GENETIC TESTING
Volume 6, Number 3, 2002

A Cost-Effective Screening Test for Detecting AZF Microdeletions on the Human Y Chromosome

PATRICIA DE C. PIERI,[1,4] DIRCEU H. PEREIRA,[2] SIDNEY GLINA,[3] JORGE HALLAK,[4] KEN McELREAVEY,[5] and CARLOS A. MOREIRA-FILHO[1]

ABSTRACT

PCR-based screening of microdeletions in the azoospermic factor (AZF) on the Yq chromosome is an accepted means of identifying a common genetic cause of male infertility, responsible for 5–15% of cases associated with a low sperm count ($\leq 5 \times 10^6$ sptz/ml). Based on an extensive analysis of the literature, we have established a cost-effective preliminary PCR-based diagnostic screening test, with a set of six pairs of primers ("set-of-6") that have the capability of detecting up to 95% of the Y microdeletion cases already published. These primers are: sY84 in AZFa, sY114, sY129, sY143 in AZFb, and sY149, sY254 in AZFc. Initially, the set-of-6 was tested with 13 other pairs of primers covering the three AZF subregions. A sample of 114 infertile men was tested and 10 (8.8%) microdeletions were found, 3 of which were among the 26 (11.5%) idiopathic azoospermic men. These results showed that all detected microdeletions would be identified using the set-of-6 only. Another sample of 34 patients was subsequently tested using the set-of-6 and 3 (8.8%) microdeletions were found in this group. A comparison of our results with those reported in the literature showed similar microdeletion detection frequencies, demonstrating that the set-of-6 primers provides a reliable, simple and cost-effective way of detecting AZF deletions.

INTRODUCTION

An association between male infertility and the loss of euchromatic portions of the long arm of the human Y chromosome (Yq11) was first proposed by Tiepolo and Zuffardi (1976), who suggested the existence of an Azoospermic factor (AZF). Since then, a growing body of evidence has demonstrated that the pathogenesis of a substantial fraction (5–15%) of male infertility cases, previously diagnosed as idiopathic, is related to microdeletions in Yq11.

Three distinct, non-overlapping subregions have been distinguished in Yq11 (Vogt *et al*., 1996, 1997), each of them bearing candidate genes or gene families related to infertility: *USP9Y* (also known as *DFFRY*) and *DBY* in AZFa (Foresta *et al*., 2000a), *RBM* in AZFb (Ma *et al*., 1993; Elliott, 2000), and *DAZ* in AZFc (Reijo *et al*., 1995). Although deletions of these subregions appear to be related to differences in the severity of disruption of spermatogenesis, the roles of these three "loci" have not been elucidated, and a clear genotype–phenotype correlation is still lacking.

Comprehensive deletion-based maps of the Y chromosome (Vollrath *et al*., 1992), as well as detailed sequence information (Blanco *et al*., 2000; Elliott, 2000; Foresta *et al*., 2000a; Saxena *et al*., 2000; Stuppia *et al*., 2000; Kuroda-Kawaguchi *et al*., 2001) have become available in recent years. Microdeletion screening of azoospermic, or severely oligozoospermic, men is currently performed by PCR amplification of discrete segments of Yq11 using specific primers that amplify the so-called sequence-tagged sites (STSs).

The overall prevalence of microdeletions should be around 12% in azoospermic and 3.4% in oligozoospermic patients (Simoni *et al*., 1998). When testicular biopsy data are available and Sertoli Cell Only (SCO) syndrome Type I is diagnosed, the highest microdeletion frequency is found (Foresta *et al*., 1998).

[1]Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brazil.
[2]Profert—Programa de Reprodução Assistida, São Paulo, SP, Brazil.
[3]Departamento de Reprodução Humana, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil.
[4]Divisão de Clínica Urológica, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, SP, Brazil.
[5]Imunogénétique Humaine, Institut Pasteur, Paris, France.

In these cases, loss of long segments of the Y chromosome is involved, often including the heterochromatic region. Although microdeletions resulting exclusively in the loss of AZFa are rare, they are apparently the smallest microdeletions that can result in SCO.

The use of the intracytoplasmic sperm injection (ICSI) procedure has by-passed natural selection, allowing the transmission of genetic alterations responsible for male infertility (ESHRE, 2000). Y-chromosome microdeletions are unequivocal examples of a 100% father-to-son transmission of a genetic trait by the ICSI method (Silber *et al*., 1998; Kamischke *et al*., 1999; Page *et al*., 1999), reinforcing the importance of this screening procedure in general andrology practice (Krausz *et al*., 2000), especially with the growing use of ICSI.

The substantial prevalence of Y microdeletions in infertile men, the worsening of sperm parameters over time in some types of microdeletions, the unequivocal risk of its transmission, and the relatively regular pattern of the deletions were compelling reasons to develop a cost-effective test using a short set of primers.

Here we report the molecular analysis of the Y chromosome in a sequential series of 148 Brazilian infertile men referred from different clinics. On the basis of literature data, we propose a quick, cheap, and reliable diagnostic protocol using an initial set of six pairs of primers ("set-of-six"), which is able to detect up to 95% of the microdeletion cases already published.

PATIENTS AND METHODS

Blood samples from 185 consecutive patients attending different infertility clinics were sent to our lab to be screened for Yq-chromosome microdeletions. Thirty-seven cases, although submitted for PCR analysis, were excluded from the study either because of a lack of complete information on semen parameters, hormone values, and karyotype, or because they did not fulfill our sperm count criteria of $\leq 5 \times 10^6$ sperm/ml. This information, as well as an informed consent, was obtained for all of the remaining 148 patients, and for the 10 normal men of proven fertility whose blood samples were drawn prior to vasectomy and used as controls.

Screening for AZF deletions was performed in blood samples from 70 azoospermic (after centrifugation) men, from 41 severe oligozoospermic ($<1 \times 10^6$ sptz/ml), or cryptozoospermic (few sperm after centrifugation), and from 37 moderate oligozoospermic (1×10^6 to 5×10^6 sptz/ml) men. Table 1 shows the idiopathic and nonidiopathic cases in each sperm count category. We considered as nonidiopathic all those cases in which obstruction, varicocele (uni- or bilateral), cryptorchidism, systemic disease, infection, or abnormal karyotypes (including terminal Yq microdeletions, low-frequency sex chromosome mosaicism, and polymorphic variants, like inversions), were present.

AZF DNA analysis

Genomic DNA was extracted from blood samples (drawn in EDTA-coated tubes) using the GFX™ Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech Inc.). DNA samples were obtained from men with a documented history of fertility and from fertile females, and used as positive and negative controls, respectively.

Based on a review of the literature (see Table 2), we chose a set of six primers—AZFa sY84; AZFb sY114, sY129, and sY143; AZFc sY149 and sY254—found to be frequently deleted in infertile men with low sperm counts. Initially, the three AZF regions were amplified by PCR using a broader set of 19 pairs of primers. For AZFa, we used the sY84 marker; for AZFb, sY113, sY114, sY124, sY129, sY132, sY142, RBM1, RBM2, and sY143; for AZFc, sY153, sY147, sY149, sY254, sY202, sY465, sY156, and sY157; and sY160 to check for the presence of the heterochromatin (Vollrath *et al*., 1992; Ma *et al*., 1992; Kobayashi *et al*., 1994; Reijo *et al*., 1995). In addition, a pair of primers that amplify SRY (sex determining region on the Y chromosome) was used in all patients to verify the presence of Y-specific DNA. A total of 114 patients were tested with this initial set of primers. Thirty-four more patients were tested using a shorter set of six pairs of primers. In these later cases, patients with AZFc microdeletions were further tested for the sY160 marker to verify the presence of heterochromatin. Patient P117 was further tested for additional markers (sY127, *EIF1AY*, sY130, and sY131).

PCRs were carried out in 25-μl reaction volumes containing: 50 ng of genomic DNA; 1.5 m*M* $MgCl_2$; dNTP mix (0.2 m*M* each), 0.5 μM of each oligonucleotide primer; and 1.5 U of recombinant *Taq* DNA polymerase in a final concentration of 1× PCR reaction buffer. All reagents were obtained from Life Technologies Gibco BRL. Each primer pair was individually tested against a patient's DNA, male and female DNA controls, and a blank. Thirty cycles of amplification were carried out in a PTC-100 MJ thermocycler as follows: 1 min at 94°C, 1 min at the annealing temperature (see Vollrath *et al*., 1992; Ma *et al*., 1992; Kobayashi *et al*., 1994; Reijo *et al*., 1995), and 1 min at 72°C. The amplification cycles were pre-

TABLE 1. MICRODELETION [Ydelq] FREQUENCIES AND INFERTILITY CHARACTERISTICS IN THE 148 PATIENTS

	Zero		*$<1 \times 10^6$ sptz/ml*		*$1–5 \times 10^6$ sptz/ml*		
Sperm count	n	n *Ydelq (%)*	n	n *Ydelq (%)*	n	n *Ydelq (%)*	*Total (Ydelq %)*
Idiopathic	30	3 (10)	17	1 (5.9)	18	2 (11.1)	65 (9.2)
Nonidiopathic	40	6[a] (15)	24	—	19	1 (5.3)	83 (8.4)
Total							148 (8.8)

[a]Four of these patients had large terminal deletions detected by karyotyping.

TABLE 2. SELECTED DATA ON Y CHROMOSOMAL MICRODELETIONS SCREENING IN INFERTILE MEN 1992–2001

Authors	Number of STSs	Number of patients	Deletions		Detection with the set-of-6 STSs proposed	
			Number	%	Number	%
Ma *et al.* (1992)	30	19	2	10.5	2	100
Reijo *et al.* (1995)	84	89	12	13.5	12	100
Hargreave *et al.* (1996)	23	117	11	9.4	11	100
Kent-First *et al.* (1996)	85	32 fathers	1	3.1	0	0
		32 sons	3	9.4	2	66.7
Najmabadi *et al.* (1996)	26	60	11	18.3	6	54.6
Nakohori *et al.* (1996)	23	153	20	13.1	18	90
Qureshi *et al.* (1996)	23	100	8	8.0	7	87.5
Reijo *et al.* (1996)	118	35	2	5.7	2	100
Vogt *et al.* (1996)	76	370	13	3.5	13	100
Girardi *et al.* (1997)	35	160	10	6.3	10	100
Kremer *et al.* (1997)	8	164	7	4.3	7	100
Mulhall *et al.* (1997)	?	83	8	9.6	6	100
Peterlin *et al.* (1997)	18	72	6	8.3	6	100
Pryor *et al.* (1997)	85	200 (all sperm count categories)	14 9 ($\leq 5.10^6$)	7.0	7/9	77.8
Selva *et al.* (1997)	12	81	2	2.5	2	100
Shirakawa *et al.* (1997)	4	25	4	16.0	4	100
Simoni *et al.* (1997)	5	168	5	3.0	5	100
van der Ven *et al.* (1997)	30	204	2	0.98	2	100
Vereb *et al.* (1997)	1 (DAZ)	168	5	3.0	5	100
Brandell *et al.* (1998)	35	286	22	7.7	22	100
Foresta *et al.* (1998)	29	18 (SCO)	10	55.5	10	100
Grimaldi *et al.* (1998)	18	67	5	7.5	5	100
Liow *et al.* (1998)	16	202	6	3.0	3	100
Oliva *et al.* (1998)	2(DAZ) + 10	186	10	5.4	10	100
Silber *et al.* (1998)	52	81	14	17.3	14	100
Stuppia *et al.* (1998)	27	126	16	12.7	5	31.2
Chang *et al.* (1999)	27	5	5		5	100
Ferlin *et al.* (1999)	38	140 ($<5 \times 10^6$)	40	28.6	33	82.5
Foresta *et al.* (1999)	31	60 criptorchidism	11	18.3	10	91
Kamischke *et al.* (1999)	8	father&son	2		2	100
Kim *et al.* (1999)	37	40	8	20	7	87.5
Kleiiman *et al.* (1999)	20	133	9	6.0	9	100
Krausz *et al.* (1999a)	6	134 unselected	3	2.2	3	100
Krausz *et al.* (1999b)	22	131	21	19.6	20	95.2
Page *et al.* (1999)	40	3 fathers & 4 sons	7		7	100
Seifer *et al.* (1999)	14	53	5	9.4	5	100
Cram *et al.* (2000)	22	86 fathers	2	2.3	2	100
		99 ICSI sons	—	—	—	—
Martinez *et al.* (2000)	9	128	9	7.0	9	100
Österlund *et al.* (2000)	13	192	4	2.1	4	100
Van Landuyt *et al.* (2000)	3 initial	402	8			
	+27	229	1	2.2	9	100
Fujisawa *et al.* (2001)	28	54	14	25.9	9	64.3
Krausz *et al.* (2001)	20	138	9	6.5	9	100
Tzschach *et al.* (2001)	13	97	—	—	—	—

Table shows number of STSs (markers) used, number of patients screened, microdeletion frequencies and percentage of possible diagnosis using the set-of-6 pairs of primers proposed in this work.

ceded by a 4-min denaturing step at 98°C, and followed by a final extension step at 72°C for 5 min. The PCR products were separated on a 1.5% agarose gel by electrophoresis in TAE (Tris, acetic acid, EDTA) buffer, stained with ethidium bromide, and photographed.

A negative result was scored when the amplification product was not obtained after three PCR attempts, two of them in less-stringent annealing conditions. For patient P117, a Southern blot was obtained to confirm the deletion of the sY129 marker.

Table 3. Clinical and Laboratory Data on the 13 Patients with Yq Microdeletions

Patient number	Age (y)	Hormones[a]		Testicular biopsy	Testicular volume (ml)		Varicocele		Sperm count (/ml)	Karyotype	del(Yq)	
		FSH (ref)	LH (ref)		R	L	R	L			AZF	STSs
P13	27	11.4 (1–10)	7 (1–8)	nd	18.2	17.6	—	—	0.1×10^6	46, XY	b-	113/114
P33	32	8.2 (0.5–10)	3.8 (1–8)	bilat.SCO	18.8	17.4	—	—	zero	46,XY	c-	149/254
P61	34	8.5 (0.5–10)	4.1 (1–8)	nd	16.2	15.4	—	—	zero	45,X,−Y,+mar	bch-	114 → 160
P62	31	4 (0.5–10)	5.8 (1–8)	nd	17.0	16.8	—	—	2.5×10^6	46,XY	c-	149/254
P66	34	17 (0.5–10)	6.2 (1–8)	bilat SCO	11.4	8.6	1	2	zero	45,X,−Y,+mar	abch-	84 → 160
P67	36	5.2 (0.5–10)	3 (2–12)	nd	22.0	20.2	—	—	zero	46,XY	b-	142/143
P76	37	6.3 (1–10)	5.9 (1–8)	nd	14.8	14.0	—	—	zero	46,XY(delqh)	bch-	114 → 160
P79	36	6.7 (0.5–10)	3.1 (1–8)	nd	17.6	23.2	—	—	zero	46,XY	c-	149/254
P91	37	11.8 (1–10)	4.9 (1–8)	unilat.SCO	13.2	12.8	—	—	zero	46,XY(delqh)	bch-	114 → 160
P117	30	5.1 (0.5–10)	2.1 (0.8–7.6)	nd	12.3	10.6	—	—	3.2×10^6	46,XY	b-	131/129
P153	27	37.8 (1–8)	20.2 (2–12)	nd	5.0	4.4	1	2	zero	46,XY	c-	149/254
P162	43	6.6 (0.5–10)	2.8 (1–8)	nd	18.2	crypt	—	crypt	3.10^6	46,Xy	c-	149/254
P175	44	4.3 (0.7–11)	1.6 (0.8–7.6)	nd	13.5	12.4	—	1	zero	46,XY	b-	114/129/143

[a]FSH and LH in mUI/ml. R, right; L, left; SCO, Sertoli Cell Only Syndrome; bilat, bilateral; unilat, unilateral; nl, normal; nd, not done; crypt, cryptorchidism.

RESULTS

A total of 148 patients were screened for the presence of Yq microdeletions, and the overall microdeletion frequency was found to be 8.8% ($n = 13$). No microdeletions were found in the 10 normal men of proven fertility used as controls (Table 1). The clinical and laboratory findings of the 13 patients with Yq deletions appear in Table 3, and the schematic representation of these deletions is depicted in Fig. 1. All of the patients with microdeletions refused to ask their male relatives to be tested to check for the *de novo* status of the microdeletions found.

Eighty-three nonidiopathic cases were studied with an overall deletion frequency of 8.4% ($n = 7$), mostly represented by large terminal deletions ($n = 4$) involving the three AZF regions (patient P66) or AZFb/AZFc (patients P61, P76, P91). All four cases with terminal deletions had no sperm in the ejaculate and testicular biopsies were read as SCO syndrome in the 2 patients with elevated follicle-stimulating hormone (FSH) levels. Three other microdeletions were found, as follows: one AZFb microdeletion in an azoospermic patient (P175) with secondary infertility, unilateral grade one varicocele and normal hormonal levels; two AZFc microdeletions, one in an azoospermic man (P153) with very high FSH (37.8 mUI/ml; normal range: 1–8 mUI/ml) and luteinizing hormone (LH) (20.1 mUI/ml; normal range: 2–12 mUI/ml) levels, small testicles, and bilateral varicocele, and another AZFb microdeletion in a moderately oligozoospermic man (P162) with left inguinal testicle and normal hormonal levels. No microdeletions were found in the 24 severe nonidiopathic oligozoospermic cases.

Varicoceles were reported in 44.6% ($n = 37$) of the nonidiopathic cases: either isolated ($n = 25$; 12 unilateral, 13 bilateral), or in association with cryptorchidism ($n = 2$), karyotype abnormalities ($n = 5$), obstruction ($n = 2$), or AZF deletions ($n = 3$).

Karyotype abnormalities were present in 19 of the 83 (22.9%) nonidiopathic cases and were mostly represented by sex chromosome mosaicism ($n = 7$), terminal Yq deletions

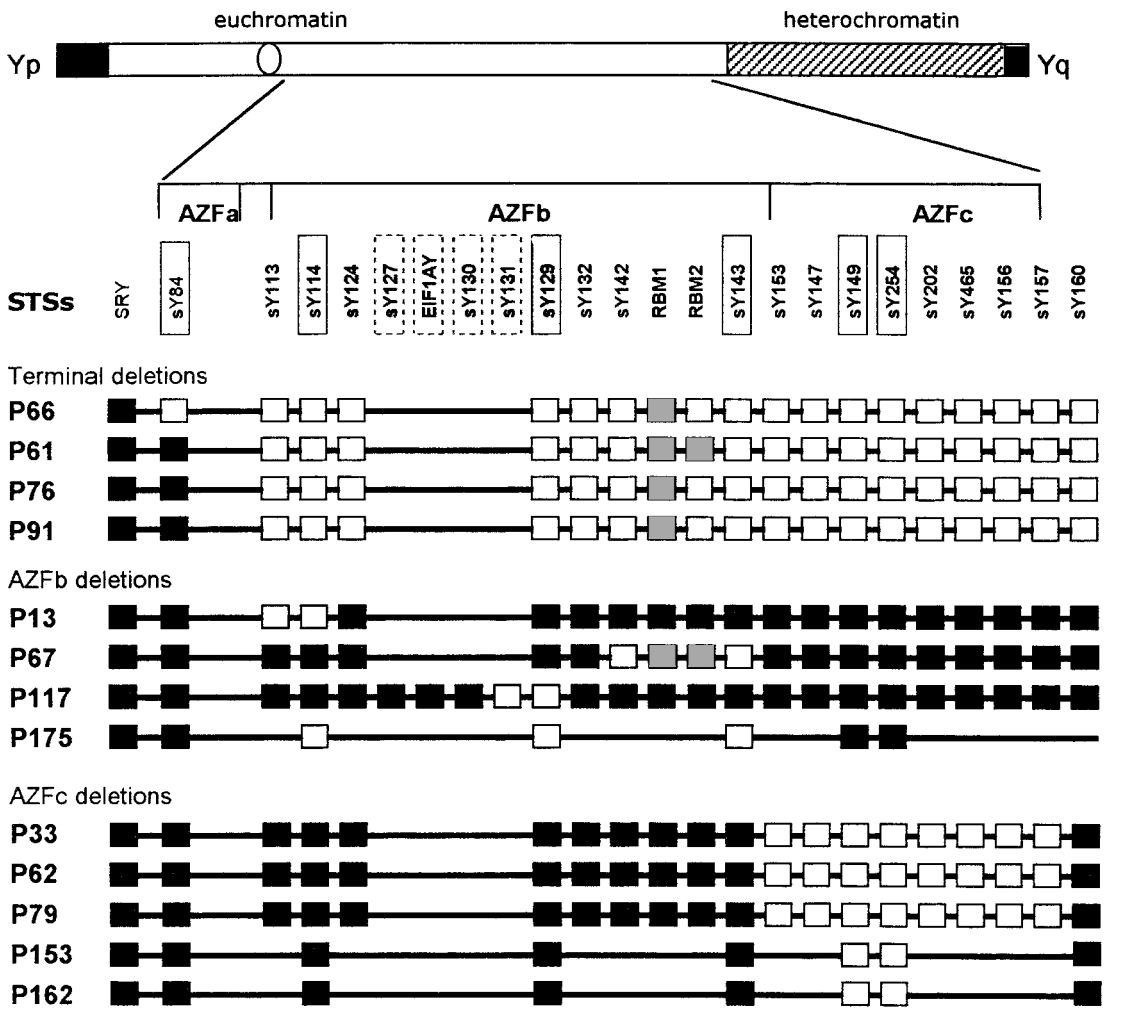

FIG. 1. A schematic representation of the Y chromosome showing the AZF regions and the Yq microdeletions found in our patients. The STSs inside solid frames represent the set-of-6 (see text); inside dotted frames are shown the STSs used only for studying patient P117. Positive PCR results for particular STSs are indicated by black boxes, absence is shown in white, and weekly positive PCR results in gray.

($n = 4$), 47,XXY ($n = 2$), and an 18;Yq translocation ($n = 1$) with no apparent loss of AZF euchromatic material. The remaining chromosomal abnormalities ($n = 5$) involved autosomal chromosomes and included a Robertosonian translocation, a bisatellited chromosome marker, a pericentromeric inversion of chromosome 2, and two mosaics for complex rearrangements.

Obstruction was the third most frequent cause of nonidiopathic infertility, present in 14 (16.9%) cases: 2 cases of CBAVD (congenital bilateral agenesis of vas deferens); one case of CUAVD (congenital unilateral agenesis of vas deferens); 3 cases of obstruction after testicular trauma; 3 cases of infection sequelae; 4 cases of epididymal cysts; one case of iatrogenic obstruction after hernia surgery.

Among the 65 idiopathic cases of infertility, six (9.2%) microdeletions were detected, three in AZFb (patients P13, P67, and P117), and three in AZFc (P33, P62, P79) region (Fig. 1). The AZFb microdeletions involved small segments and a variety of phenotypes (Table 3). One AZFb (sY113/sY114) microdeletion was detected in a patient (P13) with primary infertility and severe oligozoospermia who had a seminal analysis within the normal range (34×10^6 sptz/ml) 1 year before he was tested for Yq microdeletions. The second AZFb microdeletion was found in an azoospermic man (P67) and included the markers sY142 and sY143. The PCR for RBM1 and RBM2 [using the primers described by Ma *et al*. (1992) and modified by Kobayashi *et al*. (1994)] resulted in weak bands on the agarose gel. The third AZFb deletion was detected in a patient (P117) with moderate oligozoospermia (3.2×10^6 sptz/ml) and globozoospermia present in 96% of the sperm; this microdeletion involved the sY129 marker, confirmed by Southern blot, and further PCR investigation with other markers (sY127, *EIF1AY*; sY130, sY131) indicated a deletion of approximately 200,000 bp.

The three AZFc microdeletions in idiopathic infertile men also included a spectrum of phenotypes. One was detected in an azoospermic man (P33) with testicular histology compatible with bilateral SCO syndrome, another one was found in an azoospermic man who refused to allow testicular biopsy (P79), and the third was detected in a moderately oligozoospermic man (P62).

An isolated deletion of AZFa was not found in our patient sample. The 4 patients (P66, P61, P76, and P91) with large terminal deletions were found to be weakly positive for the multicopy RBM1 and RBM2 gene family. The azoospermic patient (P67) with the sY142-sY143 microdeletion refused testicular aspiration followed by ICSI for fertilization, choosing instead the semen donor approach.

DISCUSSION

This work shows that the microdeletion frequency found in our series of 148 infertile men using the set-of-6 approach is comparable to the frequencies reported in the literature (Table 2). The selection of the markers to compose the set-of-6 was based on an extensive review of the literature, with attention to the most frequently deleted markers in the infertile men screened. The efficiency of the set-of-6 (anticipated by the literature analysis and confirmed by the experimental work) enables us to propose its use as a routine diagnostic screening test.

As previously stated by Simoni *et al*. (1998), the criteria for selecting the patients, rather than the number of markers (STSs) used in the screening protocol, are the critical factors underlying the large variation in the Y-microdeletion frequencies reported in the literature (from 0.9% to 55.5%; see Table 2).

The use of a large number of specific Y markers is necessary only if one is interested in the precise characterization of microdeletion breakpoints or in gene mapping. In fact, to achieve an improvement in the detection of Y microdeletions, some invasive procedures are mandatory, as with the use of testicular tissue for molecular cytogenetics and gene expression studies. Such procedures are time consuming, costly, and, furthermore, not recommended by international specialists' consensus reports, such as the ESHRE Capri Workshop Group (2000) and its proposals for the initial work-up of azoospermic men.

The microdeletions missed with the set-of-6 were: (1) those found by reverse transcriptase (RT)-PCR in testicular tissue (Ferlin *et al*., 1999; Foresta *et al*., 1999); (2) those involving discontinuous STSs (Najmabadi *et al*., 1996; Stuppia *et al*., 1998; Kim *et al*., 1999; Fujisawa *et al*., 2001), or single STSs (Kent-First *et al*., 1996; Qureshi *et al*., 1996; Pryor *et al*., 1997; Fujisawa *et al*., 2001); (3) the ones involving the RBM gene family (Nakahori *et al*., 1996; Pryor *et al*., 1997) or found in infertile men with normal sperm counts (Pryor *et al*., 1997).

In this work, we initially used a panel of 19 markers. The first noticeable finding was that the patients with large terminal deletions, including three (P66) or two (P61, P76, and P91), AZF subregions, were all weakly positive for the "specific" RBM primers (Ma *et al*., 1993; Kobayashi *et al*., 1994). The same was found for another patient (P67) with an interstitial AZFb deletion. Considering that RBM is a multicopy gene family, with members spreading over the short and long arms of the Y chromosome (Elliot, 2000), these positive results could represent the PCR amplification of some members of the RBM family other than the functional copy mapped in the interval 6B, close to the AZFb/AZFc limit (Elliott *et al*., 1997). Another important piece of information on the RBM gene family is the absence of RBM2 in a large series of fertile Japanese men (Nakahori *et al*., 1994). Although the importance of the RBM gene for human fertility is recognized (Elliott, 2000), our PCR results warrant the exclusion of RBM primers for diagnostic purposes.

The choice of sY129 (also mapped in AZFb), instead of sY127 as proposed by Simoni *et al*. (1999), was due to the fact that patient P117 was positive for sY127 and negative for sY129. Further PCR investigation of patient P117, using other markers (see Fig. 1), suggested the deletion of a 200,000-bp segment flanked by markers sY130 and sY132. This segment contains a human endogenous retroviral (HERV) sequence of 8471bp (GenBank accession NT_011903). HERVs are known to influence gene expression, as tissue- and stage-specific enhancers. The closest gene to this HERV sequence is the *EIF1AY* gene, which stabilizes the binding of the initiator Met-tRNA to ribosomal subunits. The loss of this HERV sequence might have somehow impaired *EIF1AY* expression and might have contributed to the phenotype of globozoospermia presented by P117.

The sY153 marker was withdrawn from our set of primers because it was absent in an infertile man with normal sperm concentration (89×10^6 sptz/ml), described by Pryor *et al.* (1997).

We chose to screen the AZFb region at its proximal and distal limits, using the markers sY114 and sY143, respectively. Discontinuous microdeletions are mostly found in the AZFb region, the only AZF segment that is still lacking a systematic study of structure and complete nucleotide sequence. Such studies became available for AZFa and AZFc in the last few years and identify breakpoint hot spots (Blanco *et al.*, 2000; Kamp *et al.*, 2000; Kuroda-Kawaguchi *et al.*, 2001).

A major subclass of AZFa microdeletions was recently characterized as a result of intrachromosomal recombination events between two highly homologous HERV sequences. This mechanism leads to a regular pattern of euchromatic loss encompassing the AZFa *USP9Y/DBY/UTY* segment during meiosis (Blanco *et al.*, 2000; Kamp *et al.*, 2000). HERVs are a major subclass of dispersed repeats, accounting for about 1% of the human genome. Their pathogenic role is to sponsor illegitimate recombination. The segment lost through this recombination event includes sY84, a flanking marker on the 5′ end of *USP9Y* (Brown *et al.*, 1998; Sargent *et al.*, 1999). More upstream markers like sY85, sY86 [proposed by Simoni *et al.* (1999)], and sY83 would only detect potentially nonfunctional genes and highly degenerated sequences (Sargent *et al.*, 1999).

It has been suggested that both *USP9Y* and *DBY* are involved in the AZFa phenotype of SCO (Sun *et al.*, 1999; Foresta *et al.*, 2000a,b). Deletion of the entire *USP9Y* (Sargent *et al.*, 1999), or a mutation leading to a truncated protein (Sun *et al.*, 1999), is responsible for a milder AZFa phenotype (hypospermatogenesis). However, when the deletion also involves *DBY*, the effects on spermatogenesis are worse, indicating that *USP9Y* and *DBY* might work in strict dependence of one another.

Using new PCR-based assays and new STSs to study the genomic sequence of AZFc, Kuroda-Kawaguchi *et al.* (2001) found out that the AZFc region is a complex, organized, and elaborate mosaic of amplicons and palindromes, extending for 3.5 kb. All but one of 48 infertile men with AZFc deletions analyzed by these authors shared almost identical breakpoints, suggesting that such clustering of breakpoints is the result of homologous recombination between amplicons and a frequent cause of AZFc deletions (Kuroda-Kawaguchi *et al.*, 2001). The euchromatic segment most frequently lost in AZFc deletions contains four copies of the sY254 marker (included in our set-of-6 pair of primers), one for each *DAZ* gene in the cluster.

The data discussed above on AZF structure "add Y-chromosomally based infertility to a growing list of diseases resulting from illegitimate recombination between flanking repeats" (Blanco *et al.*, 2000). The diagnostic screening for Y-chromosome microdeletions could be, therefore, restricted to the euchromatic segments between specific repeats.

Some authors (Girardi *et al.*, 1997; Chang *et al.*, 1999; Österlund *et al.*, 2000; Calogero *et al.*, 2001) report that AZFc microdeletions are involved in the worsening of sperm parameters over time. This could explain, at least in part, the wide spectrum of phenotypes in AZFc microdeletions (depending on when the test is performed). It is even possible that the same could apply to AZFb deletions: we detected 2 patients with AZFb deletions and worsening of sperm parameters over time (P13), or secondary infertility associated with a varicocele (P175).

Finally, it is worthwhile to consider the cases of Y microdeletions associated with nonidiopathic infertility. Microdeletions in nonidiopathic infertility have been reported in the literature at a rather high frequency (Foresta *et al.*, 1999; Krausz *et al.*, 1999b; the present study). The deterioration of sperm parameters might be accelerated in patients with microdeletions associated with varicoceles, such as patient P175, reinforcing the need for AZF microdeletion screening and follow-up of these infertile men. It was recently reported that men with microdeletions and varicoceles do not respond to varicocelectomy (Cayan *et al.*, 2001). However, the study included only 3 men, all with signs of testicular function breakdown already established. An inexpensive Y-microdeletion screening test would be helpful in the management of varicocele, especially in those cases without testicular failure at the time of study, and in cases of subclinical or grade one varicocele in young men, for whom there is not always a clear indication for varicocelectomy.

ACKNOWLEDGMENT

This work was supported in part by FAPESP/INSERM grant no. 99/09586-9.

REFERENCES

BLANCO, P., SHLUMUKOVA, M., SARGENT, C.A., JOBLING, M.A., AFFARA, N., and HURLES, M.E. (2000). Divergent outcomes of intrachromosomal recombination on the human Y chromosome: male infertility and recurrent polymorphism. J. Med. Genet. **37,** 752–758.

BROWN, G.M., FURLONG, R.A., SARGENT, C.A., ERICKSON, R.P., LONGPIED, G., MITCHELL, M., JONES, M.H., HARGREAVE, T.B., COOKE, H.J., and AFFARA, N.A. (1998). Characterization of the coding sequence and fine mapping of the human *DFFRY* gene and comparative expression analysis and mapping to the *Sxr*^{b} interval of the mouse Y chromosome of the *Dffry* gene. Hum. Mol. Genet. **7,** 97–107.

BRANDELL, R.A., MIELNIK, A., LIOTTA, D., YE, Z., VEECK, L.L., PALERMO, G.D., and SCHLEGEL, P. (1998). AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. Hum. Reprod. **13,** 2812–2815.

CALOGERO, A.E., GAROFALO, M.R., BARONE, N., DE PALMA, A., VICARI, E., ROMEO, R., TUMINO, S., and D'AGATA, R. (2001). Spontaneous regression over time of the germinal epithelium in a Y chromosome-microdeleted patient. Hum. Reprod. **16,** 1845–1848.

CAYAN, S., LEE, D., BLACK, L.D., REIJO-PERA, R.A., and TUREK, P.J. (2001). Response to varicocelectomy in oligospermic men with and without defined genetic infertility. Urology **57,** 530–535.

CHANG, P.L., SAUER, M.V., and BROWN, S. (1999). Y chromosome microdeletion in a father and his four infertile sons. Hum. Reprod. **14,** 2689–2694.

CRAM, D.S., MA, K., BHASIN, S., ARIAS, J., PANDJAITAN, M., CHU, B., AUDRINS, P., SAUNDERS, D., QUINN, F., DEKRESTER, D., and MCLACHLAN, R. (2000). Y chromosome analysis of infertile men and their sons conceived through intracy-

toplasmic sperm injection: vertical transmission of deletions and rarity of de novo deletions. Fertil. Steril. **74,** 909–915.

ELLIOTT, D.J., MILLAR, M.R., OGHENE, K., ROSS, A., KIESEWETTER, F., PRYOR, J., McINTYRE, M., HARGREAVE, T.B., SAUNDERS, P.T.K., VOGT, P.H., CHANDLEY, A.C., and COOKE, H. (1997). Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y chromosome long arm. Proc. Natl. Acad. Sci. **94,** 3848–3853.

ELLIOTT, D.J. (2000). RBMY genes and AZFb deletions. J. Endocrinol. Invest. **23,** 652–658.

ESHRE Capri Workshop Group. (2000). Optimal use of infertility diagnostic tests and treatments. Hum. Reprod. **15,** 723–732.

FERLIN, A., MORO, E., GAROLLA, A., and FORESTA, C. (1999). Human male infertility and Y chromosome deletions: role of the AZF-candidate genes DAZ, RBM and DFFRY. Hum. Reprod. **14,** 1710–1716.

FORESTA, C., FERLIN, A., GAROLLA, A., MORO, E., PISTORELLO, M., BARBAUX, S., and ROSSATO, M. (1998). High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome. Hum. Reprod. **13,** 302–307.

FORESTA, C., MORO, E., GAROLLA, A., ONISTO, M., and FERLIN, A. (1999). Y chromosome microdeletions in cryptorchidism and idiopathic infertility. J. Clin. Endocrinol. Metabol. **84,** 3660–3665.

FORESTA, C., MORO, E., ROSSI, A., ROSSATO, M., GAROLLA, A., and FERLIN, A. (2000a). Role of AZFa candidate genes in male infertility. J. Endocrinol. Invest. **23,** 646–651.

FORESTA, C., FERLIN, A., and MORO, E. (2000b). Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. Hum. Mol. Genet. **9,** 1161–1169.

FUJISAWA, M., SHIRAKAWA, T., KANZAKI, M., OKADA, H., ARAKAWA, S., and KAMIDONO, S. (2001). Y-chromosome microdeletion and phenotype in cytogenetically normal men with idiopathic azoospermia. Fertil. Steril. **76,** 491–495.

GIRARDI, S.K., MIELNIK, A., and SCHLEGEL, P.N. (1997). Submicroscopic deletions in the Y chromosome of infertile men. Hum. Reprod. **12,** 1635–1641.

GRIMALDI, P., SCARPONI, C., ROSSI, P., MARCH, M.R., FABBRI, A., ISIDORI, A., SPERA, G., KRAUSZ, C., and GEREMIA, R. (1998). Analysis of Yq microdeletions in infertile males by PCR and DNA hybridization techniques. Mol. Hum. Reprod. **4,** 1116–1121.

HARGREAVE, T.B., CHANDLEY, A.C., ROSS, A., QURESHI, S., KUN, M., and COOKE, H. (1996). Y chromosome microdeletions and male subfertility. Andrologia **28** (Suppl 1), 19–21.

KAMISCHKE, A., GROMOLL, J., SIMONI, M., BEHRE, H.M., and NIESCHLAG, E. (1999). Transmission of a Y chromosomal deletion involving the deleted in azoospermia (DAZ) and chromodomain (CDY1) genes from father to son through intracytoplasmic sperm injection. Hum. Reprod. **14,** 2320–2322.

KAMP, C., HIRSCHMANN, P., VOSS, H., HUELLEN, K., and VOGT, P.H. (2000). Two long homologous retroviral sequences blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. Hum. Mol. Genet. **9,** 2563–2572.

KENT-FIRST, M.G., KOL, S., MUALLEM, A., OFIR, R., MANOR, D., BLAZER, S., FIRST, N., and ITSKOVITZ-ELDOR, J. (1996). The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. Mol. Hum. Reprod. **2,** 943–959.

KIM, S.W., KIM, K.D., and PAICK, J-S. (1999). Microdeletions within the azoospermia factor subregions of the Y chromosome in patients with idiopathic azoospermia. Fertil. Steril. **72,** 349–353.

KLEIMAN, S.E., YOGEV, L., GAMZU, R., HAUSER, R., BOTCHAN, A., LESSING, J.B., PAZ, G., and YAVETZ, H. (1999). Genetic evaluation of infertile men. Hum. Reprod. **14,** 33–38.

KOBAYASHI, K., MIZUNO, K., HIDA, A., KOMAKI, R., TOMITA, K., MATSUSHITA, I., NAMIKI, M., IWAMOTO, T., TAMURA, S., MINOWADA, S., NAKAHORI, Y., and NAKAGOME, Y. (1994). PCR analysis of the Y chromosome long arm in azoospermia patients: evidence for a second locus required for spermatogenesis. Hum. Mol. Genet. **3,** 1965–1967. [Erratum. Hum. Mol. Genet. 1995;4:974.]

KRAUSZ, C., BUSSANI-MASTELLONE, C., GRANCHI, S., McELREAVEY, K., SCARSELLI, G., and FORTI, G. (1999a). Screening for microdeletions of Y chromosome genes in patients undergoing intracytoplasmic sperm injection. Hum. Reprod. **14,** 1717–1721.

KRAUSZ, C., QUINTANA-MURCI, L., BARBAUX, S., SIFFROI, J.P., ROUBA, H., DELAFONTAINE, D., SOULEYREAU-THERVILLE, N., ARVIS, G., ANTOINE, J.M., ERDEI, E., TAAR, J.P., TAR, A., JEANDIDIER, E., PLESSIS, G., BOURGERON, T., DADOUNE, J.P., FELLOUS, M., and McELREAVY, K. (1999b). A high frequency of Y chromosome deletions in males with nonidiopathic infertility. J. Clin. Endocrinol. Metabol. **84,** 3606–3612.

KRAUSZ, C., QUINTANA-MURCI, L., and McELREAVEY, K. (2000). Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Hum. Reprod. **15,** 1431–1434.

KRAUSZ, C., MEYTS, R-D., FRYDELUND-LARSEN, L., QUINTANA-MURCI, L., McELREAVEY, K., and SKAKKEBAEK, N.E. (2001). Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. J. Clin. Endocrinol. Metabol. **86,** 2638–2642.

KREMER, J.A.M., TUERLINGS, J.H.A.M., MEULEMAN, E.J.H., SCHOUTE, F., MARIMAN, E., SMEETS, F.C.M., HOEFSLOOT, L.H., BRAAT, D.D.M., and MERKUS, H.M.W.M. (1997). Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic. Hum. Reprod. **12,** 687–691.

KURODA-KAWAGUCHI, T., SKALETSKY, H., BROWN, L.G., MINX, P.J., CORDUM, H.S., WATERSON, R.H., WILSON, R.K., SILBER, S., OATES, R., ROZEN, S., and PAGE, D.C. (2001). The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nature Genet. **29,** 279–286.

LIOW, L., GHADESSY, F.J., NG, S.C., and YONG, E.L. (1998). Y chromosome microdeletions, in azoospermic or near-azoospermic subjects, are located in the AZFc (DAZ) subregion. Mol. Hum. Reprod. **4,** 763–768.

MA, K., SHARKEY, S., KIRSCH, S., VOGT, P., KEIL, R., HARGREAVE, T.B., McBEATH, S., and CHANDLEY, A.C. (1992). 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. **1,** 29–33.

MA, K., INGLIS, J., SHARKEY, A., BICKMORE, W.A., HILL, R.E., PROSSER, E.J., SPEED, R.M., THOMSON, E.J., JOBLING, M., TAYLOR, K., WOLFE, J., COOKE, H.J., HARGREAVE, T.B., and CHANDLEY, A.C. (1993). A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor controlling human spermatogenesis. Cell **75,** 1287–1295.

MARTÍNEZ, M.C., BERNABÉ, M.J., GÓMEZ, E., BALLESTEROS, A., LANDERAS, J., GLOVER, G., GÍL-SALOM, M., REMOHI, J., and PELLICER, A. (2000). Screening of AZF deletions in a large series of severely impaired spermatogenesis patients. J. Androl. **21,** 651–655.

MULHALL, J.P., REIJO, R., ALAGAPPAN, R., BROWN, L., PAGE, D., CARSON, R., and OATES, R.D. (1997). Azoospermic men with deletion of the DAZ gene cluster are capable of completing spermatogenesis: fertilization, normal embryonic development and pregnancy occur when retrieved testicular spermatozoa are used for intracytoplasmic sperm injection. Hum. Reprod. **12,** 503–508.

NAJMABADI, H., HUANG, V., YEN, P., SUBBARAO, M.N.,

BHASIN, D., BANAAG, L., NASEERUDDIN, S., DEKRESTER, D.M., BAKER, H.W.G., MCLACHLAN, R.I., LOVELAND, K.A., and BHASIN, S. (1996). Substantial prevalence of microdeletions of the Y-chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. J. Clin. Endocrinol. Metab. **81,** 1347–1352.

NAKAHORI, Y., KOBAYASHI, K., KOMAKI, R., MATSUSHITA, I., and NAKAGOME, Y. (1994). A locus of the candidate gene family for azoospermia factor (YRRM2) is polymorphic with a null allele in Japanese males. Hum. Mol. Genet. **3,** 1709.

NAKAHORI, Y., KUROKI, Y., KOMAKI, R., KONDOH, N., NAMIKI, M., IWAMOTO, T., TODA, T., and KOBAYASHI, K. (1996). The Y chromosome region essential for spermatogenesis. Horm. Res. **46** (Suppl. 1), 20–23.

OLIVA, R., MARGARIT, E., BALLESCA, J.L., CARRIÓ, A., SÁNCHEZ, A., MILÀ, M., JIMÉNEZ, L., ALVAREZ-VIJANDE, J.R., and BALLESTA, F. (1998). Prevalence of Y chromosome microdeletions in oligospermic and azoospermic candidates for intracytoplasmic sperm injection. Fertil. Steril. **70,** 506–510.

ÖSTERLUND, C., SEGERSTEEN, E., ARVER, S., and POUSETTE, A. (2000). Low number of Y-chromosome deletions in infertile azoospermic men at a Swedish andrology centre. Int. J. Androl. **23,** 225–229.

PAGE, D.C., SILBER, S., and BROWN, L.G. (1999). Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. Hum. Reprod. **14,** 1722–1726.

PETERLIN, B., KUNEJ, T., ZORN, B., DROBNIÈ, S., GREGORIÈ, J., and DEBEVC, M. (1997). Sterility associated with Y chromosome abnormalities. In *Genetics of Human Male Fertility*. C. Barratt, C. DeJonge, D. Mortimer, and J. Parinaud (eds.) Éditions EDK, Paris, pp. 66–75.

PRYOR, J.L., KENT-FIRST, M., MUALLEM, A., VAN BERGEN, A.H., NOLTEN, W.E., MEISNER, L., and ROBERTS, K.P. (1997). Microdeletions in the Y chromosome of infertile men. N. Engl. J. Med. **336,** 534–539.

QURESHI, S.J., ROSS, A.R., MA, K., COOKE, H.J., MCINTYRE, M.A., CHANDLEY, A.C., and HARGREAVE, T.B. (1996). Polymerase chain reaction screening for Y chromosome microdeletions: a first step toward the diagnosis of genetically-determined spermatogenic failure in men. Mol. Hum. Reprod. **2,** 775–779.

REIJO, R., LEE, T.Y., SALO, P., ALAGAPPAN, R., BROWN, L.G., ROSENBERG, M., ROZEN, S., JAFFE, T., STRAUS, D., HOVATTA, O., DE LA CHAPELLE, A., SILBER, S., and PAGE, D.C. (1995). Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nature Genet. **10,** 383–393.

REIJO, R., ALAGAPPAN, R.K., PATRIZIO, P., and PAGE, D.C. (1996). Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet **347,** 1290–1293.

SARGENT, C.A., BOUCHER, C.A., KIRSCH, S., BROWN, G., WEISS, B., TRUNDLEY, A., BURGOYNE, P., SAUT, N., DURAND, C., LEVY, N., TERRIOU, P., HARGREAVE, T., COOKE, H., MITCHELL, M., RAPPOLD, G.A., and AFFARA, N.A. (1999). The critical region of overlap defining the AZFa male infertility interval of proximal Yq contains three transcribed sequences. J. Med. Genet. **36,** 670–677.

SAXENA, R., DE VRIES, J.W.A., REPPING, S., ALAGAPPAN, R.K., SKALETSKY, H., BROWN, L.G., MA, P., CHEN, E., HOOVERS, J.M.N., and PAGE, D.C. (2000). Four DAZ genes in two clusters found in the AZFc region of the human Y chromosome. Genomics **67,** 256–267.

SEIFER, I., AMAT, S., DELGADO-VISCOGLIOSI, P., BOUCHER, D., and BIGNON, Y.J. (1999). Screening for microdeletions on the long arm of chromosome Y in 53 infertile men. Int J. Androl. **22,** 148–154.

SELVA, J., KANAFANI, S., PRIGENT, Y., PONCET, V., and BERGERE, M. (1997). Incidence of AZF (azoospermia factor) deletions and familial forms of infertility among patients requiring intracytoplasmic spermatozoa injection (ICSI). J. Assist. Reprod. Genet. **14,** 593–595.

SHIRAKAWA, T., FUJISAWA, M., KANZAKI, M., OKADA, H., ARAKAWA, S., and KAMIDONO, S. (1997). Y chromosome (Yq11) microdeletions in idiopathic azoospermia. Int. J. Urol. **4,** 198–201.

SILBER, S.J., ALAGAPPAN, R., BROWN, L.G., and PAGE, D.C. (1998). Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. Hum. Reprod. **13,** 3332–3337.

SIMONI, M., GROMOLL, J., DWORNICZAK, B., ROLF, C., ABSHAGEN, K., KAMISCHKE, A., CARANI, C., MESCHEDE, D., BEHRE, H.M., HORST, J., and NIESCHLAG, E. (1997). Screening for deletions of the Y chromosome involving the DAZ (Deleted in AZoospermia) gene in azoospermia and severe oligozoospermia. Fertil. Steril. **67,** 542–547.

SIMONI, A., KAMISCHKE, A., and NIESCHLAG, E. (1998). Current status of the molecular diagnosis of Y-chromosomal microdeletions in the work-up of male infertility. Initiative for international quality control. Hum. Reprod. **13,** 1764–1768.

SIMONI, M., BAKKER, E., EURLINGS, M.C.M., MATTHIJS, G., MORO, E., MÜLLER, C.R., VOGT, P.H. (1999). Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. Int. J. Androl. **22,** 292–299.

STUPPIA, L., GATTA, V., CALABRESE, G., FRANCHI, P.G., MORIZIO, E., BOMBIERI, C., MINGARELLI, R., SFORZA, V., FRAJESE, G., TENAGLIA, R., and PALKA, G. (1998). A quarter of men with idiopathic oligo-azoospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11. Hum. Genet. **102,** 566–570.

STUPPIA, L., GATTA, V., FOGH, I., GASPARI, A.R., GRANDE, R., MORIZIO, E., FANTASIA, D., PIZZUTI, A., CALABRESE, G., and PALKA, G. (2000). Characterization of novel genes in AZF regions. J. Endocrinol. Invest. **23,** 659–663.

SUN, C., SKALETSKY, H., BIRREN, B., DEVON, K., TANG, Z., SILBER, S., OATES, R., and PAGE, D.C. (1999). An azoospermic man with a de novo point mutation in the Y-chromosomal gene USP9Y. Nature Genet. **23,** 429–432.

TIEPOLO, L., and ZUFFARDI, O. (1976). Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum. Genet. **34,** 119–124.

TZSCHACH, A., THAMM, B., IMTHURN, B., WEBER, W., ALEXANDER, H., GLANDER, H.-J., and FROSTER, U. (2001). Absence of Yq microdeletions in infertile men. Arch. Androl. **47,** 167–171.

VAN DER VEN, K., MONTAG, M., PESCHKA, B., LEYGRAAF, J., SCHWANITZ, G., HAIDL, G., KREBS, D., and VAN DER VEN, H. (1997). Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. Mol. Hum. Reprod. **3,** 699–704.

VAN LANDUYT, L., LISSENS, W., STOUFFS, K., TOURNAYE, H., LIEBAERS, I., and VAN STEIRTEGHEM, A. (2000). Validation of a simple Yq deletion screening programme in an ICSI candidate population. Mol. Hum. Reprod. **6,** 291–297.

VEREB, M., AGULNIK, A.I., HOUSTON, J.T., LAMB, D.J., and BISHOP, C.E. (1997). Absence of DAZ gene mutations in cases of non-obstructed azoospermia. Mol. Hum. Reprod. **3,** 55–59.

VOGT, P.H., EDELMANN, A., KIRSCH, S., HENEGARIU, O., HIRSCHMANN, P., KIESEWETTER, F., KÖHN, F.M., SCHILL, W.B., FARAH, S., RAMOS, C., HARTMANN, M., HARTSCHUH, W., MESCHEDE, D., BEHRE, H.M., CASTEL, A., NIESCHLAG, E., WEIDNER, W., GRÖNE, H.J., JUNG, A., ENGEL, W., and HAIDL, G. (1996): Human Y chromosome azoospermia factors

(AZF) mapped to different subregions in Yq11. Hum. Mol. Genet. **5,** 933–943.

VOGT, P.H., AFFARA, N., DAVEY, P., HAMMER, M., JOBLING, M.A., LAU, Y.-F.C., MITCHELL, M., SCHEMPP, W., TYLER-SMITH, C., WILLIAMS, G., YEN, P., and RAPPOLD, G.A. (1997). Report of the third international workshop on Y chromosome mapping 1997. Cytogenet. Cell Genet. **79,** 1–20.

VOLLRATH, D., FOOTE, S., HILTON, A., BROWN, L.G., BEER-ROMERO, P., BOGAN, J.S., and PAGE, D.C. (1992). The human Y chromosome: a 43-interval map based on naturally occurring deletions. Science **258,** 52–59.

Address reprint requests to:
Prof. Dr. Carlos A. Moreira-Filho
Depto. de Imunologia do ICB-USP
Av. Prof. Lineu Prestes 1730
05508-900, São Paulo, SP, Brazil

E-mail: cmoreira@icb.usp.br

Received for publication January 31, 2002; accepted July 30, 2002.