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Genetics

Y-Chromosome Microdeletions and Cytogenetic Findings in Unselected ICSI Candidates at a Danish Fertility Clinic

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***Purpose*:** To determine the frequency and type of microdeletions on the Y chromosome, and to evaluate cytogenetic findings in unselected ICSI candidates at a Danish Fertility Clinic.
***Methods*:** Genomic DNA was extracted from blood samples, which were collected prospectively from 400 ICSI candidates attending the Fertility Clinic at Aarhus University Hospital, Denmark. Twenty-five sequence tagged sites (STSs) spanning the azoospermia factor (AZF) regions of the Y chromosome were amplified in 5 multiplex sets to investigate Y microdeletions. Semen analysis, karyotype analysis, and histological evaluation of testicular biopsies were also performed.
***Results*:** Y microdeletions were detected in 3 (0.75%) of 400 unselected ICSI candidates. The frequency of Y microdeletions was found higher in azoospermic men (2%) than in oligozoospermic men (0.6%). Two patients having oligozoospermia had Y microdeletions in the AZFc region only, whereas the patient having azoospermia had Y microdeletions spanning the AZFb and AZFc regions. No microdeletion was detected in the AZFa region. Chromosomal anomalies were found in 6.1% of azoospermic men and in 2.7% of oligozoospermic men. A high frequency of cytogenetic abnormalities was found in normozoospermic men with fertilization failure (7.4%).
***Conclusions*:** The frequency of Y microdeletions both in the unselected ICSI candidates and subgroups classified as azoospermic and oligozoospermic seems rather low compared to results of previous studies, which have been quite varying. It is possible that in addition to patient selection criteria, ethnical and geographical differences may contribute to these variations. Cytogenetic evaluation of normozoospermic men with fertilization failure seems indicated because of a high frequency of cytogenetic abnormalities.

KEY WORDS: Intracytoplasmic sperm injection; karyotyping; male infertility; oligo-azoospermia; Y-chromosome microdeletions.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) has caused a revolution in the treatment of severe male infertility. Direct injection of a single spermatozoon into the oocyte enables pregnancy in couples where the man has oligozoospermia or azoospermia. Nevertheless, the etiology of male factor infertility is generally poorly understood. Recently, small interstitial deletions (microdeletions) on the Y chromosome have been suggested to be associated with a failure of spermatogenesis. Thus, several recent reports have investigated the relationship between male factor infertility and partial deletions in the azoospermia factor (AZF) region (1–14).

Because the presence of Y microdeletions is associated with impaired spermatogenesis, the obvious

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consequence is that male infertility will be transmitted to offspring, if ICSI is used. Analysis of microdeletions on the Y chromosome therefore, is recommended for patients who are candidates for ICSI treatment. The prevalence of Y microdeletions in populations of oligozoospermic or azoospermic men is well documented in several studies and reported to range from 1 to 58.3% (1–14). However, there are very few studies on the prevalence of Y microdeletions in series of unselected ICSI candidates showing a frequency between 2.2 and 6.5% (15–18). By restricting patient selection criteria to those with azoospermia or severe oligozoospermia, some microdeletions causing less severe impairment of spermatogenesis might be missed. Indeed, Y microdeletions have been reported in patients having either less severe spermatogenic defects (>5 million sperm per milliliter) or normozoospermia (2,6,7).

The wide variation in prevalence of Y deletions described in the literature is explained by several factors including methodological aspects, selection criteria of the patients and geographical or ethnical differences.

The purpose of the present study therefore was to describe the frequency and type of Y microdeletions in a population of unselected ICSI candidates at a Fertility Clinic in Denmark using a multiplex PCR protocol and to compare our results with those from other parts of the World.

MATERIAL AND METHODS

Patients

In this study 400 blood samples were collected prospectively from men in couples, who were candidates for ICSI due to male factor infertility with or without fertilization failure in previous IVF attempts. There were no exclusion criteria. All participants gave written consent according to the study protocol, which was approved by the Regional Ethics Committee.

Semen analysis was performed according to the criteria of the World Health Organization guidelines (19). On the basis of their mean sperm concentrations, participants were categorized in five groups: 1) azoospermia, 2) severe oligozoospermia (<1 million sperm per milliliter), 3) moderate oligozoospermia (1–<5 million sperm per milliliter), 4) mild oligozoospermia (5–20 million sperm per milliliter), and 5) normozoospermia (>20 million sperm per milliliter).

Explained etiology referred to patients with azoospermia that was obstructive (retrograde ejaculation, testis, epididymis or prostate infection, agenesis of the vas deferens and/or abnormal vasography results) or nonobstructive (testicular atrophy, high serum FSH concentration, abnormal testicular biopsy and/or clinical hypogonadism). Unclassified cases refer to patients with incomplete examination.

Multiplex PCR Amplification of STS

Genomic DNA was extracted from whole blood and processed using digestion of cellular proteins and subsequent salting out of the proteins with sodium chloride, and ethanol precipitation of DNA (20).

We have recently described a multiplex PCR protocol for screening of Y microdeletions, which was optimized to work under identical conditions for all 25 sequence-tagged sites (STSs) used in five multiplex PCR sets (21). Each multiplex set contains five different primer pairs covering the euchromatic region between subinterval 5 and 6 of Yq11 (AZFa, b and c regions), where microdeletions have already been reported.

Multiplex PCR analysis was performed as described previously (21). Briefly, with 500 ng of genomic DNA in a final volume of 50 μL, including a reaction buffer (Applied Biosystems, New Jersey), 0.4 mM of dNTP mix (Boehringer Mannheim, Germany), 40 pmol of each primer, and 5 IU Ampli Taq Gold DNA polymerase (Applied Biosystems, New Jersey). Amplifications were carried out on a Thermocycler (Gene Amp 9700, Applied Biosystems, New Jersey) with the following program: initial denaturation at 95°C for 10 min and subsequent series of 45 of cycles 94°C for 45 s (denaturation), 60°C for 1 min (annealing), 72°C for 2 min (extension). A final extension was carried out at 72°C for 7 min. Samples were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized using ultraviolet transillumination.

The locus designation and PCR product size of 25 STSs in five multiplex PCR sets are listed in Table I.

Internal Quality Control for the Diagnosis of Y Microdeletions

Female genomic DNA, as a negative control and a DNA sample, which was known to present all 25 PCR products after amplification in the five multiplex PCR sets as a positive control were used. Furthermore, to distinguish a negative result from a technical failure a STS for SRY (sY14), which is localized in Yp, was used as a control primer. To control for reagent

Table I. Twenty-Five STSs in Five Multiplex PCR Sets Used for Detecting of Y Microdeletions

Multiplex set	STSs	Left primer	Right primer	Base pair	Intervals
Multiplex PCR I	sY14	GAATATTCCCGCTCTCCGGA	GCTGGTGCTCCATTCTTGAG	472	1
	sY132	GAGAGTCATAATGCCGACGT	TGGTCTCAGGAAGTTTTTGC	143	6A
	sY84	AGAAGGGTCTGAAAGCAGGT	GCCTACTACCTGGAGGCTTC	326	5C
	sY152	AAGACAGTCTGCCATGTTTA	ACAGGAGGGTACTTAGCAGT	125	6C
	sY272	GGTGAGTCAAATTAGTCAATGTCC	CCTTACCACAGGACAGAGGG	93	6F
Multiplex PCR II	sY269	CTCTGGGACAAGTGTTCCTTG	CATTGGCATGAATGTGTATTCA	94	6E
	sY139	TTCAGAGGAATCATGTGGGT	AATGTTTCATCACCATTATCCC	120	6B
	sY153	GCATCCTCATTTTATGTCCA	CAACCCAAAAGCACTGAGTA	139	6C
	sY155	ATTTTGCCTTGCATTGCTAG	TTTTTAAGCCTGTGACCTGG	349	6D
	sY138	CACATGAAGCACTGGAACTG	AGGGCCTGAGTCTCCAGG	170	6A
Multiplex PCR III	sY160	TACGGGTCTCGAATGGAATA	TCATTGCATTCCTTTCCATT	236	7
	sY143	GCAGGATGAGAAGCAGGTAG	CCGTGTGCTGGAGACTAATC	311	6B
	sY144	TCATCTGCCACCATCAACAT	ACGTGTTTCTACACCTGCCC	143	6B
	sY255	GTTACAGGATTCGGCGTGAT	CTCGTCATGTGCAGCCAC	126	6D
	sY254	GGGTGTTACCAGAAGGCAAA	GAACCGTATCTACCAAAGCAGC	350	6D
Multiplex PCR IV	sY243	GTTTCTTCATAAGCAACCAAATTG	CAGATTATGCCACTGCCCTT	118	6E
	SPGY	TTTCACATACAGCCATTAAGTTTAGC	CAATTTTGATAGTCTGAACACAAGC	400	6D
	RBM1	ATGCACTTCAGAGATACGG	CCTCTCTCCACAAAACCAACA	800	6B
	sY273	GGTCTTTAAAAGGTGAGTCAAATT	AGACAGAGGGAACTTCAAGACC	93	6F
	sY164	AATGTGCCCACACAGAGTTC	TGGAAGACCAGGATTTCATG	590	5Q
Multiplex PCR V	sY117	GTTGGTTCCATGCTCCATAC	CAGGGAGAGAGCCTTTTACC	262	5M
	sY166	GAACTCCAATCATTCCCTGA	TTGGCTCTACTTTTCCCCTT	115	6F
	sY150	GGGAGAGTCACATCACTTGG	TTGAATTATCTGCCTGAGTGC	158	6C
	sY277	GGGTTTTGCCTGCATACGTAATTA	CCTAAAAGCAATTCTAAACCTCCAG	310	6D
	sY158	CTCAGAAGTCCTCCTAATAGTTCC	ACAGTGGTTTGTAGCGGGTA	231	6E

contamination, a sample with PCR reaction mixture without DNA added was used also.

In our protocol, the PCR result was confirmed by Southern blot analysis when less than three consecutive STSs were missing to circumvent the misdiagnosis of Y microdeletions due to polymorphism.

Chromosomal Analysis

Chromosome analysis in peripheral blood was performed routinely in all ICSI candidates using a standard protocol (22).

Testis Biopsy

Biopsies were performed macroscopically through a scrotal incision. The tissue sample was subdivided into two pieces; one was used for direct microscopy for the isolation of spermatozoa, the other was fixed in Bouin's solution for later histological examination. Histological evaluation of spermatogenesis was performed on hematoxylin and eosin-stained sections.

RESULTS

The age of the 400 ICSI candidates ranged from 24 to 60 years (mean age, 35 years). Of the 400 ICSI candidates 388 were Danish. Overall, Y microdeletions were found in 3 (0.75%) of the 400 unselected ICSI candidates (Table II). All three men having Y microdeletions were Danish.

According to the semen analysis, the cohort consisted of 49 azoospermic men, 149 with severe oligozoospermia, 94 with moderate oligozoospermia, 81 with mild oligozoospermia, and 27 normozoospermic men. Of the 49 azoospermic men examined,

Table II. Frequency of Y Microdeletions According to Sperm Counts

	No microdeletion	Microdeletions	Total
Azoospermia	48	1 (2%)	49
Oligozoospermia	322	2 (0.6%)	324
Severe oligozoospermia (<1 million)	147	2 (1.3%)	149
Moderate oligozoospermia (1–<5 million)	94	0 (0%)	94
Mild oligozoospermia (5–20 million)	81	0 (0%)	81
Normozoospermia (>20 million)	27	0 (0%)	27
Total	397	3	400

15 could be classified as having obstructive azoospermia, 16 as nonobstructive azoospermia, and 18 as unclassified azoospermia. Y microdeletions were demonstrated in 1 of the 49 azoospermic men (2%), 2 of the 149 severe oligozoospermic men (1.3%), and in none of the moderate oligozoospermic or normozoospermic men (0%). When azoospermic men were subclassified according to the histological and clinical findings, the frequency of Y microdeletions in nonobstuctive azoospermic men was found to be 6.2% (1/16).

Patient 1 and 2 had contiguous Y microdeletions in the AZFc region, while patient 3, who had azoospermia presented a noncontiguous deletion in the AZFb and AZFc region (Fig. 1). Table III summarizes the size of the deleted segments and clinical characteristics of these men having Y microdeletions.

Karyotype analysis of the 400 unselected ICSI candidates revealed the presence of abnormalities in 14 cases (3.5%); 9 translocations (7 robertsonian, 2 reciprocal), 2 numerical sex chromosome aberrations (one 47 XXY, one 47 XYY), 2 inversions, and 2 total deletions in the heterochromatin part of the Y chromosome (one of them has an addition of unknown DNA to the q arm of the Y chromosome) (Table IV). All men having Y microdeletions were cytogenetically normal. A high frequency of cytogenetic abnormalities (7.4%) was found in normozoospermic men with fertilization failure (2/27).

Testicular biopsies were available for 29 azoospermic men showed 5 SCOS, 5 hypoplasia, 6 atrophy, and 13 normal histology. Scrotal surgery revealed congenital aplasia of the deferentic duct in two cases.

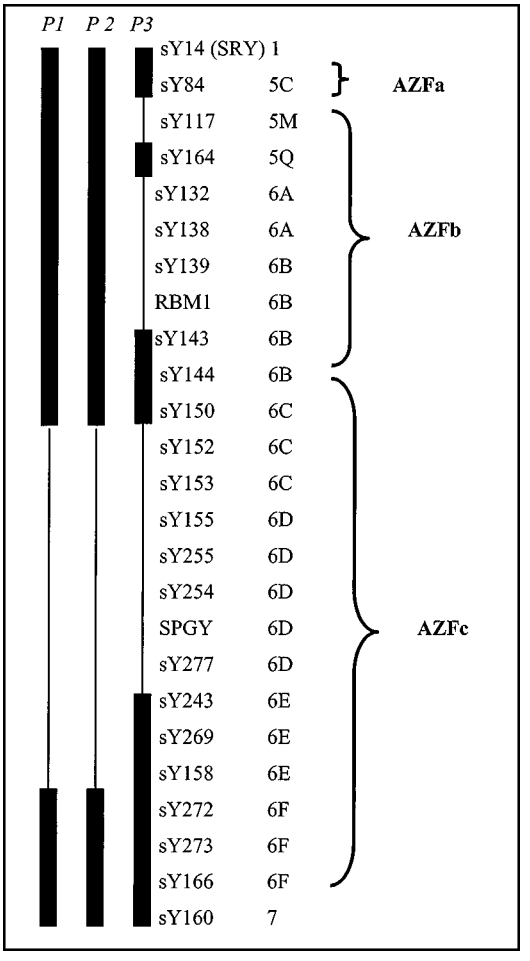

Fig. 1. Schematic presentation of Y microdeletions found for three men in this study. The positions of STSs used in the multiplex PCR are indicated. Patient number (*P1, P2, P3*) is shown on the top of the panel. The solid box indicates presence of STSs.

DISCUSSION

Using a recently developed multiplex PCR protocol we demonstrated that Y microdeletions were present in 3 of 400 ICSI candidates (0.75%). The overall frequency of chromosomal abnormalities was 3.5%.

Determination of Y microdeletions in men treated with ICSI has important clinical and ethical implications because these microdeletions and probably the related infertility can be transmitted to male offspring. However, there are very few previous studies on the prevalence of Y microdeletions in series of unselected ICSI candidates (15–18) showing frequencies of Y microdeletions varying between 2.2 and 6.5% (Table V). In the present study, the

Table III. Clinical Parameters of Three ICSI Candidate Men Having Y Microdeletions

Patient no.	Age	Sperm count (×10/6 mL)	Karyotype	Extent of deletions	Testicular biopsy
1	37	Severe oligo	46XY	AZFc[a]	Not available
2	42	Severe oligo	46XY	AZFc[b]	Not available
3	43	Azoospermia	46XY	AZFb–AZFc[c]	Sertoli-cell only

[a]STSs deleted: sY152, sY153, sY155, sY158, sY243, sY254, sY255, sY269, sY277, SPGY.
[b]STSs deleted: sY152, sY153, sY155, sY158, sY243, sY254, sY255, sY269, sY277, SPGY.
[c]STSs deleted: sY117, sY132, sY138, sY139, sY152, sY153, sY155, sY254, sY255, sY277, RBM1, SPGY.

Table IV. Presented Karyotype Analysis of 400 ICSI Candidate Men

Sperm	Chromosomal abnormalities	Total
Azoospermia	3 (6.1%)	49
	• **1** 47, XXY • **1** 45, XY, der (15; 21) (q10; q10) • **1** 46, XY, inv (10) (p11; q21)	
<1 million	3 (2%)	149
	• **1** 45, XY, der (13; 14) (q10; q10) • **1** 45, XY, der (13; 15) (q10; q10) • **1** 46, XY, t (12; 15) (q24; q24)	
1–<5 million	3 (3.1%)	94
	• **1** 46, XY, der (14; 21) (q10; q10) • **1** 46, XY, der (1; 9) (p21; q21) • **1** 45, XY, der (14; 21) (q10; q10)	
5–20 million	3 (3.6%)	81
	• **1** 45, XY, der (13; 14) (q10; q10) • **1** 46, XY, inv (Y) (p11; q21) • **1** 46, X, del (Y) (q12)	
>20 million	2 (7.4%)	27
	• **1** 47, XYY • **1** 46, X, der (Y) del (Y) (q12) add (Y)(q11.23)	

overall frequency of Y microdeletions was found to be lower not only in unselected ICSI candidates, but also in specific subgroups classified on the basis of sperm concentration. The frequency of Y microdeletions in unselected azoospermic men ranges between 2.8 and 8% in the literature (8,9,12,16,17), which seems somewhat higher than in the present population of the azoospermic men (2%). Consistent with the general trend, the present study shows that men with severe spermatogenic failure have a higher frequency of microdeletions than less severely affected men (2, 1.3, and 0.6% in azoospermic, severe oligozoospermic, and oligozoospremic men, respectively) (Table II).

Several factors may explain variations in the prevalence of Y deletions in the literature. Differences in patient selection criteria—especially the degree of spermatogenic failure—may play a role. Indeed, even varying proportions of occlusive versus spermatogenic failure in azoospermic individuals may have an impact. A high prevalence of Y microdeletions (up to 55%) has been found in azoospermic men, who were well-classified based on histological and clinical examination (1,5,6,10,14,18). Recently, two studies from Sweden and Ireland showed a low prevalence of Y microdeletions in highly selected groups of patients (12,13). Interestingly, two of the four patients with microdeletions out of the 139 nonobstructive azoospermic men reported from Sweden were actually immigrants. In these studies ethnical or geographical differences were suggested to have impact on the frequency of Y microdeletions. The low frequency of Y microdeletions also found in the present study may be explained by epidemiological variation, but we believe that more studies on the prevalence of Y microdeletions in well-defined series from

Table V. Results of Present and Previous Screenings for Y Microdeletions in Unselected ICSI Candidate Men

No. of patients	No. of STSs used	PCR method	No. with microdeletions (%)	Country	Reference
402	3	Individual	9 (2.2%)	Belgium	Van Landuyt *et al.* (17)
164	8	Multiplex	7 (4.3%)	Netherlands	Kremer *et al.* (15)
19 azoospermia			0 (0)		
111 oligozoospermia			7 (6.3%)		
34 normozoospermia			0 (0)		
134	6	Individual	3 (2.2%)	Italy	Krausz *et al.* (16)
22 azoospermia			1 (4.5%)		
42 cryptozoospermia			2 (4.7%)		
26 severe oligozoospermia			0 (0)		
27 oligozoospermia			0 (0)		
17 normozoospermia			0 (0)		
138	14	Duplex	9 (6.5%)	France	Krausz *et al.* (38)
27 azoospermic			6 (22%)		
51 cryptozoospermia			3 (5.8%)		
48 oligozoospermia			0 (0)		
12 normozoospermia			0 (0)		
400	25	Multiplex	4 (0.75%)	Denmark	Present study
49 azoospermia			1 (2%)		
149 severe oligozoospermia			2 (1.3%)		
94 moderate oligozoospermia			0 (0)		
81 mild oligozoospermia			0 (0)		
27 normozoospermia			0 (0)		

different geographical areas are needed in order to determine the impact of geographical and/or ethnical variation.

Methodological reasons for a low frequency of Y deletions in the present study is less likely. We decided to use 25 STSs, which can detect most of the microdeletions reported in previous studies (3–18). A STS-based mapping strategy allows rapid screening of a large number of infertile patients for Y microdeletions. However, it is still a question how many and which loci should be included in screening for Y microdeletions. So far, definite conclusions have not yet been made. It has been suggested that use of a larger number of STSs in a screening protocol does not necessarily increase the frequency of microdeletions detected (23). However, it is still premature to rely on a small number of STSs in the analyses due to risk of missing deletions. Thus, 20–30 STSs has been suggested to be sufficient to provide a good coverage of the important regions of the Y chromosome (24). Supporting this, it has been shown recently that more than 99% of all existing microdeletions can be detected using 48 STSs in a multiplex PCR (6). We believe that a larger number of STSs may provide additional information to evaluate the correlation between the type of Y microdeletions and clinical phenotype in infertile men, since the genetic regulation of spermatogenesis is not fully evaluated.

In the present population, the positions of the deletions and the associated phenotypes in the three patients with Y microdeletions are similar to those previously reported in the literature. Two patients having severe oligozoospermia were deleted in the AZFc region only. Patient 3, who had azoospermia, presented a deletion from AZFc to AZFb. A high frequency of Y microdeletions in the AZFa region has been reported to occur in patients with Sertoli cell-only syndrome (SCOS) (14). However, we did not detect microdeletions in the AZFa region in five azoospermic men with SCOS.

It is worth noting that the deleted STSs in patient 3 are not contiguous. In the literature, multiple, noncontiguous deletions along a particular Y chromosome have been reported in many studies (7,14,25–31). Although it is possible that these represent more than one separate microdeletion in the same patient, as has been previously reported (25,26,28), it is also likely that the order of these STSs may not correct in the published map. The STS map of Y chromosome has been revised several times and its accuracy is still questioned (25,32–37). Another explanation of the noncontiguous deletions is that some STSs may represent repetitive or polimorphic sequences. As we have discussed clearly in our previous study (21) that, there are controversial conclusions on which STSs are polymorphic or repetitive in the literature (6,8,24,26,29). In the present study, sY144 and sY164, which are claimed to be repetitive, result in the noncontiguous deletion in patient 3. However, it is important to note that Y microdeletions have been detected by using these STSs, which possibly rules out the assumption that these STSs represent repetitive sequences (32,38).

In the present study, the percentage of abnormal karyotype in the azoospermic, oligozoospermic, and normozoospermic men is comparable with data in the literature (3,39,40). A high frequency of cytogenetic abnormalities was found in normozoospermic men with fertilization failure (7.4%). Because karyotype analysis were performed in the small number of normozooospermic men ($n = 27$), it is rather difficult to draw any ultimate conclusion. However, it is important to note that, this finding is in agreement with recent study in which 430 normozoospermic men were investigated for cytogenetic abnormalities (40). Therefore, we suggest that karyotyping should be performed for all ICSI candidates including normozoospermic men, who are enrolled into ICSI treatment.

Screening for Y microdeletions in a Danish population of ICSI candidates showed a prevalence, which may seem low, but at a level similar to the prevalence of genetic mutations causing other diseases (usually less than 0.5%). Therefore, this frequency of Y microdeletions seems high enough to suggest routine screening of all ICSI candidates—irrespective of their sperm concentration—until the role of Y microdeletions in spermatogenesis has been sufficiently evaluated.

In conclusion, screening for Y microdeletions in a Danish population of ICSI candidates showed a low prevalence compared to results of previous studies, which have been quite varying. It is possible that in addition to patient selection criteria, ethnical and geographical differences may contribute to these variations. Further studies on the prevalence of Y microdeletions in patients from different ethnical groups and/or different geographical areas could be helpful to elucidate a potential epidemiological variation.

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REFERENCES

1. Najmabadi H, Huang V, Yen P, Subbarao MN, Bhasin D, Banaag L, Naseeruddin S, de Krester DM, Baker HW, McLachlan RI: Substantial prevalence of microdeletions in infertile men with idiopathic azoospermia and oligozoospermia detected by a sequence-tagged site-based mapping strategy. J Clin Endocrinol Metab 1996;81:1347–1352
2. Chang PL, Sauer MV, Brown S: Y chromosome microdeletion in a father and his four infertile sons. Hum Reprod 1999;14:2689–2694
3. Van der Ven K, Montag M, Peschka B, Leygraaf J, Schwanitz G, Haidl G, Krebs D, van der Ven H: Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. Mol Hum Reprod 1997;3:699–704
4. Simoni M, Gromoll J, Dworniczak B, Rolf C, Abshagen K, Kamischke A, Carani C, Meshede D, Behre Hm, Horst J, Nieschlah E: Screening for microdeletions of the Y chromosome involving the DAZ (deleted in azoospermia) gene in azoospermia and severe oligozoospermia. Fertil Steril 1997;67:542–547
5. Kim SW, Kim KD, Paick JS: Microdeletions within the azoospermia factor subregions of the Y chromosome in patients with idiopathic azoospermia. Fertil Steril 1999;72:349–353
6. Kent-First M, Muallem A, Shultz J, Pryor J, Roberts K, Nolten W, Meisner L, Chandley A, Gouchy G, Jorgensen L, Havighurst T, Grosch J: Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. Mol Reprod Dev 1999;53:27–41
7. Kerr NJ, Zhang J, Sin FY, Benny P, Sin IL: Frequency of microdeletions in the azoospermia factor region of the Y-chromosome of New Zealand men. N Z Med J 2000;10:468–470
8. Liow SL, Ghadessy FJ, Ng SC, Yong EL: Y chromosome microdeletions, in azoospermic or near-azoospermic subjects, are located in the AZFc (DAZ) subregion. Mol Hum Reprod 1998;4:763–768
9. Qureshi SJ, Ross AR, Ma K, Cooke HJ, Intyre MA, Chandley AC, Hargreave TB: Polymerase chain reaction screening for Y chromosome microdeletions: A first step towards the diagnosis of genetically-determined spermatogenic failure in men. Mol Hum Reprod 1996;2:775–779
10. Silber SJ, Alagappan R, Brown LG, Page DC: Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. Hum Reprod 1998;13:3332–3337
11. Jiang MC, Lien YR, Chen SU, Ko TM, Ho HN, Yang YS: Transmission of de novo mutations of the deleted in azoospermia genes from a severely oligozoospermic male to a son via intracytoplasmic sperm injection. Fertil Steril 1999;71:1029–1032
12. Osterlund C, Segersteen E, Arver S, Pousette A: Low number of Y-chromosome deletions in infertile azoospermic men at a Swedish andrology center. Int J Androl 2000;23:225–229
13. Friel A, Houghton JA, Maher M, Smith T, Noel S, Nolan A, Egan D, Glennon M: Molecular detection of Y chromosome microdeletios: An Irish study. Int J Androl 2001;24:31–36
14. Foresta C, Ferlin A, Garolla A, Moro E, Pistorello M, Barbaux S, Rossato M: High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome. Hum Reprod 1998;13:302–307
15. Kremer JA, Tuerlings JH, Meuleman EJ, Schoute F, Mariman E, Smeets DF, Hoefsloot LH, Braat DD, Merkus HM: Microdeletions of the Y chromosome and intracytoplasmic sperm injection: From gene to clinic. Hum Reprod 1997;12:687–691
16. Krausz C, Bussani-Mastellone C, Granchi S, McElreavey K, Scarselli G, Forti G: Screening for microdeletions of Y chromosome genes in patients undergoing intracytoplasmic sperm injection. Hum Reprod 1999;14:1717–1721
17. Van Landuyt L, Lissens W, Stouffs K, Tournaye H, Liebaers I, Van Steirteghem A: Validation of a simple Yq deletion screening programme in an ICSI candidate population. Mol Hum Reprod 2000;6:291–297
18. Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K, Skakkebaek NE: Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: Microdeletions are specific for spermatogenic failure. Clin Endocrinol Metab 2001;86:2638–2642
19. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cevical Mucusa Interaction, 3rd edn. Cambridge, Cambridge University Press, 1992
20. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;11:1215
21. Bor P, Hindkjaer J, Ingerslev HJ, Kolvraa S: Multiplex PCR for screening of microdeletions on the Y chromosome. J Assisted Reprod Gen 2001;18:209–216
22. Rooney DE, Czepulkowiski BH (eds): Human Cytogenetics—A Practical Approach. Oxford, UK, IRL Press, 1984
23. Simoni M, Bakker E, Eurlings MCM, Matthijs G, Moro E, Muller CR, Vogt PH: Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. Int J Androl 1999;22:292–299
24. Pryor JL, Roberts KP: Principles of sequence-tagged site selection in screening for Y deletions. Hum Reprod 1998;13:1768
25. Ma K, Sharkey A, Kirsch S, Vogt P, Keil R, Hargreave TB, McBeath S, Chandley AC: Towards the molecular localisation of the AZF locus: Mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. Hum Mol Genet 1992;1:29–33
26. Foresta C, Ferlin A, Garolla A, Rossato M, Barbaux S, De Bortoli A: Y-chromosome deletions in idiopathic severe testiculopathies. J Clin Endocrinol Metab 1997;82:1075–1080
27. Fujisawa M, Shirakawa T, Kanzaki M, Okada H, Arakawa S, Kamidono S: Y-chromosome microdeletion and phenotype in

cytogenetically normal men with idiopathic azoospermia. Fertil Steril 2001;76:491–495
28. Kato H, Komori S, Nakata Y, Sakata K, Kanazawa R, Handa M, Kobayashi S, Koyama K, Isojima S: Screening for deletions in interval D16-22 of the Y chromosome in azoospermic and oligozoospermic Japanese men. J Hum Genet 2001;46:110–114
29. Ferlin A, Moro E, Garolla A, Foresta C: Human male infertility and Y chromosome deletions: Role of the AZF-candidate genes DAZ, RBM and DFFRY. Hum Reprod 1999;14:1710–1716
30. Stuppia L, Gatta V, Calabrese G, Guanciali Franchi P, Morizio E, Bombieri C, Mingarelli R, Sforza V, Frajese G, Tenaglia R, Palka G: A quarter of men with idiopathic oligo-azoospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11. Hum Genet 1998;102:566–570
31. Grimaldi P, Scarponi C, Rossi P, March MR, Fabbri A, Isidori A, Spera G, Krausz C, Geremia R: Analysis of Yq microdeletions in infertile males by PCR and DNA hybridization techniques. Mol Hum Reprod 1998;4:1116–1121
32. Rejio R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O, Chapelle A, Silber S, Page DC : Diverse spermatogenetic defects in human caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nature Genet 1995;10:383–393
33. Affara N, Bishop C, Brown W, Cooke H, Davey P, Ellis N, Graves JM, Mitchell M, Rappold G, Tyler-Smith C, Yen P, Lau YF: Report of the Second International Workshop on Y Chromosome Mapping 1995. Cytogenet Cell Genet 1996;73:33–76
34. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewette F, Kohn FM, Schill WB, Farah S, Meschede D, Behra HM, Castel A, Nieschlag E, Weidner W, Grone HJ, Jung A, Engel W, Haidl G: Human Y chromosome azoospermia factors (AZF) mapped different subregions in Yq11. Hum Mol Genet 1996;5:933–943
35. Vollrath D, Foote S, Hilton A, Brown LG, Beer-Romero P, Bogan JS, Page DC: The human Y chromosome: A 43-interval map based on naturally occurring deletions. Science 1992;258:52–59
36. Foote S, Vollrath D, Hilton A, Page DC: The human Y chromosome: Overlapping DNA clones spanning the euchromatic region. Science 1992;2:60–66
37. Yen PH: A long-range restriction map of deletion interval 6 of the human Y chromosome: A region frequently deleted in azoospermic males. Genomics 1998;54:5–12
38. Krausz C, Qintana-Murci L, Barbaux S, Siffori JP, Rouba H, Delafontaine D, Souleyreau-Therville N, Arvis G, Antoine JM, Erdei E, Taar JP, Tar A, Jeandidier E, Plessis G, Bourgeron T, Dadoune JP, Fellous M, McElreavey K: A high frequency of Y chromosome Deletions in males with nonidiopathic infertility. J Endoc Metab 1999;84;3606–3612
39. Retief A, Van Zyl J, Menkveld M: Chromsome studies in 496 infertile males with a sperm count below 10 million per ml. Hum Genet 1984;66:162–164
40. Gekas J, Thepot F, Turleau C, Siffroi JP, Dadoune JP, Wasels R, Benzacken B: Chromosomal factors of infertility in candidate couples for ICSI: An equal risk of constitutional aberrations in women and men. Hum Reprod 2001;16: 82–90