Asian Journal of Andrology (2020) 22, 368–371
www.asiaandro.com; www.ajandrology.com

Open Access

ORIGINAL ARTICLE

Male Infertility

Y chromosome microdeletion screening using a new molecular diagnostic method in 1030 Japanese males with infertility

Masashi Iijima[1], Kazuyoshi Shigehara[1], Hideki Igarashi[2], Koichi Kyono[3], Yasuo Suzuki[4], Yuji Tsuji[5,6], Yoshitomo Kobori[7], Hideyuki Kobayashi[8], Atsushi Mizokami[1]

The azoospermia factor (AZF) region is important for spermatogenesis, and deletions within these regions are a common cause of oligozoospermia and azoospermia. Although several studies have reported this cause, the present research, to the best of our knowledge, is the first large-scale study assessing this factor in Japan. In this study, 1030 male patients with infertility who were examined for Y chromosome microdeletion using the polymerase chain reaction-reverse sequence-specific oligonucleotide (PCR-rSSO) method, a newly developed method for Y chromosome microdeletion screening, were included. The study enrolled 250 patients with severe oligospermia and 717 patients with azoospermia. Among the 1030 patients, 4, 4, 10, and 52 had AZFa, AZFb, AZFb+c, and AZFc deletions, respectively. The sperm recovery rate (SRR) of microdissection testicular sperm extraction in patients with AZFc deletions was significantly higher than that in those without AZF deletions (60.0% *vs* 28.7%, $P = 0.04$). In patients with gr/gr deletion, SRR was 18.7%, which was lower than that in those without gr/gr deletion, but was not statistically significant. In conclusion, our study showed that the frequency of Y chromosome microdeletion in male patients in Japan was similar to that reported in patients from other countries, and SRR was higher in patients with AZFc deletion.

Asian Journal of Andrology (2020) **22**, 368–371; doi: 10.4103/aja.aja_97_19; published online: 11 October 2019

Keywords: azoospermia factor; Japanese infertile men; sperm recovery rate; Y chromosome microdeletion

INTRODUCTION

The male factor can account for up to 50% of infertility when included in combination with the female factor. A majority of male infertility cases are related to the dysfunction of spermatogenesis. Moreover, genetic abnormalities are one of the obvious causes of testicular dysfunction. Y chromosome microdeletion and the numerical and structural abnormality of chromosomes are clinically important genetic abnormalities associated with male infertility. The guidelines published by the American Society for Reproductive Medicine or European Academy of Andrology (EAA) and European Association of Urology state that "genetic tests for severe oligozoospermia and azoospermia are recommended."[1,2] Previously, we had been examining Y chromosome microdeletion using classical methods; however, the sensitivity of the markers recommended by the EAA guidelines was low when assessed using these methods in Japanese cases. Thus, we developed a polymerase chain reaction-reverse sequence-specific oligonucleotide (PCR-rSSO) with microbead suspension array for Y chromosome microdeletion screening in the Japanese population that is currently being widely used in Japan.[3] Using this new method, minor deletions, including gr/gr deletions, can be detected along with major deletions using 21 sequence-tagged site (STS) markers. It is well known that gr/gr deletion is present in a third of the Japanese male population. To the best of our knowledge, this is the first multicenter study investigating the clinical data of Japanese male patients with infertility examined using the new Y chromosome microdeletion detection method.

PATIENTS AND METHODS

In this study, 1072 male patients with infertility who underwent testing using the GENOSEARCH™ AZF Deletion kit (MBL, Nagoya, Japan) for detecting Y chromosome microdeletions between April 2014 and December 2016 at eight hospitals in Japan (Kanazawa University Hospital in Kanazawa; Suzuki Lady's Hospital in Kanazawa; Kyono ART Clinic Sendai in Sendai; Kyono ART Clinic Takanawa in Tokyo; Ebisu Tsuji Clinic in Tokyo; Tenjin Tsuji Clinic in Fukuoka; Dokkyo Medical University Kosigaya Hospital in Kosigaya; and Toho University Medical Center Omori Hospital in Tokyo) were enrolled. We analyzed hormone levels (*e.g.*, luteinizing hormone [LH], follicle-stimulating hormone [FSH], and total testosterone), patient backgrounds (comorbidity, medical histories, surgical histories, and drug histories), chromosome karyotypes, semen parameters, and results of testicular sperm extraction (TESE). Patients with ejaculatory dysfunction, a history of chemotherapy or radiation therapy, or hypogonadism were excluded from the analysis.

[1]Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8641, Japan; [2]Kyono ART Clinic, Sendai 980-0014, Japan; [3]Kyono ART Clinic, Takanawa, Tokyo 108-0074, Japan; [4]Suzuki Lady's Hospital, Kanazawa 921-8033, Japan; [5]Ebisu Tsuji Clinic, Tokyo 150-0021, Japan; [6]Tenjin Tsuji Clinic, Fukuoka 810-0001, Japan; [7]Dokkyo Medical University Koshigaya Hospital, Saitama 343-8555, Japan; [8]Department of Urology, Faculty of Medicine, Toho University, Tokyo 143-8541, Japan.
Correspondence: Dr. M Iijima (easymercy1980@staff.kanazawa-u.ac.jp)
Received: 04 March 2019; Accepted: 30 July 2019

Y chromosome microdeletions were assessed at a clinical laboratory (SRL, Inc., Tokyo, Japan) using the GENOSEARCH™ AZF Deletion kit based on the PCR-rSSO method[3] which combines multiplex PCR and hybridization. This kit was developed to solve the problem that the sensitivity of STS markers recommended by EAA is insufficient for the Japanese population, by reselecting STS markers and improving detection methods. Genomic DNA extracted from the patients' peripheral blood lymphocytes was amplified using multiplex PCR and detected with a bead suspension array. In this method, the following 20 STS probes in the Y chromosome were selected: sY1324, sY1316, and sY1714 in the AZFa region; sY1024, sY1967, sY1309, sY3199, sY1233, sY3010, sY2990, sY1197, sY1191, sY1307, sY1291, sY2858, and sY1206 in the AZFb and AZFc regions; sY14 and sY3118 in the short arm of the Y chromosome; and sy1251 and sy3159 outside the AZF region in the long arm of the Y chromosome.

TESE was performed at each hospital by several doctors. The results of microdissection TESE (microTESE) for nonobstructive azoospermia (NOA) with an FSH level of >10 IU l^{-1} and without Klinefelter syndrome (KS) were compared between patients with and without Y chromosome microdeletions.

Statistical analysis was performed using the SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were analyzed using an unpaired *t*-test, and categorical variables were analyzed using the Chi-square test. $P < 0.05$ was considered statistically significant. The study was a retrospective study, and instead of obtaining informed consent, we informed the patient by posting a written description of the study. This study was approved by the Ethics Committee of the Kanazawa University (Kanazawa, Japan) and complies with the Code of Ethics of the World Medical Association (Declaration of Helsinki 1964, revised in 2013).

RESULTS

Among 1072 patients who were tested using the PCR-rSSO method for Y chromosome microdeletion screening, 42 patients with ejaculatory dysfunction, a history of chemotherapy or radiation therapy, or hypogonadism were excluded. Of these, 250 had severe oligozoospermia (sperm count $<500 \times 10^6$ ml^{-1}), 717 had azoospermia, and the result of semen analysis was unknown among 63 patients. Chromosomal karyotyping revealed that 797 patients had no abnormalities, 83 had KS, 16 had Y chromosome structural abnormalities, 6 had Robertsonian translocations, and 11 had other abnormalities. Y chromosome structural abnormalities included 46,X,del(Y)(q11.23); 46,X,+mar, 46,X,?Y; 46,XY,dic(Y;13); 46,X,+mar/45X; and 46,XY,inv(Y). **Table 1** shows the deletion patterns for all 1030 patients. Each deletion pattern in Y chromosome long arm is shown in **Figure 1**. In the 250 patients with severe oligospermia, AZFc deletion was observed in 20 (8.0%) patients. Among the 83 patients with KS, AZFc deletion was found in 1 (1.2%) patient. **Table 2** shows the clinical data of 249 patients who underwent micro TESE (patients with KS and FSH level <10 IU l^{-1} were excluded). Patients with AZFc deletion had significantly higher sperm recovery rate (SRR) compared with those without AZFc deletion ($P < 0.05$). The SRR of patients with gr/gr deletion tended to be lower than that of those without deletion, but without significant difference ($P = 0.09$). **Table 3** shows the data of patients with oligozoospermia with AZFc deletions and all patients with azoospermia (including FSH level <10 IU l^{-1}) who underwent micro TESE. In addition, LH and FSH levels were significantly higher in patients with azoospermia than those in patients with oligospermia ($P < 0.05$); however, patients with azoospermia in whom sperms could not be recovered were significantly older than those with oligozoospermia ($P < 0.05$).

Table 1: Distribution of deletion patterns

Deletion pattern	*Total, n (%)*	*Azoospermia, n (%)*	*Severe oligozoospermia, n (%)*
AZFa	4 (0.4)	4 (0.6)	0 (0)
AZFb+c	10 (1.0)	9 (1.3)	0 (0)
AZFb	4 (0.4)	4 (0.6)	0 (0)
AZFc	52 (5.0)	28 (3.9)	20 (8.0)
Ym-3 (AZFb partial)	1 (0.1)	1 (0.1)	0 (0)
Ym-6 (P3 + P2 + P1)	2 (0.2)	2 (0.3)	0 (0)
Ym-8 (b1/b3)	3 (0.3)	2 (0.3)	0 (0)
Ym-9 (P3)	1 (0.1)	1 (0.1)	0 (0)
Ym-11 (b2/b3)	14 (1.4)	10 (1.4)	4 (1.6)
Ym-12 (gr/gr)	343 (33.3)	241 (33.6)	83 (33.2)
Yq I (distal to AZFa)	9 (0.9)	8 (1.1)	0 (0)
Yq II (distal to AZFb)	5 (0.5)	5 (0.7)	0 (0)
Yq III (distal to AZFb)	3 (0.3)	3 (0.4)	0 (0)
Yq IV (distal to AZFb)	3 (0.3)	3 (0.4)	0 (0)
Yq V (distal to AZFb)	1 (0.1)	1 (0.1)	0 (0)
Yq VI (distal to AZFc)	1 (0.1)	1 (0.1)	0 (0)
No deletion	565 (54.9)	386 (53.8)	143 (57.2)
Unknown	9 (0.9)	8 (1.1)	0 (0)
Total	1030	717	250

The pattern of deletion was defined in our previous study[3]. AZF: azoospermia factor

DISCUSSION

Frequency of major AZF deletions

AZF is a region comprising numerous genes that are important for spermatogenesis and is located in the male-specific region of the Y chromosome long arm. Due to the palindrome structure in this region, chromosomal homologous recombination causes microdeletion, which is cannot be detected in the G-band. Tiepolo and Zuffardi[4] first reported AZF in 1976, and subsequently, the deletion patterns were classified into AZFa, AZFb, and AZFc by Vogt *et al*.[5] in 1996. Many studies on AZF have been conducted; however, there are only a few reports involving approximately 1000 patients. The frequency of AZF deletions in large-scale studies with approximately 1000 patients is shown in **Table 4**, demonstrating that the frequency of deletion varies depending on the ethnic composition of the population. Among the 717 patients with azoospermia in the present study, AZFa, AZFb, AZFc, AZFb+c, and AZFa+b+c deletions were observed in 0.6%, 0.6%, 3.9%, 1.3%, and 1.1% of the patients, respectively, which were similar to those reported in previous studies.[6–10] In patients with severe oligozoospermia, the AZFc deletion was observed in 8.0% of patients, which was higher than that in patients with azoospermia. To detect AZFc deletions, the EAA/European Molecular Quality Network (EMQN) guidelines[1] recommended Y chromosome microdeletion screening at a sperm count of $<5 \times 10^6$ ml^{-1}, whereas Johnson *et al*.[9] reported that even if this level was reduced to 0.5×10^6 ml^{-1}, the sensitivity to detect AZFc deletion did not decrease. However, in the present study, one patient had a sperm count of 2×10^6 ml^{-1}; therefore, the threshold $<0.5 \times 10^6$ ml^{-1} is not considered appropriate for screening for the Japanese population. Y chromosome microdeletion screening is strongly recommended in cryptozoospermia because 12 out of 20 patients with severe oligospermia with AZFc deletions had cryptozoospermia in our study.

Frequency of minor AZF deletions

The PCR-rSSO method used in this study enabled the detection of minor deletions in addition to major deletions. In the present study, b1/b3, b2/b3, and gr/gr deletions, known as the partial deletions of

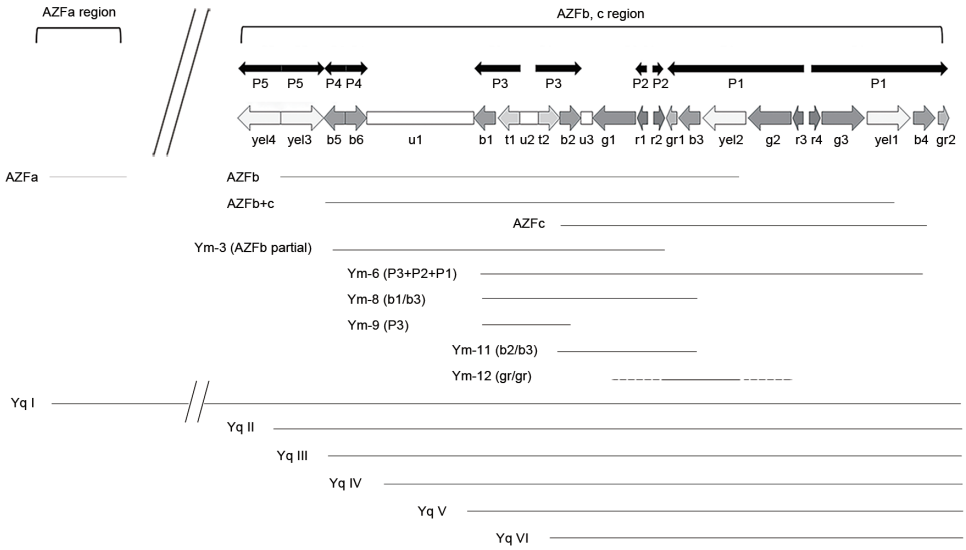

Figure 1: Deletion patterns in Y chromosome long arm detected by PCR-rSSO method. Major deletions: AZFa, AZFb, AZFb+c, and AZFc; minor deletions: Ym-3, Ym-6, Ym-8, Ym-9, Ym-11, and Ym-12; Y chromosome long arm terminal deletions: Yq I, Yq II, Yq III, Yq IV, Yq V, and Yq VI. All deletion patterns are defined in our previous study.[3] AZF: azoospermia factor; PCR-rSSO: polymerase chain reaction-reverse sequence-specific oligonucleotide.

Table 2: Clinical data of patients who underwent testicular sperm extraction

Deletion pattern	*Patient (n)*	*Age (year)*	*LH (IU l^{-1})*	*FSH (IU l^{-1})*	*TT (ng ml^{-1})*	*TESE outcome*	
						SRR (%)	*P*
AZFb + c	1	37	3.9	14.2	3.3	0	0.53
AZFc	10	36.0±6.1 (26–43)	8.3±3.1 (4.7–14.0)	25.6±10.2 (15.7–48.7)	4.4±1.8 (2.1–7.4)	60.0	0.04*
Ym-6 (P3 + P2 + P1)	1	34	7.3	13.3	8.5	0	0.53
Ym-8 (b1/b3)	2	36.5±1.5 (35–38)	16.3±3.9 (12.4–20.2)	37.9±1.0* (36.9–38.9)	5.5±1.5 (3.9–7.0)	50.0	0.51
Ym-11 (b2/b3)	5	29.0±2.1* (26–31)	10.2±5.4 (4.3–18.5)	22.6±7.7 (15.3–35.5)	5.2±2.5 (1.3–8.0)	40.0	0.58
Ym-12 (gr/gr)	91	36.2±5.5 (23–50)	8.5±3.7 (3.6–23.1)	22.7±7.6* (11.2–53.3)	4.0±1.7 (1.4–13.0)	18.7	0.09
Yq I (distal to AZFa)	1	33	7.4	22.9	4.4	0	0.53
Yq II (distal to AZFb)	1	29	13.1	27.4	3.2	0	0.53
Yq V (distal to AZFb)	1	38	13.1	37.3	3.7	0	0.53
No deletion	136	34.9±6.3 (24–62)	9.6±6.7 (2.7–51.0)	25.3±11.0 (10.0–71.2)	4.3±2.1 (0.2–13.5)	28.7	-

Values are shown as mean±s.d. (range) and statistically compared between no deletion group and each deletion group. Patients with an FSH level of >10 IU l^{-1} and KS were excluded. *Significant difference (P<0.05). LH: luteinizing hormone; FSH: follicle-stimulating hormone; TT: total testosterone; SRR: sperm retrieval rate; s.d.: standard deviation; KS: Klinefelter syndrome; AZF: azoospermia factor; -: no deletion" is the control group.

Table 3: Clinical data of patients with azoospermia factor c deletion

	Patient (n)	*Age (year)*	*LH (IU l^{-1})*	*FSH (IU l^{-1})*	*TT (ng ml^{-1})*
Oligozoospermia	20	32.6±3.5 (27–41)	4.7±1.6 (1.0–7.0)	8.7±4.2 (2.5–16.9)	5.8±2.1 (2.7–10.6)
Azoospermia (TESE sperm positive)	10	34.6±6.3 (26–44)	7.2±4.0* (1.7–14.0)	21.2±13.8* (3.6–48.7)	4.4±1.9 (2.0–7.4)
Azoospermia (TESE sperm negative)	5	37.0±5.8* (30–43)	8.2±3.3* (4.8–12.3)	20.0±7.3* (10.3–30.1)	4.1±1.1 (3.0–5.4)

Values are shown as mean±s.d. (range) and statistically compared between each group. *Significant difference (P<0.05) compared with patients with oligozoospermia. LH: luteinizing hormone; FSH: follicle-stimulating hormone; TT: total testosterone; SRR: sperm retrieval rate; s.d.: standard deviation; TESE: testicular sperm extraction

Table 4: Prevalence of Y chromosome microdeletions in reported studies with azoospermia

Study	*Patients (n)*	*AZFa*	*AZFb*	*AZFc*	*AZFb+c*	*AZFa+b+c*
Present study	717	0.6	0.6	3.9	1.3	1.1
Johnson *et al*.[9]	405	0.2	1.0	7.9	2.0	NA
Akinsal *et al*.[10]	1043	0.1	0.4	1.6	0.6	0.7
Zhu *et al*.[8]	984	0.7	0.6	6.7	1.7	1.0
Stahl *et al*.[7]	1153	0.3	1.4	4.3	2.7	1.6
Ferlin *et al*.[6]	625	1.0	1.1	4.0	1.9	0.3

Each study included over 1000 male patients suffering from infertility in total. NA: not available. AZF: azoospermia factor.

AZFc, were detected in 0.3%, 1.4%, and 33.3% patients, respectively. Analysis of approximately 20 000 patients[11] revealed that the frequency of b1/b3, b2/b3, and gr/gr deletions was 1.1%, 0.1%, and 2.4%, respectively. In this report, although the frequency of b1/b3 and gr/gr deletions was 0.25% and 4.1% and the odds ratio was 1.9 and 2.5 times higher than those without deletion in the severe spermatogenic failure (SSF) with the sperm concentration <5 × 10^6 ml^{-1}, b2/b3 deletion was not a risk factor of SSF. Meanwhile, influences of minor deletion differ depending on the population; Krausz and Casamonti[12] reported that b2/b3 deletion has a strong correlation with male infertility. The prevalence of gr/gr deletion is less than 10% in many ethnic groups,[13–15]

but about 33% in the Japanese population with haplogroup D as one of the major groups.[16] In the present study, the frequency of gr/gr deletion was 33.3%, which is similar to that reported above.

Spermatogenesis in each AZF deletion

Okuyama *et al*.[17] reported that the SRR of Japanese patients with NOA was 28.9%, which was similar to that observed in the present study. SRR was 0 for patients with AZFa, AZFb, and AZFb+c deletions in the present study; however, the number of patients analyzed was small. In addition, the SRR of patients with AZFc deletion tends to be as high as approximately 50%–70%.[1,7,18] Similarly, in the present study, a significantly higher SRR was observed in patients with AZFc deletion than that in those without deletion ($P = 0.04$). For AZFc deletion, Hopps *et al*.[18] demonstrated that there was no significant difference in age between males with oligozoospermia and those with azoospermia; similarly, there was no significant difference in age between men with azoospermia in whom sperm retrieval was successfully performed using TESE and men with azoospermia in whom sperm retrieval was not successfully performed using TESE. Our results showed that patients with oligozoospermia were significantly ($P < 0.05$) younger than those with azoospermia in whom sperm retrieval was not performed using TESE (**Table 3**). In comparison with the results of intracytoplasmic sperm injection (ICSI) using testicular and ejaculated sperms in patients with AZFc deletion, the result of ICSI with ejaculated sperm is slightly better.[19,20] These results indicate that an early diagnosis of AZFc at younger age can achieve pregnancy without artificial technology. Patients with AZFc deletion have a good chance of fertilizing an egg. Moreover, male offspring of such patients inherit this deletion.[21] Therefore, genetic counseling should be performed, so that the second generation of men with AZFc deletions can receive early treatment.

Sin e*t al*.[16] reported that the frequency of gr/gr deletion was not significantly different between the control group and the infertile male group in the Japanese population. However, when analyzed according to the gr/gr deletion subtype in the normozoospermic group, the sperm concentration in subjects with *CDY1b* deletion, which is abundant in haplogroup O, was significantly lower than that with *CDY1a* deletion.[16] Ghorbel *et al*.[22] also reported that *CDY1b* deletion was significantly more frequent in infertile patients than that in fertile men and is a risk factor of male infertility in Tunisian men.

In the present study, no significant difference ($P = 0.09$) was observed in SRR using TESE between men with and without gr/gr deletions. Subgroup analysis by *CDY1* copy number might lead to different results.

This is the first large-scale study assessing Y chromosome microdeletions in Japanese males. The frequency of AZF deletions was similar to that reported in previous studies. Besides, SRR determined using TESE was significantly higher in men with AZFc deletion than that in those without the deletion. Further studies are needed to reveal the effect of gr/gr deletion on SRR. Sperms could not be retrieved from the patients with complete AZFa, AZFb, and AZFb+c deletions.

AUTHOR CONTRIBUTIONS

MI conceived the study and coordinated and drafted the manuscript. HI, KK, YS, YT, YK, and HK collected data and created databases. KS performed statistical analysis. AM supervised the study. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

REFERENCES

1 Krausz C, Hoefsloot L, Simoni M, Tuttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. *Andrology* 2014; 2: 5–19.
2 Evaluation of the azoospermic male: a committee opinion. *Fertil Steril* 2018; 109: 777–82.
3 Iijima M, Koh E, Izumi K, Taya M, Maeda Y, *et al*. New molecular diagnostic kit to assess Y-chromosome deletions in the Japanese population. *Int J Urol* 2014; 21: 910–6.
4 Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet* 1976; 34: 119–24.
5 Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, *et al*. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 1996; 5: 933–43.
6 Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, *et al*. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. *J Clin Endocrinol Metab* 2007; 92: 762–70.
7 Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, *et al*. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. *Fertil Steril* 2010; 94: 1753–6.
8 Zhu XB, Gong YH, He J, Guo AL, Zhi EL, *et al*. Multicentre study of Y chromosome microdeletions in 1,808 Chinese infertile males using multiplex and real-time polymerase chain reaction. *Andrologia* 2017; 49: e12662.
9 Johnson M, Raheem A, De Luca F, Hallerstrom M, Zainal Y, *et al*. An analysis of the frequency of Y-chromosome microdeletions and the determination of a threshold sperm concentration for genetic testing in infertile men. *BJU Int* 2019; 123: 367–72.
10 Akinsal EC, Baydilli N, Dundar M, Ekmekcioglu O. The frequencies of Y chromosome microdeletions in infertile males. *Turk J Urol* 2018; 44: 389–92.
11 Rozen SG, Marszalek JD, Irenze K, Skaletsky H, Brown LG, *et al*. *AZFc* deletions and spermatogenic failure: a population-based survey of 20,000 Y chromosomes. *Am J Hum Genet* 2012; 91: 890–6.
12 Krausz C, Casamonti E. Spermatogenic failure and the Y chromosome. *Hum Genet* 2017; 136: 637–55.
13 Visser L, Westerveld GH, Korver CM, van Daalen SK, Hovingh SE, *et al*. Y chromosome gr/gr deletions are a risk factor for low semen quality. *Hum Reprod* 2009; 24: 2667–73.
14 Navarro-Costa P, Gonçalves J, Plancha CE. The AZFc region of the Y chromosome: at the crossroads between genetic diversity and male infertility. *Hum Reprod Update* 2010; 16: 525–42.
15 Bansal SK, Jaiswal D, Gupta N, Singh K, Dada R, *et al*. Gr/gr deletions on Y-chromosome correlate with male infertility: an original study, meta-analyses, and trial sequential analyses. *Sci Rep* 2016; 6: 19798.
16 Sin HS, Koh E, Shigehara K, Sugimoto K, Maeda Y, *et al*. Features of constitutive gr/gr deletion in a Japanese population. *Hum Reprod* 2010; 25: 2396–403.
17 Okuyama N, Obata R, Oka N, Nakamura Y, Hattori H, *et al*. Long-term clinical outcomes of testicular sperm extraction and intracytoplasmic sperm injection for infertile men. *Reprod Med Biol* 2018; 17: 82–8.
18 Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, *et al*. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. *Hum Reprod* 2003; 18: 1660–5.
19 Patrat C, Bienvenu T, Janny L, Faure AK, Fauque P, *et al*. Clinical data and parenthood of 63 infertile and Y-microdeleted men. *Fertil Steril* 2010; 93: 822–32.
20 Kim MJ, Choi HW, Park SY, Song IO, Seo JT, *et al*. Molecular and cytogenetic studies of 101 infertile men with microdeletions of Y chromosome in 1,306 infertile Korean men. *J Assist Reprod Genet* 2012; 29: 539–46.
21 Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the *AZFc* region of the Y chromosome, and of 18 children conceived via ICSI. *Hum Reprod* 2002; 17: 2813–24.
22 Ghorbel M, Baklouti-Gargouri S, Keskes R, Chakroun N, Sellami A, *et al*. Deletion of *CDY1b* copy of Y chromosome *CDY1* gene is a risk factor of male infertility in Tunisian men. *Gene* 2014; 548: 251–5.