Genetic screening for chromosomal abnormalities and Y chromosome microdeletions in Chinese infertile men

Li Fu · Da-Ke Xiong · Xian-Ping Ding · Chuang Li ·
Li-Yuan Zhang · Min Ding · Shuang-Shuang Nie ·
Qiang Quan

Received: 27 December 2011 / Accepted: 27 February 2012 / Published online: 14 March 2012
© Springer Science+Business Media, LLC 2012

Abstract

Purposes To investigate the frequency and type of both chromosomal abnormalities and Y chromosome microdeletions and analyze their association with defective spermatogenesis in Chinese infertile men.

Methods This is a single center study. Karyotyping using G-banding and screening for Y chromosome microdeletion by multiplex polymerase chain reaction (PCR) were performed in 200 controls and 1,333 infertile men, including 945 patients with non-obstructive azoospermia and 388 patients with severe oligozoospermia.

Results Out of 1,333 infertile patients, 154 (11.55%) presented chromosomal abnormalities. Of these, 139 of 945 (14.71%) were from the azoospermic and 15 of 388 (3.87%) from the severe oligozoospermic patient groups. The incidence of sex chromosomal abnormalities in men with azoospermia was 11.53% compared with 1.03% in men with severe oligozoospermia ($P < 0.01$). Also 144 of 1,333 (10.80%) patients presented Y chromosome microdeletions. The incidence of azoospermia factor (AZF) microdeletion was 11.75% and 8.51% in patients with azoospermia and severe oligozoospermia respectively. Deletion of AZFc was the most common and deletions in AZFa or AZFb or AZFabc were found in azoospermic men. In addition, 34 patients had

chromosomal abnormalities among the 144 patients with Y chromosome microdeletions. No chromosomal abnormality and microdeletion in AZF region were detected in controls.

Conclusions There was a high incidence (19.80%) of chromosomal abnormalities and Y chromosomal microdeletions in Chinese infertile males with azoospermia or severe oligozoospermia. These findings strongly suggest that genetic screening should be advised to infertile men before starting assisted reproductive treatments.

Keywords Male infertility · Chromosomal abnormality · Y chromosome microdeletion · Azoospermia · Severe oligozoospermia

Introduction

Infertility affects about 15% of all the couples attempting to generate pregnancy [7], approximately 50% of which can be attributed to male factors [34]. Over 50% of all infertile males with azoospermia or severe oligozoospermia and genetic abnormalities are thought to account for 15%–30% of male factor infertility [12]. Patients which harbour genetic abnormalities should be provided comprehensive counseling prior to opting for assisted reproductive technique (ART), which can reduce the potential risk of transmission of genetic aberrations to the descendants. Although the underlying etiology is still poorly understood, the primary genetic causes of male infertility that can be passed on to the offspring are cytogenetic abnormalities and Y chromosome microdeletions [4].

Chromosomal abnormalities are confirmed as one of the frequent causes of male infertility, the incidence of which has been shown to be as high as 20% in azoospermic males, with the sex chromosomes more commonly involved [34]. On the other hand, up to 8% of infertile men with severe

Capsule This study uses G-banding and multiplex polymerase chain reaction to investigate the frequency and type of both chromosomal abnormalities and Y chromosome microdeletions in Chinese infertile men.

L. Fu · D.-K. Xiong · X.-P. Ding (✉) · C. Li · L.-Y. Zhang ·
M. Ding · S.-S. Nie · Q. Quan
Institute of Medical Genetics, Key Laboratory of Bio-resources
and Eco-environment, Ministry of Education,
College of Life Science, Sichuan University,
Chengdu 610064, China
e-mail: brainding@scu.edu.cn

oligozoospermia were found to have one or more chromosomal abnormalities, most of which were structural aberration of the autosome, such as robertsonian translocations, balanced translocations, inversions (pericentric or paracentric) [10].

The microdeletion of the azoospermia factor (AZF) region in the Y chromosome was discovered as another frequent genetic cause associated with male infertility. Molecular analysis of infertile men with severe oligozoospermia or azoospermia has identified that AZF region was divided into three nonoverlapping subregions (AZFa, AZFb and AZFc) [32], which encode spermatogenic genes such as USP9Y, RBM and DAZ [26]. Further, Repping and colleagues [24] reported that AZFb and AZFc regions overlapped. Extensive studies have been carried on Y microdeletions in azoospermic and oligozoospermic patients showing an incidence which ranges from 7% to 21% and 0% to 14%, respectively [8, 9, 14].

The aim of this study was to evaluate the frequency and type of chromosomal abnormalities and Y chromosome microdeletions and to analyze the relationship between chromosomal abnormalities and deletions of Yq microdeletion in infertile azoospermic or severe oligozoospermic Chinese men.

Materials and methods

Patients

Patients who were recruited consecutively from the Affiliate Hospital of Sichuan Genitalia Hygiene Research Center (Chengdu, China) between July 2004 and June 2011 were prospectively enrolled into the study. A total of 1,333 infertile Chinese men with non-obstructive azoospermia ($n=945$) or severe oligozoospermia ($n=388$, sperm count $<5 \times 10^6/\text{ml}$) aged between 17 and 43 (mean $\pm$ SD = 29.15 ± 3.18 year). Semen analysis was performed according to World Health Organization recommendations [21]. All subjects underwent semen analysis at least three times. Other possible causes of spermatogenic failure such as endocrine or obstructive causes were excluded. A total of 20 healthy women and 180 men who had proven paternity without assisted reproductive technologies were selected as controls. All participants gave informed consent according to the protocol approved by the institutional ethical review boards of Sichuan University.

Cytogenetic analysis

Karyotyping was performed using the standard G-banding. At least 20 metaphases were analyzed for each patient and control. In cases of karyotype abnormality, more than 30 metaphases were analyzed to confirm the result. We took full advantage of the C-banding for karyotyping when necessary. All chromosomal abnormalities were reported in

accordance with the current international standard nomenclature [25].

Molecular analysis

Genomic DNA was extracted from the whole blood by means of H.Q.&Q.Blood DNA Kit (AnHui U-gene Biotechnology Co.,Ltd, China). The amount of DNA was quantified by spectroscopic methods. Primers covering only hot spot regions were chosen, and the primers' sequences and the size of related PCR products is shown in Table 1. A series of six sequence-tagged sites (STS) from the AZF region on Yq11 were used to detect submicroscopic deletions. These included sY86 and USP9Y (AZFa), sY127 and sY134 (AZFb), sY254 and sY255 (AZFc). In addition, SRY (sY14) gene was used as an internal control. Fertile male, female samples and deionized water were used as positive, negative and blank controls. Two multiplex PCR reactions were designed as follows: multiplex1 contained STSs for (USP9Y, sY134, sY255), Multiplex2 for (SRY, sY86, sY127, sY254). All primers were manufactured by Invitrogen (America). Multiplex PCR amplifications were performed in a total volume of 25 μL buffered solution containing about 200 nanograms of genomic DNA, 2.5 mM of dNTPs, 1.5 mM Mg^{2+} , 10 pM of each primer and 2.5 U Taq polymerase. Thermocycling consisted of 10 min at 95°C for 1 cycle; 30 s at 95°C , 30 s at 59°C , and 30 s at 72°C for 35 cycles; with a final extension of 5 min at 72°C . Finally the samples were stored at 4°C .

PCR samples were analyzed on a 2% agarose gel prepared in $1 \times$ TAE buffer containing ethidium bromide with 120 V for

Table 1 Seven sequence-tagged sites (STSs) primer sequences used for screening Y chromosome microdeletions

STSs	Region	Primer sequence 5'→3'	PCR products (bp)
SY14F (SRY)	Yp	GAATATTCCCCTCTCCGGA	472
SY14R (SRY)		GCTGGTGCTCCATTCTTGAG	
sY86F	AZFa	AGACTATGCTTCAGCAGGTC	156
sY86R		CCAGTCTTTGGGATTCTTT	
USP9YF	AZFa	AGCCTGTTTCACTGTTTGGG	169
USP9YR		ATACCCTCAAGGAACCTCAAG	
sY127F	AZFb	AGCACCCACTGGAATCTACC	195
sY127R		CATGGCTACACAGACAGGGA	
sY134F	AZFb	GTCTGCCTCACCATAAAACG	301
sY134R		ACCACTGCCAAAACCTTTCAA	
sY254F	AZFc	GGGTGTTACCAGAAGGCAAA	380
sY254R		GAACCGTATCTACCAAAGCAGC	
sY255F	AZFc	GTTACAGGATTCTGGCGTGAT	123
sY255R		CTCGTCATGTGCAGCCAC	

45 min at room temperature. The samples that were detected to have deletion of STSs were further confirmed by another single primer reaction for three times.

Statistical analysis

χ^2 test was used to compare differences between the two studied groups. Statistical analysis was carried out with SPSS11.0 statistical software (SPSS Inc., Chicago, Illinois, USA). Data were considered statistically significant when $P < 0.05$.

Results

Cytogenetic evaluation

Karyotyping was performed for 200 controls and 1,333 infertile men with azoospermia ($n=945$) or severe oligozoospermia ($n=388$). No chromosome abnormalities were detected in controls. As shown in Table 2, 154 of the 1,333 (11.55%) infertile patients had chromosomal abnormalities, including 139 of the 945 (14.71%) patients with azoospermia and 15 of the 388 (3.87%) patients with severe oligozoospermia. Significant differences in the incidence of sex chromosomal abnormalities (11.53% vs. 1.03%, $P < 0.01$), but not in autosomal chromosomal abnormalities (3.17% vs. 2.84%, $P > 0.05$) were detected, between patients with azoospermia and patients with severe oligozoospermia.

One hundred and thirteen patients presented sex chromosomal abnormalities accounting for 73.38% of all abnormal karyotypes. About 56.64% of these men bore a 47,XXY karyotype whereas the other 43.36% represented either by 47,XXY/46,XY mosaicism or higher grade sex chromosomal aneuploidy. The remaining 41 patients presented autosomal abnormalities, which accounted for 26.62% of all abnormal karyotypes. Of the autosomal abnormalities, 48.78% were chromosomal translocations, whereas the other 51.22% were chromosomal polymorphism or inversions (pericentric or paracentric). Aberration of chromosome 1 and 14 were little more frequent among autosomal abnormalities, which were present in 27 (65.85%) of 41 patients with autosomal abnormalities.

Y chromosome microdeletion screening

Prevalence of *Yq* microdeletions

A total of 1,533 cases, including 1,333 azoospermic ($n=945$) or severe oligozoospermic ($n=388$) patients and 200 controls, were analyzed for the incidence of Y chromosome deletions. As shown in Table 3, 144 of 1,333 (10.80%) infertile males presented Y chromosome microdeletions. No

microdeletions were detected in controls. The frequency of microdeletions was 11.75% (111/945) in the azoospermic group compared with 8.51% (33/388) in the severe oligozoospermic group ($P > 0.05$).

Type of microdeletions

In this study, the type of Y chromosome microdeletions analyzed included AZFa, AZFb, AZFc, AZFab, AZFac, AZFbc and AZFabc. As shown in Table 4, deletion of AZFc was the most frequent AZF microdeletion in both azoospermic and severe oligozoospermic patients. It was present in 78 of 144 (54.17%) of all AZF microdeletions. More than one third of the carriers with AZFc deletion had mature sperms detected in their semen and the great majority of patients (84.85%) with severe oligozoospermia presented deletion in AZFc region. In addition, 15 infertile patients (10.42%) presented deletion in the AZFb region (13 azoospermic and 2 severe oligozoospermic males) and 13 azoospermic males (9.03%) in the AZFa region. Only azoospermic patients presented AZFa microdeletions. The larger types of microdeletions involving 2 or 3 complete AZF regions included 2.08% of the AZFab, 2.08% of the AZFac, 18.75% of the AZFbc and 3.47% of the AZFabc regions, respectively.

Karyotyping in patients with Y chromosome microdeletions

As shown in Table 5, there were 34 patients (23.61%) with chromosomal abnormalities out of the total 144 patients with Y chromosome microdeletions. The abnormal karyotypes among these 34 patients included 46,XY,Yq-; 46,XX; 45,X/46,XY; 45,X,delY; 46,XY,delYp/45,X,delY, all of them related to the sex chromosome abnormalities. A total of 23 patients with 46,XY,Yq- presented combined deletions in AZFb and AZFc regions, and the another 4 patients with 46,XY,Yq- showed the AZFc region deletions. Four cases with 46,XX carried combined AZFabc deletions. Furthermore, combined deletions involving AZFb,c regions were detected in one 45,X/46,XY and one 46,XY,delYp/45,X,delY patient. Combined deletions involving the three AZF regions were detected in one 45,X,delY patient. The molecular analysis of SRY gene detected the presence of this gene in all four patients with 46,XX and in the patient with 45,X,delY.

Discussion

Several previous studies have reported a wide range (2% to 16%) of chromosomal abnormalities in infertile patients [12, 15, 30, 36]. Reasons for this discrepancy might be ethnic differences or the criteria applied for patient selection. In

Table 2 The abnormal karyotypes in the infertile patients with non-obstructive azoospermia or severe oligozoospermia

Abnormal karyotypes		Non-obstructive azoospermia (<i>n</i> =945)(%)	Severe oligozoospermia (<i>n</i> =388)(%)
Sex chromosome abnormalities		109	4(1.03)
	47,XXY	62(6.56)	2(0.52)
	46,XY,Yq-	29(3.07)	
	46,XY, Yqh ⁺	8(0.85)	1(0.26)
	46,XX	4(0.42)	
	46,XY(94%)/47,XXY(6%)	2(0.21)	1(0.26)
	46,XY,delYp(83%)/45,X,delY (17%)	1(0.11)	
	45,X,delY	1(0.11)	
	45,X(90%)/46,XY(10%)	1(0.11)	
	46,XY,Yp++	1(0.11)	
Autosome abnormalities		30	11(2.84)
	45,XY,t (13;14)	5(0.53)	1(0.26)
	45,XY,t (14;21)	1(0.11)	1(0.26)
	46,XY,t (1;17)	2(0.21)	1(0.26)
	46,XY,t (1;11)	2(0.21)	
	45,XY,t (14;15)		1(0.26)
	46,XY,t (1;7)		1(0.26)
	46,XY,t (1;22)	2(0.21)	
	46,XY,t (1;16)	2(0.21)	
	46,XY,t (2;21)	1(0.11)	
	45,XY,t (13;18)	1(0.11)	
	45,XY,der(22;22)		1(0.26)
	46,XY,15ps ⁺	3(0.32)	1(0.26)
	46,XY,22ps ⁺	2(0.21)	
	46,XY,13ps	2(0.21)	
	46,XY,14ps ⁺	2(0.21)	
	46,XY,21ps ⁺		1(0.26)
	46,XY,1qh ⁺	2(0.21)	1(0.26)
	46,XY,dup(9p)	1(0.11)	
	46,XY,inv(1)(q31;pter)	2(0.21)	1(0.26)
	46,XY,inv(9)(p11;q13)		1(0.26)
Total		139(14.71)	15(3.87)

Table 3 Incidence of Y chromosome microdeletions testing detected in the past 7 years

Year	Nonobstructive azoospermia (n)	Severe oligozoospermia (n)	Total number (<i>n</i>)	Deletion n(%)
2004.7–2005.6	75	27	102	11(10.78)
2005.7–2006.6	97	34	131	11(8.40)
2006.7–2007.6	119	51	170	14(8.24)
2007.7–2008.6	129	31	160	18(11.25)
2008.7–2009.6	138	92	230	23(10.00)
2009.7–2010.6	169	69	238	29(12.18)
2010.7–2011.6	218	84	302	38(12.58)
Total	945	388	1333	144(10.8)
Deletion n(%)	111(11.75)	33(8.51)	144(10.80)	<i>P</i> >0.05

Table 4 Frequency of different Yq AZF microdeletions($n=144$)

	Nonobstructive azoospermia(n)	Severe oligozoospermia (n)	Total $n(\%)$
AZF α	13	0	13(9.03)
AZF β	13	2	15(10.42)
AZF γ	50	28	78(54.17)
AZF $\alpha\beta$	3	0	3(2.08)
AZF $\alpha\gamma$	2	1	3(2.08)
AZF $\beta\gamma$	25	2	27(18.75)
AZF $\alpha\beta\gamma$	5	0	5(3.47)
Deletion $n(\%)$	111(11.75)	33(8.51)	$P>0.05$

this study, the total prevalence of chromosomal aberrations was 11.55% in infertile men that was within the range of the published data. The frequency of chromosomal abnormalities was 14.71% in patients with azoospermia and 3.87% in patients with severe oligozoospermia. These results are also similar to the published data of 13.7%–15.4% in azoospermic patients and 1.7%–4.6% in oligozoospermic patients [20].

Karyotypic abnormalities identified in this work included autosome abnormalities and sex chromosome abnormalities. The most common sex chromosomal aberration was Klinefelter's syndrome, which accounted for 56.64% (64/113) of the abnormalities, followed by Y chromosome terminal deletions (Yq-) and structural autosomal abnormalities. The incidence of sex chromosomal abnormality in men with azoospermia (11.53%) was significantly ($P<0.01$) higher than that in men with severe oligozoospermia (1.03%), which was largely due to the high incidence of patients with Klinefelter's syndrome. There was no significant difference ($P>0.05$) in the incidence

of autosome abnormalities between infertile patients with azoospermia(3.17%) and that with severe oligozoospermia (2.84%). The vast majority of autosome abnormalities in the infertile population was chromosome translocations, which could cause the loss of genetic material at the break points of genes and corrupt the genetic message [5]. Our findings support the previous notion that abnormalities in sex chromosomes are primarily found in azoospermic patients, while balanced autosomal are the most frequent abnormalities in oligozoospermic males [10].

Interestingly, out of the 41 patients with autosome abnormalities, 27(65.85%) had abnormal karyotypes involving chromosome 1 or 14, suggesting that some genes present on these chromosomes might play an important role in spermatogenesis. In fact, several genes closely related to spermatogenesis have been located in chromosome 1, such as SCP-1, tsMCAK, MTHFR, MSH4, and MMP-23 gene [22, 31]. On the other hand, Lian and colleagues [17] found that expression of miRNAs in region of 14q32.31 could be important in spermatogenesis. However, the detailed mechanisms of how abnormalities involving chromosomes 1 or 14 work deserve further investigation.

The overall incidence of chromosomal abnormality was as high as 11.55% in our infertile population. Therefore, we suggest that karyotyping should be requested for all infertile men with azoospermia or severe oligozoospermia in the Chinese population, also for all patients seeking ART treatment.

In 1976, Tiepolo [29] provided the first evidence that deletion of long arm of Y chromosome is associated with infertility in men. Subsequently, numerous studies on the association of the Y chromosome AZF microdeletion with male infertility were reported. These studies revealed that Y chromosome microdeletions ranged from 1% to 55% among infertile men with azoospermia or severe oligozoospermia [1, 13, 18, 37]. We investigated AZF microdeletion in 1,333 infertile patients and found the total prevalence of AZF microdeletion was 10.80%. The frequency of AZF microdeletion was 11.75% in patients with azoospermia and 8.51% in patients with severe oligozoospermia. These results are similar to the published data of 10%–15% in

Table 5 The results of chromosome abnormalities and AZF microdeletions

STS deleted	Karyotype	Total number (n)
sY254,sY255	46,XY,Yq-	4
sY127,sY134,sY254,sY255	46,XY,Yq-	23
USP9Y,sY86,sY127,sY134,sY254,sY255	46,XX	4
sY127,sY134,sY254,sY255	45,X/46,XY	1
USP9Y,sY86,sY127,sY134,sY254,sY255	45,X,delY	1
sY127,sY134,sY254,sY255	46,XY,delYp/45,X,delY	1
sY127	46,XY	2
USP9Y,sY86	46,XY	13
sY127,sY134	46,XY	13
sY254,sY255	46,XY	74
sY127,sY134,sY254,sY255	46, XY	2
USP9Y,sY86, sY127,sY134	46,XY	3
USP9Y,sY86, sY254,sY255	46,XY	3
Total		144

azoospermic patients and 5%–10% in oligozoospermic patients [33]. However, in a review of some literature [2, 11, 27], authors observed a high frequency (51.6%) of microdeletions among azoospermic patients [2]. The low frequency (less than 5%) was found in men with severe oligozoospermia [11, 27]. These variations in deletion frequencies could be explained by ethnic or geographical differences, the selection criteria of the patients and sample size.

Deletions of the different AZF regions occurred with different frequency. Confirming previous data [1, 15, 26], our findings showed that classical AZFc deletions represented the most frequent finding (54.17% of deletions in our cases), followed by the AZFbc region (18.75%), AZFb (10.42%) and AZFa (9.03%). Our data revealed that there was a slightly higher frequency of the AZFa and AZFb microdeletions in infertile patients than previous studies [14, 26], which was mainly due to a high rate of partial deletions in AZFa region. It could be explained by patient selection criteria differences, ethnic variances and environmental influences.

The progresses in molecular biology of the Y chromosome in the past 10 years have been demonstrated that each AZF subregion acts at a different phase of spermatogenesis. It is suggested that complete deletion of AZFa region may result in complete Sertoli cell-only syndrome and azoospermia [26, 32]. Deletions of the AZFb region may cause SCO syndrome or arrest of spermatogenesis at the primary spermatocyte stage [35]. Deletions in the AZFc region produce a wide range of phenotypes from normal to oligozoospermia and azoospermia [26]. A deletion of the AZFc region may also predispose men to Y chromosome loss, leading to sexual reversal. Several studies have found this deletion to be a premutation for 45,X [16, 36] and for the mosaic phenotype 45,X/46,XY [23]. This is in accordance with the results of the present study in which AZFa deletions were only detected in azoospermic patients, but AZFb or AZFc deletions were found in both, patients with severe oligozoospermia and patients with azoospermia. In this study, mature sperm was observed in 35.90% (28/78) semen samples from severe oligozoospermic (according to Table 4) patients with deletions of the AZFc region. In addition, the present study showed that combined deletions involving the three AZF regions, AZFa,b regions or partial AZFb deletions were also only detected in azoospermic patients.

A total of 34 patients in this study had chromosomal abnormalities among the 144 patients with Y chromosome microdeletions, all of which were related to the sex chromosome abnormality and azoospermia. All patients with 46,XX and 45,X,delY in this study presented the SRY gene. SRY is considered to determine sexual identity towards male development [3, 28]. It is well-known fact that 46,XX or 45,X maleness is a rare syndrome. The frequency of 46,XX males was estimated in 1/20,000 male neonates [6]. SRY sequences were present in approximately 90% of these cases [19]. The majority of the XX males carry SRY gene due to an

illegitimate recombination between X and Y chromosomes. The molecular analysis of SRY gene is characterized by its simplicity, rapidity. Therefore, it is quite useful to add this analysis to the clinical infertility investigation and subsequent prenatal diagnosis. Including chromosome abnormalities or Y chromosome deletions, the rate of genetic abnormalities was 19.80% (264/1,333) in our patients. These data demonstrate that patients with chromosomal abnormalities may be found to have microdeletions of the Yq and confirm that those are important genetic etiologies resulting in infertility in Chinese male population.

Nowadays, more and more infertile men choose the ART, such as ICSI/IVF to have their offspring. With the help of ART, it is possible for the patients with severe impaired spermatogenesis to father children. However, this technologies will increase the risk of transmitting their genetic disorder to their descendants. Thus, before assisted reproduction, an understanding of the genetic defects for the infertile male is essential to avoid the vertical transmission of anomalies to the offspring.

As a conclusion, our study shows the incidence of chromosomal abnormality and Yq microdeletion in Chinese infertile male with azoospermia or severe oligozoospermia. Our results are in agreement with previous reports performed in different societies and ethnic groups. It is strongly emphasized to screen chromosomal abnormalities and Yq microdeletions in infertile patients during diagnosis and before ART.

References

1. Akin H, Onay H, Turker E, Ozkinay F. Primary male infertility in Izmir/Turkey: a cytogenetic and molecular study of 187 infertile Turkish patients. *J Assist Reprod Genet.* 2011;1–5.
2. Ali M, Hayat M. Screening of ‘Y’ chromosome microdeletions in Iranian infertile males. *J Hum Reprod Sci.* 2008;1:2–9.
3. Brennan J, Capel B. One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet.* 2004;5(7):509–21.
4. Carrell D, De Jonge C, Lamb D. The genetics of male infertility: a field of study whose time is now. *Syst Biol Reprod Med.* 2006;52(4):269–74.
5. Carrell DT. Contributions of spermatozoa to embryogenesis: assays to evaluate their genetic and epigenetic fitness. *Reprod Biomed Online.* 2008;16(4):474–84.
6. Chapelle A. The etiology of maleness in XX men. *Hum Genet.* 1981;58(1):105–16.
7. De Kretser D. Male infertility. *Lancet.* 1997;349(9054):787.
8. De Kretser D, Baker H. Infertility in men: recent advances and continuing controversies. *J Clin Endocrinol Metab.* 1999;84(10):3443.
9. El Awady MK, El Shater SF, Ragaa E, Atef K, Shaheen IM, Megiud NA. Molecular study on Y chromosome microdeletions in Egyptian males with idiopathic infertility. *Asian J Androl.* 2004;6(1):53–8.
10. Elghezel H, Hidar S, Braham R, Denguezli W, Ajina M, Saâd A. Chromosome abnormalities in one thousand infertile males with nonobstructive sperm disorders. *Fertil Steril.* 2006;86(6):1792–5.

11. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, Lenzi A, Foresta C. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. *J Clin Endocrinol Metab*. 2007;92(3):762.
12. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. *Reprod BioMed Online*. 2007;14(6):734–45.
13. 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(1):152.
14. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev*. 2001;22(2):226–39.
15. Hofherr SE, Wiktor AE, Kipp BR, Dawson DB, Van Dyke DL. Clinical diagnostic testing for the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience. *J Assist Reprod Genet*. 2011;1–8.
16. Jaruzelska J, Korcz A, Wojda A, Jedrzejczak P, Bierla J, Surmacz T, Pawelczyk L, Page DC, Kotecki M. Mosaicism for 45, X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion. *J Med Genet*. 2001;38(11):798.
17. Lian J, Zhang X, Tian H, Liang N, Wang Y, Liang C, Li X, Sun F. Altered microRNA expression in patients with non-obstructive azoospermia. *Reprod Biol Endocrinol*. 2009;7:13.
18. Mahanta R, Gogoi A, Roy S, Bhattacharyya I, Sharma P. Prevalence of Azoospermia Factor (AZF) deletions in idiopathic infertile males in North-East India. *Int J Hum Genet*. 2011;11(2):99–104.
19. Margarit E, Soler A, Carrio A, Oliva R, Costa D, Vendrell T, Rosell J, Ballesta F. Molecular, cytogenetic, and clinical characterisation of six XX males including one prenatal diagnosis. *J Med Genet*. 1998;35(9):727–30.
20. Martinez-Garza SG, Gallegos-Rivas MC, Vargas-Maciell M, Rubio-Rubio JM, de los Monteros-Rodriguez ME, Gonzalez-Ortega C, Cancino-Villarreal P, de Lara LGV, Gutierrez-Gutierrez AM. Genetic screening in infertile Mexican men: chromosomal abnormalities, Y chromosome deletions, and androgen receptor CAG repeat length. *J Androl*. 2008;29(6):654.
21. Organization WH. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999.
22. Paquis-Flucklinger V, Santucci-Darmanin S, Paul R, Saunier A, Turc-Carel C, Desnuelle C. Cloning and expression analysis of a meiosis-specific MutS homolog: the HumanMSH4Gene. *Genomics*. 1997;44(2):188–94.
23. Patsalis PC, Sismani C, Quintana-Murci L, Taleb-Bekkouché F, Krausz C, McElreavey K. Effects of transmission of Y chromosome AZFc deletions. *Lancet*. 2002;360(9341):1222–4.
24. Repping S, Skaletsky H, Lange J, Silber S, Van der Veen F, Oates RD, Page DC, Rozen S. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet*. 2002;71(4):906–22.
25. Shaffer LG, Tommerup N. ISCN 2005: an international system for human cytogenetic nomenclature (2005): recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. S Karger Pub. 2005.
26. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. *Int J Androl*. 2004;27(4):240–9.
27. Simoni M, Tuttelmann F, Gromoll J, Nieschlag E. Clinical consequences of microdeletions of the Y chromosome: the extended Münster experience. *Reprod BioMed Online*. 2008;16(2):289–303.
28. Sinclair A, Berta P, Palmer M, Hawkins J, Griffiths B. A gene from the human sex-determining region encodes a protein with. *Nature*. 1990;346(6281):240–4.
29. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet*. 1976;34(2):119–24.
30. Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, Van Steirteghem A, Liebaers I. Cytogenetics of infertile men. *Hum Reprod*. 1996;11 suppl 4:1.
31. Velasco G, Pendás AM, Fueyo A, Knäuper V, Murphy G, López-Otín C. Cloning and characterization of human MMP-23, a new matrix metalloproteinase predominantly expressed in reproductive tissues and lacking conserved domains in other family members. *J Biol Chem*. 1999;274(8):4570.
32. Vog P, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, Köhn F, Schill W, Farah S, Ramos C. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet*. 1996;5(7):933.
33. Wang RX, Fu C, Yang YP, Han RR, Dong Y, Dai RL, Liu RZ. Male infertility in China: laboratory finding for AZF microdeletions and chromosomal abnormalities in infertile men from North-eastern China. *J Assist Reprod Genet*. 2010;27(7):391–6.
34. Wong WY, Thomas CMG, Merkus JMW, Zielhuis GA, Steegers-Theunissen RPM. Male factor subfertility: possible causes and the impact of nutritional factors. *Fertil Steril*. 2000;73(3):435–42.
35. Yang Y, Ma M, Xiao C, Li L, Li S, Zhang S. Massive deletion in AZFb/b+ c and azoospermia with sertoli cell only and/or maturation arrest. *Int J Androl*. 2008;31(6):573–8.
36. Zhang F, Lu C, Li Z, Xie P, Xia Y, Zhu X, Wu B, Cai X, Wang X, Qian J. Partial deletions are associated with an increased risk of complete deletion in AZFc: a new insight into the role of partial AZFc deletions in male infertility. *J Med Genet*. 2007;44(7):437–44.
37. Zhu YJ, Liu SY, Wang H, Wei P, Ding XP. The prevalence of azoospermia factor microdeletion on the Y chromosome of Chinese infertile men detected by multi-analyte suspension array technology. *Asian J Androl*. 2008;10(6):873–81.