T, *et al*. Genetic and epigenetic risks of intracytoplasmic sperm injection method. *Asian J Androl* 2006; 8: 643–73.

19 Foresta C, Garolla A, Bartoloni L, Bettella A, Ferlin A. Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. *J Clin Endocrinol Metab* 2005; 90: 152–6.
20 Peters DG, Yatsenko SA, Surti U, Rajkovic A. Recent advances of genomic testing in perinatal medicine. *Semin Perinatol* 2015; 39: 44–54.
21 Tuck-Muller CM, Chen H, Martinez JE, Shen CC, Li S, *et al*. Isodicentric Y chromosome: cytogenetic, molecular and clinical studies and review of the literature. *Hum Genet* 1995; 96: 119–29.
22 Siffroi JP, Le Bourhis C, Krausz C, Barbaux S, Quintana-Murci L, *et al*. Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. *Hum Reprod* 2000; 15: 2559–62.
23 Patsalis PC, Skordis N, Sismani C, Kousoulidou L, Koumbaris G, *et al*. Identification of high frequency of Y chromosome deletions in patients with sex chromosome mosaicism and correlation with the clinical phenotype and Y-chromosome instability. *Am J Med Genet* 2005; 135: 145–9.
24 Jaruzelska J, Korcz A, Wojda A, Jedrzejczak P, Bierla J, *et al*. Mosaicism for 45,X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion. *J Med Genet* 2001; 38: 798–802.
25 Patsalis PC, Sismani C, Quintana-Murci L, Taleb-Bekkouche F, Krausz C, *et al*. Effects of transmission of Y chromosome AZFc deletions. *Lancet* 2002; 360: 1222–4.
26 Madon PF, Athalye AS, Parikh FR. Polymorphic variants on chromosomes probably play a significant role in infertility. *Reprod Biomed Online* 2005; 11: 726–32.
27 Guo T, Qin Y, Gao X, Chen H, Li G, *et al*. The role of male chromosomal polymorphism played in spermatogenesis and the outcome of IVF/ICSI-ET treatment. *Int J Androl* 2012; 35: 802–9.
28 Belangero SI, Christofolini DM, Bianco B, Gava MM, Wroclawski ER, *et al*. Male infertility related to an aberrant karyotype, 46,XY,9ph,9qh+. *Fertil Steril* 2009; 91, 2732.e1–3.
29 Eiben B, Leiopoldt M, Rammelsberg O, Krause W, Engel W. High incidence of minor chromosomal variants in teratozoospermic males. *Andrologia* 1987; 19: 684–7.
30 Nakamura Y, Kitamura M, Nishimura K, Koga M, Kondoh N, *et al*. Chromosomal variants among 1790 infertile men. *Int J Urol* 2001; 8: 49–52.
31 Sahin FI, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O, *et al*. Chromosome heteromorphisms: an impact on infertility. *J Assist Reprod Genet* 2008; 25: 191–5.
32 Minocherhomji S, Athalye AS, Madon PF, Kulkarni D, Uttamchandani SA, *et al*. A case–control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype. *Fertil Steril* 2009; 92: 88–95.

Supplementary Table 1: The incidence and characteristics of chromosomal aberrations and azoospermia factor microdeletions in the study

Chromosomal abnormalities	*n*	*AZF microdeletions (n)*
47,XXY	184	AZFc (2)
46,X,del(Y)(q11-q12)	26	AZFc (5)
		AZFb+c (15)
		AZFa+b+c (6)
45,XY,der(13;14)(q10;q10)	17	
46,XX	15	AZFa+b+c (9)
47,XYY	10	
45,XY,der(14;21)(q10;q10)	8	
47,XY,+mar	6	
46,X,inv(Y)(p11.2q11.23)	5	
46,X,+mar	3	AZFc (1)
		AZFa+b+c (2)
46,X,der(X)t(X;Y)(p22.3; p11.2)	2	AZFa+b+c (2)
46,XY,inv(5)(p13p14)	2	
47,XX,inv(Y)(p11.2q11.23)	2	
47,XXY[27]/46,XX[3]	2	
48,XXYY	1	
45,X,dic(Y;14)(q12;p11.2)	1	
45,X[12]/46,X,del(Y)(q12)[8]	1	AZFb+c (1)
45,X[25]/46,X,del(Y)(q12)[5]	1	AZFb+c (1)
45,X[26]/46,XY,Y≤21[14]	1	AZFb+c (1)
45,X[34]/46,X,Yqh-[16]	1	AZFb+c (1)
45,X[6]/46,XY[16]	1	AZFc (1)
45,X[6]/46,XY[44]	1	
45,XY,der(13;21)(q10;q10)	1	
45,XY,der(13;22)(q10;q10)	1	
45,XY,der(14;15)(q10;q10)	1	
45,XY,der(15;15)(q10;q10)	1	
45,XY,der(18)t(18;21)(p11.3;q21),-21	1	
45,XY,der(2)t(2;21)(q37;q11.2),-21	1	
45,XY,inv(9)(p12q13),rob(14;21)(q10;q10)	1	
46,X,+mar[22]/46,X,del(Y)(q12)[8]/45,X[2]	1	
46,X,+mar[36]/45,X[11]/46,XY[3]	1	
46,X,del(Y)(q12)[17]/45,X[13]	1	AZFb+c (1)
46,X,del(Y)(q12)[8]/45,X[7]	1	AZFb+c (1)
46,X,idic(Y)(p11.3)	1	
46,X,idic(Y)(p11.3)[45]/45,X[4]	1	
46,X,t(Y;1)(q11.21;p22)	1	
46,X,t(Y;10)(q12;q24)	1	
46,X,t(Y;20)(q12;p11.2)	1	
46,X,Yqh-[14]/45,X[6]	1	AZFc (1)
46,X,Yqh-[21]/45,X[9]	1	AZFb+c (1)
46,X,Yqh-[23]/45,X[7]	1	AZFb+c (1)
46,X,Yqh-[44]/45,X[6]	1	AZFb+c (1)
46,X,Yqh-[45]/45,X[5]	1	AZFb+c (1)
46,XY,1qh+[28]/45,X,1qh+[22]	1	
46,XY,der(6)t(6;14)(q12;p12),der(14)t(6;14) inv(14)(p12q12)	1	
46,XY,dup(9)(q13q21)	1	
46,XY,inv(1)(p13q12)	1	
46,XY,inv(1)(p13q21)	1	
46,XY,inv(1)(p32q25)	1	
46,XY,inv(12)(p13q15)	1	
46,XY,inv(13)(q12q32)	1	
46,XY,inv(14)(q22q24)	1	
46, XY, t (1;5)(p35;q35)	1	
46, XY, t (1;8)(q21;q24.2)	1	
46,XY,t(11;22)(q23;q11.2)	1	
46,XY,t(12;13)(p11.2;q14)	1	
46,XY,t(12;13)(q24.2;q12)	1	
46,XY,t(13;17)(q12;q11.2)	1	
46,XY,t(14;19)(p12;q12)	1	
46,XY,t(15;16)(q11.2;q22)	1	
46,XY,t(16;22)(p13.2;q11.2)	1	
46,XY,t(2;15)(p25;q21)	1	
46,XY,t(2;9)(p23;q22.3)	1	
46,XY,t(20;22)(p13;q12)	1	
46,XY,t(3;19)(q21;q13.3)	1	
46,XY,t(3;21)(q21;q21)[10]/46,XY[40]	1	
46,XY,t(3;4)(q27;q21)	1	
46,XY,t(3;5)(q13.2;p15.2)	1	
46,XY,t(4;13)(q12;q14)	1	
46,XY,t(4;21)(q21;q21)	1	
46,XY,t(4;22)(q31.3;q11.2)	1	
46,XY,t(4;7)(q32;q34)	1	
46,XY,t(5;14)(q22;p12)	1	
46,XY,t(5;21)(q22;q11.2)	1	
46,XY,t(6;12)(p21.3;p13)	1	
46,XY,t(6;15)(p21.3;p11.2)	1	
46,XY,t(7;11)(q11.2;q22)	1	
46,XY,t(7;14)(p13;q22)	1	
46,XY,t(7;9)(p22;q22)	1	
46,XY,t(9;13)(p21;q14)	1	
46,XY,t(9;9)(p12;q21)	1	
46,Y,t(X;6)(q28;p22)	1	
46,Y,t(X;7)(p22.2;p15),9qh+	1	
47,XXY[28]/46,XX[52]	1	
47,XXY[8]/45,X[3]/46,XY[2]/48,XXXY[1]/46,XX[16]	1	
47,XY,+21	1	
47,XY,i(X)(q10)	1	
47,XYY[40]/46,XY[10]	1	
47,XYY[6]/46,XY[44]	1	
48,XY,+mar×2	1	
Total	370	54

Chromosomal polymorphisms	*n*	*AZF microdeletions (n)*
46,XY,Y≤21	61	AZFc (4)
		AZFb+c (3)
46,XY,1qh+	40	
46,X,Yqh-	35	AZFc (2)
		AZFb+c (14)
46,XY,inv(9)(p12q13)	33	
46,XY,Y≥18	10	
46,XY,16qh+	8	
46,XY,9qh+	6	
46,X,Yqh+	4	
46,XY,21pstk+	4	
46,XY,22pstk+	4	
46,XY,14pstk+	3	

Contd...

Supplementary Table 1: Contd...

Chromosomal abnormalities	*n*	*AZF microdeletions (n)*
46,XY,inv(19)(p13.1q13.4)	1	
46,XY,inv(2)(p11.2q13)	1	
46,XY,inv(7)(q22q34)	1	
46,XY,inv(8)(p11.2q21.3)	1	
46,XY,inv(9)(p12q13),t(17;19)(q12;q13.3)	1	
46,XY,t(1;10)(q25;q23.2)	1	
46,XY,t(1;12)(p13;q24.1)	1	
46,XY,t(1;13)(p22;q22)	1	
46,XY,t(1;16)(p32;q22)	1	
46,XY,t(1;19)(q44;q13.3)	1	
46,XY,t(1;2)(p34.1;q37)	1	
46,XY,t(1;20)(q24;q13.3)	1	
46, XY, t (1;5)(p35;q35)	1	
46, XY, t (1;8)(q21;q24.2)	1	
46,XY,15pstk+	2	
46,XY,21ps+	2	
46,XY,21pstkstk	2	
46,XY,13pstk-	1	
46,XY,13pstk+	1	
46,XY,14ps+	1	
46,XY,14pstkstk	1	
46,XY,15pstkstk	1	
46,XY,22pstk-	1	
46,XY,22pstkstk	1	
46,XY,inv(9)(p12q13)×2	1	
Total	222	23