

SUBMICROSCOPIC DELETIONS OF THE Y CHROMOSOME ARE NOT LIMITED TO AZOOSPERMIC MEN, BUT ARE ALSO DETECTED IN INFERTILE MEN WITH IDIOPATHIC OLIGOZOOSPERMIA

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ABSTRACT

It is now agreed that 10-25% of infertile men with azoospermia have submicroscopic deletions of the Y chromosome long arm (Yq), consistent with the proposed location of the azoospermia locus (AZF) in Yq 11.23. However, it is not known whether Yq microdeletions are unique to men with azoospermia or whether they are also observed in infertile men with less severe defects of spermatogenesis (oligozoospermia). The objective of this study was to determine the prevalence of Yq deletions in infertile men with idiopathic oligozoospermia.

DNA was extracted from blood lymphocytes of 45 oligozoospermic men (sperm densities <20 million/mL) in whom known causes of infertility had been excluded. All subjects were typed for the 27 Y-specific STSs that have been mapped to deletion interval 6. An STS was considered negative if no PCR product was observed in 3 reactions, in which a fertile male gave a specific PCR product and a normal female DNA did not. Whenever sufficient DNA was available, deletions detected by PCR were verified by Southern hybridization.

Of the 45 oligozoospermic men, 4 (9%) had deletions of one or more STSs. These deletions were verified by Southern hybridization. All 4 deletions were located in distal interval 6 (6C and 6D) and included the DAZ (Deleted in Azoospermia) gene, a Y-specific gene that has been proposed as a candidate for male infertility. All four patients had sperm densities of less than 1 million/mL and three out of these 4 patients had mean testis volume of less than 15 mL. Two of the infertile men with Y deletions had a testicular biopsy; testicular histology in both of these patients was consistent with germ cell arrest, spermatocyte stage.

Yq microdeletions are not unique to infertile men with azoospermia; but are observed

also in infertile men with oligozoospermia. Taken together with previously reported studies on azoospermic men, these results indicate that Yq deletions are predominantly observed in, but are not limited to, infertile men with relatively severe defects of spermatogenesis.

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INTRODUCTION

Spermatogenesis is a complex process comprising of mitotic and meiotic divisions, and terminal differentiation of haploid, round spermatids into elongated, motile spermatozoa. The precise molecular mechanisms that regulate germ cell development remain largely unknown. Therefore, it is not surprising that the pathogenic defects that result in failure of the spermatogenic process and consequently infertility/subfertility in the male partner are unclear in the majority of infertile couples. Indeed after all the readily recognizable causes of infertility have been excluded, idiopathic oligo/azoospermia emerges as the largest single category, accounting for one-third to one-half of infertile men.¹⁻⁵

Of the various candidate genes considered important in the pathogenesis of male infertility, genes on the long arm of the Y chromosome appear to be most promising at the present time.⁶ Previous studies have shown that men with macroscopic deletions of the long arm of the Y chromosome (Yq) are azoospermic.⁷⁻¹⁶ Based on observations that men with macroscopic deletions of the long arm of the Y chromosome (Yq) are azoospermic, Tiepolo and Zuffardi were the first to propose the location of an azoospermia factor, AZF, loci on the long arm of the Y chromosome.¹⁷ Vergnaud et al., based on further deletion mapping studies, narrowed the location of the AZF locus to deletion interval 6 (also referred to as Yq 11.23).¹⁸ Although clinical experience and published data indicate that large deletions of the Y chromosome that are detectable on karyotyping are uncommon in infertile men, several groups including our own have reported the existence of submicroscopic deletions of Yq region (microdeletions), not large enough to be detectable by karyotyping, in a subgroup of infertile men, currently classified as having idiopathic azoospermia.¹⁹⁻²⁷ There is consensus that in approx. 10-20% of infertile men with idiopathic azoospermia, infertility is associated with Yq microdeletions.²⁷⁻²⁸ These microdeletions can be detected by PCR-based sequence-tagged site mapping or by Southern hybridization.²⁹ We recently published the validation of a PCR-based tagged mapping strategy to detect these microdeletions in infertile men.²⁷ Using a deletion mapping approach, two Y-specific gene families have been cloned and proposed as candidates for the putative azoospermia factor, namely the RBM (RNA Binding Motif containing gene), and the Deleted in Azoospermia, DAZ, gene family.^{22,27,28,30-33} The physiologic

function and the role of these gene families in the pathogenesis of male infertility remain unclear at the present time.^{22,34}

Previous studies had left several questions unanswered. Most of the published studies have focused on azoospermic men and it is not clear whether Yq deletions are limited to men with azoospermia or are also present in infertile men with less severe defects of spermatogenesis. Therefore, the first objective of this study was to determine the prevalence of Yq deletions in a well-characterized subset of infertile men with idiopathic oligozoospermia. A second objective was to determine the prevalence of DAZ deletions in individuals with Yq deletions. We were interested in finding out whether deletions of the DAZ gene(s) were present in all oligozoospermic men with Y deletions or whether additional Y-specific genes were implicated in other subsets of male infertility. We used a previously validated STS-mapping method to detect the Yq deletions in 45 infertile men with oligozoospermia. Whenever sufficient DNA was available, deletions detected by PCR were verified by Southern hybridization.

MATERIAL AND METHODS

Subjects

Blood samples were collected from infertile men following protocols approved by the Monash Medical Center Human Ethics Committee, and the Institutional Review Boards of the Harbor-UCLA Medical Center, Charles R. Drew University of Medicine and Science, and Karolinska Institute. Peripheral blood or DNA samples from 45 infertile men, 24-41 years of age, who were oligozoospermic, were available for this study. Each individual had undergone evaluation for infertility, and known causes of infertility or hypogonadism had been excluded. Patients with clinical phenotype consistent with Klinefelter's syndrome (very small testes, eunuchoid proportions, gynecomastia and hypergonadotropic hypogonadism) were also excluded. Isolation of DNA from blood samples was performed by the Mini-Prep (Quiagen Inc., Chatsworth, CA) procedure³⁵ or a modified phenol chloroform method.³⁶ Serum FSH, LH, and testosterone were measured at the Monash University by specific radioimmunoassays. In Los Angeles and Stockholm, serum testosterone levels were measured by an iodinated immunoassay, and serum and FSH levels were measured by an iodinated immunoassay, and by specific and sensitive fluoroimmunoassays

STS MAP OF OLIGOZOOSPERMIC INDIVIDUALS

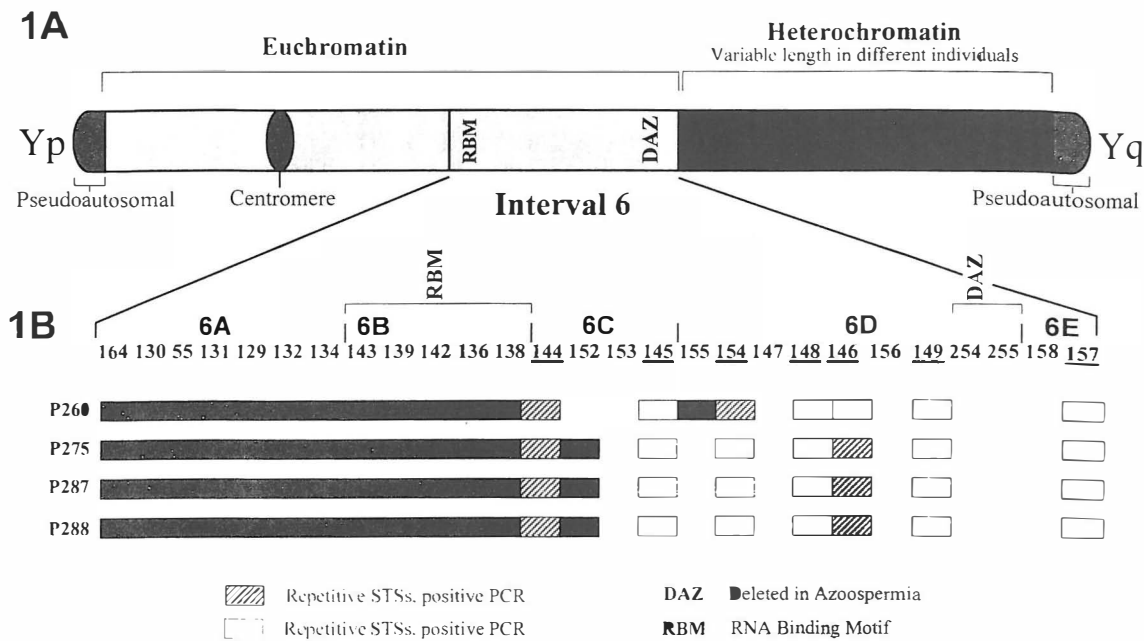


Fig. 1. A list of sequence-tagged sites corresponding to interval 6 of the Y-chromosome. A. Drawing of the Y-chromosome. B. A list of the STSs used for typing patients is shown across the top; a list of 4 individuals bearing deletions in different locations of interval 6 is shown below. STSs that are underlined have been confirmed by Southern analysis. Solid bars represent the presence of STS.

ing reagents obtained from Delphia-Wallace Corporation (Turko, Finland).³⁷ Based on the normal ranges established for these hormones in the Los Angeles³⁷ and Monash Laboratories, 57% of the subjects had elevated serum FSH levels, 3% had elevated LH levels and 30% had low testosterone levels. Eleven patients had chromosomal studies and normal 46,XY karyotypes. Testicular biopsy information was available in 13 subjects. Six patients had germ cell arrest, one had a Sertoli Cell Only (SCO) phenotype, 4 had hypospermatogenesis, one had SCO and hypospermatogenesis, and one biopsy was reported as "normal". No biopsy was done solely for the purpose of this research.

To ascertain if microdeletions were associated only with idiopathic oligozoospermia, we also typed DNA samples from 8 infertile men with oligozoospermia associated with maldescent of the testes.

STSs

Twenty five STSs, used to map the Yq interval 6, were selected from the STS map of Vollrath et al.³⁸ based on the following criteria: first, these STSs were Y-specific and did not have homologues on the X chromosome or autosomes; second, these STSs had been localized to Yq deletion interval 6.^{29,38} Two additional STSs, sY254 and sY255, corresponding to DAZ sequences, were also examined. The STSs

used in this study are listed in Figure 1. The PCR primer pairs for these 27 STSs have been previously published.³⁸

We have previously demonstrated that Y deletions occur extremely infrequently, if at all, in normal fertile men.

PCR conditions

Each patient sample was typed for all 27 STSs using polymerase chain reaction (PCR). For each PCR, one μ L (100 ng to 200 ng) of patient DNA (extracted from peripheral blood cells) was used. A mix containing 8.6 μ L water, 2 μ L (25 μ M) $MgCl_2$, 2 μ L 10X buffer, 1 μ L (10 mM) of each dNTP, 1 μ L (1 mM) each of the forward and reverse primers, and 2 units of Amp Taq DNA polymerase (Perkin-Elmer Cetus Corporation, Emeryville, CA) was added to the patient's DNA. The PCR reaction was carried out at different annealing temperatures depending on the specificity of the STS primers. An annealing temperature of 55°C was used for all reactions except for STSs 164, 139, 148, 154, 155, 156, 158, and 157, which had variable annealing temperatures. Two μ L of tracking dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol) was added to each sample before running it on a 3% agarose/TAE gel. For STS products longer than 400 bp, 1% gel was used instead.

Criteria used for defining microdeletions

Each set of PCR reactions included at least two nor-

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Table I. Clinical phenotype of oligozoospermic men with Yq deletions.

Number	Age	Sperm density million/mL	Testis volume		Hormone levels			Karyotype	Histology
			Rt.	Lt.	T	LH	FSH		
1	31	<1	30	30			5.5		GCA
2	39	<1	8	8	9.9	4.9	7.2	46, XY	NA
3	29	<1	15	15	20.3	4	14	NA	GCA
4	30	<1	12	12	21.1	28	11	NA	NA

T, serum testosterone levels nmol/L; LH, serum luteinizing hormone U/L; FSH, serum follicle-stimulating hormone, U/L.

NA, not available; GCA, germ cell arrest at the primary spermatocyte stage.

Sperm densities are in million/mL.

LH, normal range is 2-12 IU/L at Monash and 2-13 IU/L in Los Angeles Laboratories; FSH, normal range is 1-9 IU/L at Monash and 1-11 IU/L in Los Angeles Laboratories; T (testosterone), normal range is 10-23 nmol/L at Monash and 7.3-30 nmol/L in Los Angeles Laboratories.

Testicular volumes measured by Pradeer orchidometer, refer to the volumes of right (Rt.) and left (Lt.) testes, respectively.

mal male (positive control) and a normal female control (negative control). A PCR reaction was considered satisfactory if the normal male DNA produced the PCR product of the specific size and the normal female did not. A patient sample was considered "positive" for an STS if it produced the PCR product of the expected size under the same condition, and negative if a product of the expected size was not obtained after 3 PCR attempts.

Verification of microdeletions by Southern analysis

All Y deletions detected by PCR were verified by Southern analysis. For the Southern analysis, 10 μ L of genomic DNA was digested with EcoRI, run on 0.7% agarose gel, transferred to nylon membrane and hybridized with 32p-labeled probe.³⁹ The PCR products for each STS were labeled and used as probe.²⁷

RESULTS

The validation of the STS mapping strategy has been previously published.

STS typing of infertile men

We studied 45 infertile men with oligozoospermia using this STS-based mapping strategy. Four patients (9%) failed to amplify one or more STS. Figure 1 lists the STSs that were found to be deleted in these 4 patients. All 4 of these deletions are distal (i.e. they are located in intervals 6C and 6D). These deletions overlap with the DAZ (deleted in azoospermia) gene region. Indeed, sY254 repressing DAZ sequences was deleted in all 4 of these patients. All of the deletions included multiple STSs.

Southern hybridization data

In each of the 4 patients with Yq deletions, the deletions

were verified by Southern hybridization, similar to our previous publication.²⁷

Phenotypes of men with oligozoospermia and Yq microdeletions

The characteristics of these men are shown in Table I. All the oligozoospermic men with microdeletions had sperm densities of less than 1 million/mL (Table I). In 2 of these patients, testicular histology was available; the biopsy appearance consisted of maturation arrest at the primary spermatocyte stage in both of these patients. Testicular volumes were less than 15 μ L in 3 of these 4 patients. Karyotype was available only in one patient with microdeletion; this patient had a normal 46, XY karyotype. Serum FSH levels were elevated in 2 patients.

Family studies

DNA was available from the father and brother of one oligozoospermic man with Y deletion. Southern analysis of these patients' genomic DNA with DAZcDNA probe revealed a Y deletion that included the DAZ gene. Examination of the father's genomic DNA reveals the presence of all the DAZ bands (Figure 2). These data indicate that in this patient, the deletion occurred *de novo* and was not inherited from his father.

DISCUSSION

Our data suggest significant (9%) prevalence of Yq microdeletions in infertile men with idiopathic oligozoospermia. This prevalence is similar to the 10-20% prevalence of Yq deletions previously reported in azoospermic men by others and us.^{27,28} However, it is notable that all 4 infertile patients with Y deletions had a sperm density of less than 1 million/mL. Taken together with the previous data on pre-

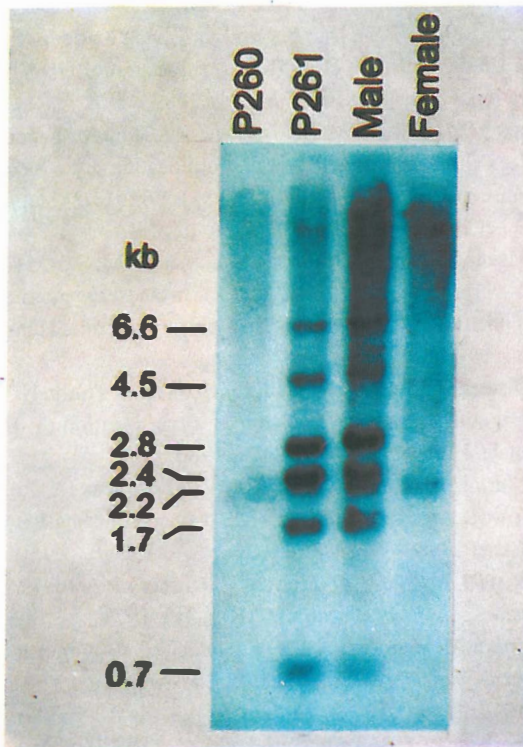


Fig. 2. This figure represents an example of Southern analysis using the DAZ probe, that was kindly provided by David Page, to screen patients for DAZ deletions. Patient P 260 is an oligozoospermic individual that has a deletion in DAZ gene, but his father (P 261) showed no detectable deletion in DAZ gene. Male and female controls are also shown.

dominantly azoospermic men, these results suggest that Yq deletions are observed mostly in infertile men with more severe defects of spermatogenesis (azoospermia or severe oligozoospermia). It is also remarkable that 3 out of 4 men with deletions in this study, and 6 of 7 azoospermic men with Y deletions (on whom data were available) in our previous study²⁷ had mean testis volumes of less than 15 mL.

Because all the deletions were in the distal region and only a small number of patients had microdeletions, we are unable to ascertain a correlation between the location or the size of the microdeletions and the clinical phenotype; this issue needs further examination in a larger sample of infertile men. Also, to-date only infertile men with azoospermia and oligozoospermia have been examined. Further studies are needed on larger samples of infertile men with other infertility phenotypes, such as morphology and motility defects and obstructive lesions to establish genotype-phenotype correlation.

The deletions in these subjects appear not to be contiguous. This observation has also been reported previously by others and us. Some of the intervening STSs represent repetitive sequences, and the PCR product may represent amplification from a different site. It is also possible that the order of these STSs may not be correct in the published

map. This conclusion is supported by known discrepancies between the deletion map and the YAC contiguous map in some STS location.²⁹ We did not have access to blood samples from the fathers or brothers of all the patients. However, DNA samples were available from the father and one brother of one individual with Yq deletion. In this patient, Southern analysis of the infertile son's DNA revealed a large-deletion resulting in deletion of the DAZ bands. On the other hand, the father and brother had all the expected bands (Figure 2). Thus the Y deletion in this patient must have occurred *de novo*, and was not inherited from his father, although vertical transmission from father to son has also been reported.⁴⁰

It is now agreed that genes on the Y chromosome play an important role in germ cell development. Growing bodies of data from several laboratories have demonstrated the association of Y microdeletions with azoospermia. The study reported in this manuscript adds oligozoospermia to the infertility phenotypes that are associated with Y deletions. While this manuscript was in preparation, Reijo et al. reported the occurrence of Y deletion in a group of oligozoospermic men.⁴¹ These data only demonstrate associations; a cause and effect relationship between Y deletions and infertility has not been established. Two Y-specific gene families have been cloned and been proposed as candidates for putative azoospermia factor.^{22,27,28,30-33} The RBM (RNA Binding Motif containing) gene family is a multiple copy gene family. Most of these copies are located in the proximal part of deletion interval 6. At least two members of this gene family are expressed uniquely in the testis. The genomic structure and organization of the RBM gene family has been recently published by Najmabadi et al. and reveals a 15 kb gene with 12 exons.³³ There is a high degree of homology between exons VI, VII, VII, IX and X and also between some of the introns within the same gene.⁴² The role of the RBM gene family as a candidate for the AZF has been challenged by several observations. First, large deletions of the RBM gene are infrequent in infertile men.³³ More importantly, RBM sequences have been shown to be present even in infertile men with Y deletions. These data do not exclude the formal possibility that point mutations, small deletions or rearrangement of the RBM gene may be present in some of the patients. Reijo et al. reported the cloning of a novel, RNA-binding protein gene, DAZ (deleted in azoospermia).³² Although this gene was originally reported to be a single-copy gene, additional copies have been identified in the distal region of Yq. The presence of the RNA binding motif suggests a role in RNA processing. However, its exact role in spermatogenesis remains to be determined. Deletions of the Y-chromosome that include the DAZ gene appear to be the most frequent deletions in infertile men with azoospermia and oligozoospermia. Although all the deletions described in this manuscript are in the DAZ region, microdeletions that do not overlap with the DAZ region have

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been reported by several groups.³³ These findings raise the possibility that gene(s) in Yq deletion interval 6 might be associated with subsets of male infertility. Whether there are other Y-specific genes in interval 6 or whether point mutations in *DAZ* or *RBM* genes are responsible for infertility remains to be seen. A recent case control study of subfertile men suggested that subfertility might be inherited as an autosomal recessive disorder.⁴³ Thus it is likely additional Y-specific and autosomal genes will be implicated in subsets of male infertility.²²

The occurrence of chromosomal deletions in infertile men with oligozoospermia has significant implications from the perspective of assisted reproductive technologies. Because intracytoplasmic sperm injection may allow partners of these oligozoospermic men with Yq deletions to achieve pregnancy, this genetic defect will be transmitted to the offspring. The expression of infertility in the offspring as a consequence of these therapeutic maneuvers will not become apparent for several decades. This raises issues of informed consent and ethical concerns. The substantial prevalence of microdeletions detected in this study suggests that infertile men with oligozoospermia who are undergoing intracytoplasmic sperm injection should be screened for Yq deletions.^{28,40} Considering the ease and accessibility of the PCR technology, the STS-based screening for microdeletions could become potentially available for screening in clinical cytogenetic laboratories. At the very minimum, infertile couples with azoospermia and/or oligozoospermia who are considering assisted reproductive technologies, particularly intracytoplasmic sperm injection, should be counseled about the potential for transmitting genetic disorders to the offspring.²⁸

REFERENCES

1. Bhasin S, De Krester DM, Baker HWG: Pathophysiology and natural history of male factor infertility. *J Clin Endocrinol* 79: 1525-1529, 1994.
2. Santen RJ, Swerdloff RS: Male Reproductive Dysfunction: Diagnosis and Management of Hypogonadism, Infertility and Impotence. New York: Marcel Dekker, pp. 342-372, 1986.
3. De Krester DM, Burger HGG, Fortune D, Hudson B, Long AR, Paulsen CA, et al: Hormonal, histological, and chromosomal studies in adult males with testicular disorders. *J Clin Endocrinol Metab* 35: 392-401, 1972.
4. Dublin L, Amelar RD: Etiologic factors in 1294 consecutive cases of male fertility. *Fertil Steril* 22: 469-474, 1971.
5. Howards SS: Treatment of male infertility. *New England Journal of Medicine* 332: 312-317, 1995.
6. Burgoyne PS: The role of the mammalian Y-chromosome in spermatogenesis. *Develop* 101(suppl): 133-141, 1987.
7. Andersson M, Page DC, Pettay D, Suert I, Turleau C, deGrouchy J, et al: Y; autosome translocations and mosaicism in etiology of 45, X maleness: assignment of fertility factor to distal Yq 11. *Hum Genet* 79: 2-7, 1988.
8. Andersson M, Page DC, Brown LG, Elving K, de la Chapelle A: Characterization of a (Y; 4) translocation of DNA hybridization. *Hum Genet* 78: 377-381, 1988.
9. Cantu JM, Diaz M, Moller M, Jimenez-Sainz M, Sandoval L, Vaca G, et al: Azoospermia and duplication 3qter as distinct consequences of a familial t(X; 3)(q26; q13.2). *Am J Med Genet* 20: 677-684, 1985.
10. Maeda T, Ohno M, Ishibashi A, Samejima M, Sasaki K: Ring Y-chromosome: 45, X/46, X, r(Y) chromosome mosaicism in a phenotypically normal male with azoospermia. *Hum Genet* 34: 99-102, 1976.
11. Disteche CM, Brown L, Salli J, Friedman C, Thulin HC, Hoar DI, et al: Molecular detection of a translocation (Y-15) in a male. *Hum Genet* 74: 372-377, 1986.
12. Martin MJ, Rodriguez T, Allre V, Abrisqueta JA, Rojo JM: Yq-deletion (a11.21) in a H-Y azoospermic male. *Clinical Genet* 28: 80-83, 1985.
13. Neu RI, Matthew MS, Barlow J, Gardner LI: A 46, X Yq-male azoospermia. *Fertil Steril* 24: 811-813, 1973.
14. Smith A: An infertile male with balanced Y; 19 translocation. Review of Y; autosome translocation. *Ann Genet* 22: 189-194, 1979.
15. Sadagawa I, Nakada T, Adachi Y, Kato T, Sawamura T, Ishigooka M, et al: Y; autosome translocation associated with azoospermia. *Scan J Urol Nephrol* 27: 285, 1993.
16. Guichaoua MR, Speed RM, Ruciani JM, Delafontaine D, Chandley AC: Infertility in human males with autosomal translocations II. Meiotic studies in three reciprocal rearrangements, one showing tertiary nonosomy in a 45-chromosome individual and his father. *Cytogenet Cell Genetic* 60: 96-101, 1992.
17. Tiepolo L, Zuffardi O: Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y-chromosome long arm. *Hum Genet* 34: 119-124, 1975.
18. Vergaud E, Page DC, Brown L, Rouyer F, Noel B, et al: Deletion map of the human Y-chromosome based on DNA hybridization. *Am J Hum Genet* 39: 109-124, 1986.
19. Chandey AC, Cooke HG: Human male fertility Y-linked genes and spermatogenesis. *Hum Mol Genet* 3: 1449-1452, 1994.
20. Kotecki M, Jaruzelska J, Skowronska M, Fichna P: Deletion mapping of interval 6 of the human Y-chromosome. *Hum Genet* 87: 234-236, 1994.
21. Caamurri L, Novelli G, Gennarelli M, Cantarelli M, Dallapiccolo G: Yq deletions and AZF locus: molecular analysis in two fetuses with non-familial homozygous Yq rearrangements. *Genet Counseling* 4: 223, 1993.
22. Bhasin S, Ma K, de Kretser DM: Y-chromosome microdeletions and male infertility. *Ann Med* 29: 261-263, 1997.
23. Marachio P, Tupler R, Daintte E, Cortinovi M, Tiepolo L: Molecular analysis of human Y, 11 translocation in an azoospermic male. *Cytogenet Cell Genetic* 65: 256-262, 1994.
24. Vogt P, Chandley AC, Hargreave TV, Keil R, Ma K, Sharkey A: Microdeletions in interval 6 of the Y-chromosome of male

- with idiopathic setility point disruption of AZF, a human spermatogenesis gene. *Hum Genet* 89: 491-496, 1992.
25. Nagafuchi S, Namiki M, Nakahor Y, Kondoh N, Okuyama A, Naagome Y: A minute deletion of Y-chromosome in men with azoospermia. *J Urol* 150: 1155-1157, 1993.
 26. Ma K, Sharkey A, Kirsh S, Vogt P, Keil R, Hargreave TB, et al: Towards the molecular localization of the AZF locus: mapping of microdeletions in azoospermic men within fourteen subintervals of interval 6 of human Y-chromosome. *Hum Mol Genet* 1: 29-33, 1992.
 27. Najmabadi H, Huang V, Yen P, Subbarao MN, Bhasin D, Banaag L, Naseeruddin S, De Krester DM, Baker HWG, McLachlan RI, Loveland KA, Bhasin S: Substantial prevalence of microdeletions of the Y-chromosome in infertile men with idiopathic azoospermia detected using a sequence-tagged site based mapping strategy. *J Clin Endocrinol Metab* 81: 1347-1352, 1996.
 28. Krausz C, Mc Elreavy K: Y chromosome and male infertility. *Front-Biosci* 15: 1-8, 1999.
 29. Foote S, Vollrath D, Hilton A, Page D: The human Y chromosome: overlapping DNA clones spanning the euchromatin region. *Science* 258: 60-66, 1992.
 30. Ma K, Inglis JD, Sharkey A, Bickmore WA, Hill RE, Prosser EJ, et al: A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell* 75: 1287-1295, 1993.
 31. Kim YJ, Baker BS: Isolation of PRM-type RNA binding protein genes and the analysis of their relatedness by using a numerical approach. *Am Soc for Microbiol* 13: 174-183, 1993.
 32. Reijo R, Lee TY, Salo P, et al: Diverse spermatogenetic defects in human caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet* 10: 383-392, 1995.
 33. Najmabadi H, Chai N, Kapali A, Subbarao M, Bhasin D, Woodhouse E, Yen P, Bhasin S: Genomic structure of Y-specific ribonucleic acid binding motif-containing gene: a putative candidate for a subset of male infertility. *J Clin Endocrinol Metab* 81: 2159-2164, 1996.
 34. Cooke HJ: Y chromosome and male infertility. *Rev-Reprod* 4: 5-10, 1999.
 35. Higuchi R: Rapid, efficient DNA extraction for PCR from cells or blood. *Amplifications* 2: 1-3, 1989.
 36. Geever R, Wilson L, Nallaseth F, Milner P, Bittner M, Wilson J: Direct identification of sickle cell anemia by blot hybridization. *Proc Natl Acad Sci USA* 78: 5081-5085, 1981.
 37. Bhasin S, Swerdloff RS, Steiner BS, et al: A biodegradable testosterone microcapsule formulation provides uniform eugonadal levels of testosterone for 10-11 weeks in hypogonadal men. *J Clin Endocrinol Metab* 74: 75-83, 1992.
 38. Vollrath D, Foote S, Hilton A, Brown L, B  er-Romero P, Bogan J, et al: The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science* 258: 52-59, 1992.
 39. Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual*. ed. 2, New York: Cold Spring Harbor Laboratory Press, pp. 382-89, 1989.
 40. Mulhall JP, Reijo R, Alagappan R, Brown L, Page D, Carson R, Oates RD: Azoospermic men with deletion of the DAZ gene cluster are capable of completing spermatogenesis: fertilization, normal embryonic development & pregnancy occur when retrieved testicular spermatozoa are used for intracytoplasmic sperm injection. *Human Reproduction* 21: 503-508, 1997.
 41. Reijo R, Alagappan RK, Patrizio P, Page DC: Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet* 347: 1290-1293, 1996.
 42. Chai NN, Zhou H, Hernandez J, Najmabadi H, Bhasin S, Yen PH: Characterization of several YRRM genes in deletion interval 6 of the human Y chromosome. Submitted to *Am J Hum Genet*.
 43. Lilford R, Jones MA, Bishop DT, Thronoton J, Muller R: Case-control study of whether subfertility in men is familial. *BMJ* 309: 570-573, 1994.

