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Frequency of Y chromosome microdeletions and chromosomal abnormalities in infertile Thai men with oligozoospermia and azoospermia

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Abstract

Aim: To investigate the possible causes of oligozoospermia and azoospermia in infertile Thai men, and to find the frequencies of Y chromosome microdeletions and cytogenetic abnormalities in this group. **Methods:** From June 2003 to November 2005, 50 azoospermic and 80 oligozoospermic men were enrolled in the study. A detailed history was taken for each man, followed by general and genital examinations. Y chromosome microdeletions were detected by multiplex polymerase chain reaction (PCR) using 11 gene-specific primers that covered all three regions of the azoospermic factor (AZFa, AZFb and AZFc). Fifty men with normal semen analysis were also studied. Karyotyping was done with the standard G- and Q-banding. Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and testosterone were measured by electrochemiluminescence immunoassays (ECLIA). **Results:** Azoospermia and oligozoospermia could be explained by previous orchitis in 22.3%, former bilateral cryptorchidism in 19.2%, abnormal karyotypes in 4.6% and Y chromosome microdeletions in 3.8% of the subjects. The most frequent deletions were in the AZFc region (50%), followed by AZFb (33%) and AZFbc (17%). No significant difference was detected in hormonal profiles of infertile men, with or without microdeletions. **Conclusion:** The frequencies of Y chromosome microdeletions and cytogenetic abnormalities in oligozoospermic and azoospermic Thai men are comparable with similarly infertile men from other Asian and Western countries. *(Asian J Androl 2007 Jan; 9: 68–75)*

Keywords: azoospermia factor; azoospermia; male infertility; oligozoospermia; Y chromosome microdeletions

1 Introduction

Men with a defect in sperm production represent 40–50% of all infertile men. However, the origin of reduced male fertility is still unknown in approximately 30% of cases. Azoospermia and severe oligozoospermia can be associated with many conditions such as sperm duct obstruction, cryptorchidism, endocrine disorders, infection, chromosome abnormalities and microdeletions of the Y chromosome[1, 2].

The incidence of cytogenetic abnormality has been estimated at 5.8% in infertile men and only 0.5% in the

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normal population [3]. Y chromosome microdeletions have been implicated in the pathogenesis of spermatogenic failure because of the loss of genes controlling spermatogenesis [2, 4]. These genes are located on Yq within the region termed azoospermia factor (AZF). AZF has been further subdivided into three non-overlapping regions: AZFa, AZFb and AZFc [2, 5].

Recently, it has been made possible for men with a cytogenetic abnormality or microdeletions on the Y chromosome to father children by using intracytoplasmic sperm injection (ICSI) treatment. However, there is a chance that an abnormality will be transmitted to the male offspring of these men, causing infertility in future generations [4].

The main purpose of this study is to investigate the possible causes of azoospermia and oligozoospermia among infertile Thai men. The prevalence and types of both Y chromosome microdeletions and cytogenetic abnormalities were analyzed using gene-based multiplex polymerase chain reaction (PCR) and standard cytogenetic methods.

2 Materials and methods

The Ethics Committee of the Faculty of Medicine at Chiang Mai University approved this study. We explained the purposes and the scope of the study to all eligible subjects and invited them to participate. Informed written consent was obtained from each participant.

2.1 Subjects

Couples who failed to conceive after 1 year of unprotected sexual intercourse were defined as being infertile. The male partners in such couples, who attended the Infertile Clinic at Maharaj Nakorn Chiang Mai University Hospital, were recruited into the study. They were asked to complete a comprehensive questionnaire relating to their medical and surgical history (such as cryptorchidism in childhood, genital infections, trauma and operations), lifestyle habits (such as smoking, alcohol and drug use), and exposure to gonadotoxins (such as radiation therapy and drugs used for cancer chemotherapy). Two physicians (either Dr Teraporn Vutyavanich or Dr Supachai Sirisukkasem) performed a general physical and genital examination. Testicular number, location and volume were recorded, followed by palpation of the epididymis and the scrotal part of the vas deferens. Men suspected of having vas deferens obstruction by palpation and those with previous vasectomy were excluded from the study. Examination for the presence of varicocele was carried out by clinical examination only.

A complete semen analysis was performed in all cases according to the World Health Organization Guidelines [6]. Semen samples were obtained after a 2- to 7-day period of sexual abstinence. At least two abnormal semen analyses had to be presented before a diagnosis of oligozoospermia could be made. Azoospermia was verified in at least two ejaculates by pellet analysis after semen centrifugation (1 000 × *g* for 20 min). In addition, blood samples were obtained for DNA extraction, chromosome analysis and hormonal evaluations.

2.2 Hormonal evaluations

Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and testosterone were measured by electrochemiluminescence immunoassays (ECLIA) using Roche Elecsys 1010 (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Normal reference ranges for men were: FSH 1.5–12.4 IU/L, LH 1.7–8.6 IU/L, PRL 4.1–18.4 ng/mL and testosterone 2.8–8.0 ng/mL. The intra- and inter-assay coefficients of variation (CV) were, respectively: 1.4–2.0% and 2.9–5.3% for FSH; 0.8–1.8% and 1.9–5.2% for LH; 2.1–3.6% and 4.9–5.8% for PRL; and 0.9–4.6% and 1.6–7.4% for testosterone.

2.3 Cytogenetic evaluations

Chromosome analyses were performed in the Genetic Division, Department of Anatomy, Faculty of Medicine, Chiang Mai University. Briefly, cultures of peripheral blood lymphocytes were treated with 0.1 μg/mL of colcemid (Seromed, Berlin, Germany) after a 72-h incubation period. The prometaphase chromosomes were spread and stained using standard G- and Q-banding techniques. At least 20 metaphases per subject were analyzed.

2.4 Molecular genetic evaluations

Four multiplex PCR, consisting of 11 gene-specific primers, were performed to investigate Y chromosome microdeletions. They covered all three regions of the AZF: AZFa (*DFFRY* and *DBY*); AZFb (*SMCY, EIF1AY, RBM1* and *PRY*) and AZFc (*TTY2*, *DAZ* [*sY283*], *DAZ* [*sY277*], *CDY* and *BPY2*). The *SRY* (*sY14*) gene was used as an internal control to test the presence of the Y chromosome. Most of the primers used in our study

have been described in previous studies [7, 8], but some were newly designed or adapted to optimize our multiplex PCR condition. Primer sequences are shown in Table 1.

Patient genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) by using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) before being analyzed by multiplex PCR. The PCR was carried out in a 25-µL reaction volume containing: 200 ng genomic DNA, 1.5 mmol/L $MgCl_2$, 200 µmol/L dNTP, 0.1–0.5 µmol/L of each primer, PCR buffer with adjuvants (Q-solution; Qiagen, Hilden, Germany) and 1 U HotStarTaq DNA polymerase (Qiagen, Hilden, Germany). Thermocycling (Px2 Thermal Cycler; Thermo Electron Corporation, Bremen, Germany) for multiplex PCR set No. 1 was carried out for 15 min at 95ºC for initial denaturation, followed by 40 cycles of denaturation at 94ºC for 1 min, annealing at 57ºC for 1 min, extension at 72ºC for 1.5 min and a final extension at 72ºC for 10 min. Multiplex PCR sets No. 2, No. 3 and No. 4 were performed to-

Table 1. The sequences, gene location and product sizes of 12 primers in 4 multiplex PCRs in this study. The *SRY* (*sY14*) gene was used as an internal control to test the presence of the Y chromosome. AZF, azoospermia factor.

Multiplex PCR set No.	Genes product	Region	Left primer	Right primer	Primer concentration (µmol/L)	Size (bp)
1	*sY14(SRY)*[7]	Yp	GAA TAT TCC CGC TCT CCG GA	GCT GGT GCT CCA TTC TTG AGT	0.2	470
	SMCY [7]	AZFb	CCT CCA GAC CTG GAC AGA AT	TGT GGT CTG TGG AAG GTG TCA	0.1	362
	RBM1[7]	AZFb	ATG CAC TTC AGA GAT ACG GGA	CTC TCT CCA CAA AAC CAA CAT G	0.4	800
	EIF1AY [7]	AZFb	CTC TGT AGC CAG CCT CTT CTG	GAC TCC TTT CTG GCG GTT AC	0.1	84
2	*sY14(SRY)*[7]	Yp	GAA TAT TCC CGC TCT CCG GA	GCT GGT GCT CCA TTC TTG AGT	0.2	470
	DBY	AZFa	ATC GAC AAA GTA GTG GTT CCA G	AGA TTC AGT TGC CCC ACC AG	0.1	689
	DAZ (sY283)	AZFc	ACT TGA TGC CTC TTG ACA CTG AT	GTT ATT TGA AAA GCT ACA CGG G	0.1	314
	PRY	AZFb	GAG CAC ACC ACA CCA GAA ACA	CTC AGA CTG ACC TCG GAC TGT	0.1	80
3	*sY14(SRY)*[7]	Yp	GAA TAT TCC CGC TCT CCG GA	GCT GGT GCT CCA TTC TTG AGT	0.2	470
	DFFRY	AZFa	ATG TGG ACT ATA ATT TCT TCC CTT	CTG TCG TTC CCT CCT ACT GG	0.4	130
	CDY1	AZFc	TGG GCG AAA GCT GAC AGC A	TTG GGT GAA AGT TCC AGT CAA	0.2	79
4	*sY14(SRY)*[7]	Yp	GAA TAT TCC CGC TCT CCG GA	GCT GGT GCT CCA TTC TTG AGT	0.2	470
	TTY2	AZFb	GAC AAC TCT GAC AGC CAG GG	TCA GAA CTC CCA AAC AGG C	0.3	87
	BPY2 [7]	AZFc	GGG ATT ATC ACA TAT TGC GG	ATG ATA GTC GCG TCA GCT GG	0.2	370
	DAZ (sY277)[7]	AZFc	GGG TTT TGC CTG CAT ACG TAA TTA	CCT AAA AGC AAT TCT AAA CCT CCA G	0.2	312

gether under the same PCR conditions as follows: initial denaturation at 95ºC for 15 min; followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 62ºC for 1 min, extension at 72ºC for 1.5 min and a final extension at 72ºC for 10 min.

PCR products were separated on 2.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized using gel documentation system. In each multiplex PCR assay, samples from two normal fertile men, without Y chromosome microdeletions, were used as normal controls. Two samples from healthy women and double distilled water served as negative controls. Fifty healthy men with normal semen analysis were also studied for Y chromosome microdeletions. A sample was considered to contain 'no-deletion' for a given microdeletion when the PCR product of the expected size was present. It was considered a 'deletion' if a product of the expected size was not present after three successive PCR reactions.

2.5 Statistical analysis

Statistical analysis was carried out by the Statistical Package for Social Science for Windows, version 11.0 (SPSS; Chicago, IL, USA). The unpaired *t*-test, Mann–Whitney *U*-test and Chi-squared test were used as appropriate. $P < 0.05$ was considered significantly different.

3 Results

From June 2003 to November 2005, 130 infertile Thai men were enrolled in the study. Fifty had azoospermia (where no sperm was found in the ejaculate even after centrifugation) and 80 had oligozoospermia (54 and 26 with a sperm count of 1–5 million or >5–10 million spermatozoa/mL, respectively). The ages (mean ± SD) of infertile men and normal controls were 34.6 ± 6.3 years (range 22–52 years) and 32.3 ± 5.0 years (range 23–42 years), respectively. There was no significant difference in the mean ages of men with oligozoospermia and azoospermia (34.2 ± 5.9 *vs.* 35.9 ± 6.5 years, respectively). The average duration of infertility was 4.7 years (range 1–22 years). There was no significant difference in the duration of infertility in those who had oligozoospermia (4.6 ± 4.2 years) and azoospermia (5.0 ± 4.2 years). Significantly more azoospermic men (49/50 or 98%) had primary infertility compared to oligozoospermic men (60/80 or 75%; $P < 0.001$). Most of the oligozoospermic and azoospermic men in this study were employees (27.7%), civil servants (22.3%), businessmen (21.5%) and farmers (10.8%). None reported previous exposure to gonadotoxins, such as radiation treatment or cancer chemotherapy.

3.1 Clinical findings

Sixteen of 50 azoospermic men (32%) and 23 of 80 oligozoospermic men (28.8%) had a history of postpubertal mumps. However, only 10 (10/50 or 20%) of the azoospermic men and 19 of the oligozoospermic men (19/80 or 23.8%) had a previous history of orchitis. Of the 10 azoospermic men with a history of orchitis, four had postpubertal mump orchitis (40%), and two had orchitis from severe testicular trauma (20%). Four were related to sexually transmitted diseases (40%). Six of the 19 oligozoospermic men (31.6%) with a history of orchitis had postpubertal mump orchitis and one had orchitis from severe testicular trauma (5.3%). Twelve (63.2%) were related to sexually transmitted diseases (STD) (Table 2).

Unilateral cryptorchidism was present in two (2/50 or 4%) and 1 case (1/80 or 1.25%) of azoospermic and oligozoospermic men, respectively. Bilateral cryptorchidism was detected in seven (7/50 or 14%) and 18 cases (18/80 or 22.5%) of azoospermic and oligozoospermic men, respectively (Table 2). Varicocele was present in 4% (2/50) of azoospermic and 7.5% (6/80) of oligozoospermic men.

The prevalence of past and present smoking was 40% (20/50) in the azoospermic men, 32.5% (26/80) in the oligozoospermic men, and only 10% (5/50) in men with normal semen analysis. Only one male in the oligozoospermic group reported the use of more than 20 cigarettes per

Table 2. Clinical and laboratory findings in 50 azoospermic and 80 oligospermic men. STD, sexually transmitted diseases; * each patient also had unilateral cryptorchidism (total = 3 cases); ** one patient had single *PRY* gene deletion (partial AZFb deletion); # this patient, with *PRY* gene deletion, also had STD-related orchitis.

	Azoospermic group	Oligozoospermic group
Orchitis	10	19
Mump orchitis	4*	6*
STD-related	4*	12**
Testicular trauma	2	1
Bilateral cryptorchidism	7	18
Abnormal karyotype	5	1
AZF gene deletion	5	1#

day. Ten (10/50 or 20%) of the azoospermic men and 16 (16/80 or 20%) of the oligozoospermic men reported regular use of a mild to moderate amount of alcohol.

3.2 Cytogenetic analysis

Cytogenetic abnormalities were found in six out of 130 infertile men, corresponding to a frequency of 4.6%. Two of them had translocations involving chromosomes 7 and 14 or 16 (46,XY, t[7;14] and 46,XY, t[7;16]). Four cases had 47,XXY karyotype (Klinefelter's syndrome). Five cases had azoospermia and one severe oligozoospermia, corresponding to 10% (5/50) and 1.25% (1/80) frequencies of cytogenetic abnormalities among azoospermic and oligozoospermic men, respectively. The karyotypes and sperm count of these men are summarized in Table 3.

3.3 Hormone analysis

The levels of FSH and LH in azoospermic men (14.6 ± 11.0 and 8.9 ± 6.0 IU /L, respectively) were significantly higher than those in the oligozoospermic men (7.9 ± 5.1 and 6.5 ± 3.8, respectively, $P = 0.001$). However, the levels of PRL (17.8 ± 9.4 *vs.* 18.9 ± 11.5 ng/mL; $P = 0.481$) and testosterone (3.9 ± 1.7 *vs.* 4.5 ± 2.6 ng/mL; $P = 0.757$) were not significantly different. There was no significant difference in the mean levels of PRL, FSH, LH and testosterone in azoospermic and oligozoospermic men, with ($n = 6$) or without Y chromosome microdeletions ($n = 124$; $P = 0.187$–0.766; Table 4).

3.4 Microdeletion analysis

Y chromosome microdeletion was not detected in any of the 50 men with normal semen analysis. Six of 130 DNA samples from infertile men (4.6%) showed microdeletions of one or more genes on the Y chromosome. Five (No. 36, 45, 67, 91 and 116) were azoospermic (5/50, 10%) and the remaining case (No. 96) was oligozoospermic (1/80, 1.25%) (Figure 1). This case (No. 96) had a single *PRY* deletion (partial AZFb region) and also a history of orchitis from a sexually transmitted disease. The other five men had deletions involving more than three genes and they did not have a history of orchitis. Case No. 36, 45 and 116 had deletions of *DAZ* (*sY277*), *DAZ* (*sY283*) and *BPY2*, which were located in the AZFc region. Case No. 91 had deletions of *SMCY, EIF1AY, RBM1* and *PRY* genes, which were located in the AZFb

Table 3. Chromosome abnormalities in azoospermic and oligozoospermic men.

Case No.	Karyotype	Sperm count (million/mL)
14	46,XY, t(7;14)	0
16	46,XY, t(7;16)	0
25	47,XXY	0
35	47,XXY	0
52	47,XXY	0
114	47,XXY	3.2

Table 4. Hormone profiles in azoospermic and oligozoospermic men with Y chromosome microdeletions and comparison of men with and without AZF microdeletions. Values are mean ± SD, if applicable. FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin.

Case No.	Age (year)	PRL (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)
36 (azoospermia)	38	9.1	6.8	4.7	5.6
45 (azoospermia)	31	12.5	20.4	16.5	2.6
67 (azoospermia)	33	13.8	17.6	9.5	2.5
91 (azoospermia)	28	22.9	6.6	6.7	4.2
96 (oligozoospermia)	35	16.5	12.3	7.2	4.5
116 (azoospermia)	39	18.1	13.0	10.1	5.2
Azoospermia ($n = 50$)					
with AZF deletion ($n = 5$)	33.8 ± 4.6	15.3 ± 5.4	12.9 ± 6.2	9.5 ± 4.5	3.8 ± 1.3
without AZF deletion ($n = 45$)	34.2 ± 5.9	18.1 ± 9.7	14.8 ± 11.5	8.9 ± 6.2	3.9 ± 1.8
Oligozoospermia ($n = 80$)					
with AZF deletion ($n = 1$)	35	16.5	12.3	7.2	4.5
without AZF deletion ($n = 79$)	35.9 ± 6.5	18.9 ± 11.5	7.9 ± 5.1	6.5 ± 3.7	4.5 ± 2.7

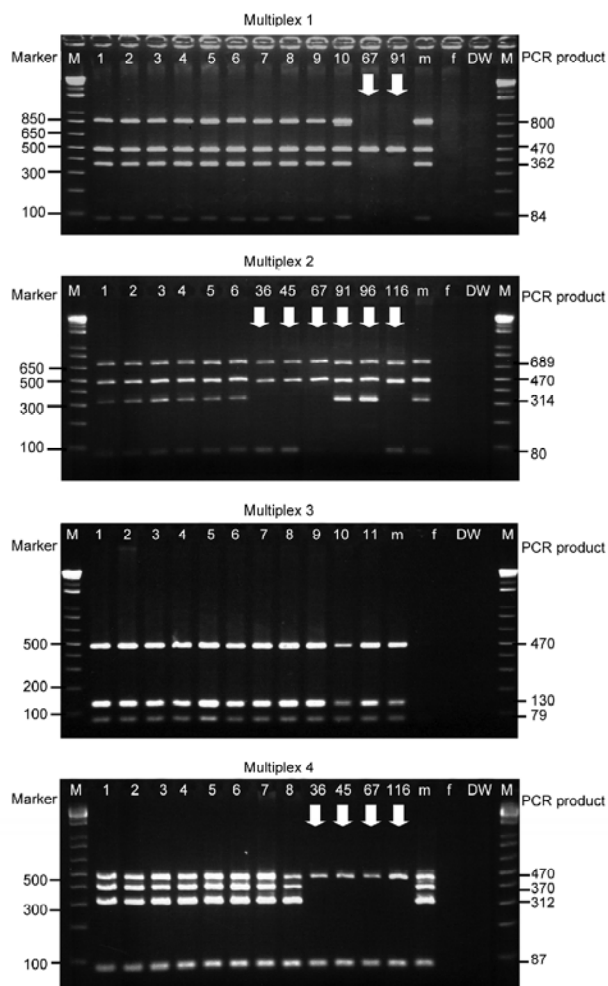

Figure 1. Products of multiplex PCRs in men with and without Y chromosome microdeletions. In multiplex PCR set No. 1, case No. 67 and 91 had *RBM1, SMCY* and *EIF1AY* microdeletions. In multiplex PCR set No. 2, case No. 36, 45 and 116 had *DAZ (sY283)* microdeletions, case No. 67 had *DAZ (sY283)* and *PRY* microdeletions, case No. 91 and 96 had *PRY* microdeletions. None had microdeletions in multiplex PCR set No. 3. In multiplex PCR set No. 4, case No. 36, 45, 67 and 116 had *BPY2* and *DAZ (sY277)* microdeletions. M, DNA marker; m, normal male control; f, female control; DW, double-distilled water.

Region	Genes	Case No.					
		36	45	67	91	96	116
Yp	*SRY*	■	■	■	■	■	■
AZFa	*DFFRY*	■	■	■	■	■	■
	DBY	■	■	■	■	■	■
AZFb	*SMCY*	■	■	□	□	■	■
	EIF1AY	■	■	□	□	■	■
	RBM1	■	■	□	□	■	■
	PRY	■	■	□	□	□	■
	TTY2	■	■	■	■	■	■
AZFc	*DAZ (sY277)*	□	□	□	■	■	□
	DAZ (sY283)	□	□	□	■	■	□
	BPY2	□	□	□	■	■	□
	CDY1	■	■	■	■	■	■
Phenotype		A	A	A	A	O	A

Figure 2. Schematic diagram of Y chromosome microdeletions in six infertile men. Solid boxes indicated the presence of genes and open boxes indicated gene deletions. Phenotypes are indicated as A for azoospermia and O for oligozoospermia, respectively.

region. Case No. 67 had a wide range of deletions involving *SMCY, EIF1AY, RBM1* and *PRY* genes in the AZFb region and *DAZ*(*sY277*), *DAZ*(*sY283*) and *BPY2* gene in the AZFc region. In this study, we found no men with microdeletions of the AZFa region (Figure 2).

3.5 Histological findings

Testicular histology was available in two of the six men with microdeletions. Case No. 36 (AZFc microdeletions) had few mature spermatozoa in a testicular biopsy. Case No. 91, with extensive AZFb microdeletions, had maturation arrest with only few spermatids, but no mature spermatozoa.

4 Discussion

In our present study, azoospermia and oligozoospermia could be explained by previous orchitis in 22.3%, former bilateral cryptorchidism in 19.2%, abnormal karyotypes in 4.6% and Y chromosome microdeletions in 3.8% of the subjects. The cause(s) of azoospermia and oligozoospermia in the other 50.1% was unknown. It has become more evident in recent years that a significant proportion (up to 35%) of such men may have mutations and polymorphism of the androgen receptor (*AR*) gene. The increase in length of a trinucleotide repeat (CAG) tract in the transactivation domain of the AR has been reported to be associated with an increased risk of defective spermatogenesis, especially in Asian popu-

lations [9]. As we did not study the AR, the conclusion as to whether defective AR played a significant role in our cases remains speculative. It is also remarkable that significantly more men with azoospermia and oligozoospermia reported past or present use of cigarettes compared to men with normal semen analysis. Further study should be done to ascertain whether cigarette smoking is gonadotoxic to male germ cells.

The frequency of an abnormal karyotype in this study was within the previously reported range of 2.2–14.3% for infertile men [3,4]. The most common abnormality was Klinefelter's syndrome (4/6 or 66%), which was in agreement with a previous study by Foresta *et al.* [10].

The levels of FSH, LH, PRL and testosterone were undistinguishable in oligozoospermic and azoospermic men, with and without microdeletions. This was in agreement with a large study by Tomasi *et al.* [11], who found no difference in the function of pituitary-testicular axis in men with and without Y-chromosome microdeletions.

None of the 28 cases with a history of cryptorchidism (25 bilateral and three unilateral cryptorchidism) had Y chromosome microdeletions. This was consistent with the study by Fagerli *et al.* [12], who found no Y chromosome microdeletions (using 17 different STS markers) in any of the 38 men with cryptorchidism in their study. However, deletions have been reported by Simoni *et al.* [13] as a chance association in men with occasional varicocele or a history of maldescended testis.

There is a wide variation in the reported frequencies of Y-chromosome microdeletions: from 4.25% [5] to 23% [14] and 0.1% [15] to 8.5% [8] in azoospermic and oligozoospermic men, respectively. The reasons for this discrepancy might be small sample sizes in many studies, different inclusion criteria, the type and number of primers used (5–118, mean 24.5 primers) and ethnic variations [1, 5, 16]. Based on previous reports [1, 4, 7, 8, 14–19], we estimated the average frequency of microdeletions at 12.5% and 4.7% in azoospermic and oligozoospermic men, respectively. In order to be 90% confident that the frequencies of microdeletions were between 4.5% to 20.5% (average 12.5%) in azoospermic men and 0.7% to 8.7% (average 4.7%) in oligozoospermic men, we needed to screen at least 47 azoospermic and 76 oligozoospermic men.

Gene-based primers were used in this study because STS primers were not specific for the genes that mapped the AZF intervals. The results of this study should, therefore, be more reliable and more informative than previous studies that used STS markers. We included primers that amplified the only two known genes in the AZFa region, four representative genes out of seven functional genes in the AZFb region and four of eight functional genes in the AZFc region [2]. These primers were selected based on a previous report that showed deletions in Asian populations [7]. It is possible that we underestimated the prevalence of Y chromosome microdeletions because some genes known and others still unknown were not screened. Also, as we excluded men with obstructive azoospermia by history and clinical examination only, some such cases could have been inadvertently included, thus lowering the frequency of microdeletions in our study. Nevertheless, the frequencies of microdeletions in our azoospermic (10.00%) and oligozoospermic men (1.25%) were still within the range reported by many recent studies of Asian populations [1, 7, 8, 17]

Because testicular histology was available in only two cases, a precise genotype/phenotype correlation was not possible in this study. However, men with AZFc microdeletions are usually described to have a low number of mature spermatozoa in their testicular tissues [1, 2]; this was also the case (No. 36) in our study. Case No. 91, with AZFb microdeletions involving *SMCY*, *EIF1AY*, *RBM1* and *PRY,* had spermatogenic arrest, which was compatible with previous reports in men with extensive AZFb microdeletions [2].

Four of our six men had the presence of Y chromosome microdeletions confined to the AZFc region in common, specifically in the DAZ locus. Our study confirmed earlier reports that azoospermic men had a higher frequency of microdeletions than oligozoospermic men, and that men with deletions in the AZFc region were the leading group [2, 7, 8, 16]. One particular case, with an average sperm count of 8.4 million/mL, had a *PRY* gene microdeletion. It is still doubtful whether this subject's condition was caused by gene deletion or previous orchitis. A recent study by Stouffs *et al.* [20] suggested that the *PRY* gene probably played no role in spermatogenesis, but that it might be involved in apoptosis of spermatids and spermatozoa.

In conclusion, the pattern and prevalence of Y chromosome microdeletions and chromosomal abnormalities in oligozoospermic and azoospermic Thai men were comparable with infertile men from other Asian and Western countries. Testing for Y microdeletions and chromosomal abnormalities was useful in azoospermic men with

no apparent abnormalities.

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