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Genetic evaluation of infertile men

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Recently, microdeletions in the azoospermic factor region of the Y chromosome, in addition to chromosomal anomalies, have been detected in men with azoospermia or severe oligozoospermia. In this study we evaluated the molecular and cytogenetic defects of infertile men. The frequency of Y microdeletions among 105 azoospermic, 28 oligozoospermic and 32 fertile men was tested on lymphocyte DNA using a series of 20 sequence-tagged sites. In addition, microdeletions were evaluated on testicular-derived DNA among 26 azoospermic patients who underwent testicular biopsy and in whom no sperm cells could be identified. Karyotype analysis was performed on 72 of the infertile patients. Deletions were detected in 6.7% azoospermic and 3.6% oligozoospermic men. No deletions were identified among the fertile men. Identical results were obtained with DNA derived either from lymphocytes or testicular tissue. The frequency of chromosomal aberrations in the 72 infertile patients tested (62 azoospermic, 10 oligozoospermic) was 16.6%, with a high percentage of gonosome anomalies. Additional andrological parameters (hormone values, cryptorchidism) failed to identify men at risk for having microdeletions before the test. Our findings support the recommendation to perform genetic defect screening among infertile men before their enrolment in an intracytoplasmic injection/in-vitro fertilization programme.

Key words: infertility/karyotype/microdeletions/testis

Introduction

Among couples with fertility problems, male factor is the aetiology in about 50% of cases (de Kretser, 1997). In these instances, oligozoospermia or azoospermia (AZO) are frequently observed. Genetic macroscopic defects (detected by cytogenetic methods) and genetic microscopic defects (detected by molecular biology methods) have been identified in such infertile patients (Jaffe and Oates, 1994). The genetic information for the control of spermatogenesis carried on the Y chromosome was first stated by Tiepolo and Zuffardi (1976). Cytogenetic analysis of infertile men revealed that 0.5% had macroscopic deletions of the distal long arm of the Y chromosome (Yq). The presence of an azoospermic factor (AZF) in the long arm of the Y chromosome was proposed and further mapped to Yq11.22-23 (Andersson *et al.*, 1988). With the advent of the polymerase chain reaction (PCR) and construction of a Y chromosome sequence-tagged site (STS) map (Foote *et al.*, 1992), microdeletions were detected at a frequency of 5–18% in the AZF region of oligozoospermic and azoospermic men (Bardoni *et al.*, 1991; Ma *et al.*, 1992; Vogt *et al.*, 1992; Kobayashi *et al.*, 1994; Najmabadi *et al.*, 1996; Reijo *et al.*, 1996). The microdeletions were mapped to three close subregions, labelled AZFa, AZFb and AZFc (Kobayashi *et al.*, 1994; Vogt *et al.*, 1996).

To date, two candidate genes have been identified: the RNA-binding motif (*RBM*) gene was isolated from the AZFb region, and the deleted in azoospermia (*DAZ*) gene was identified in several copies in the AZFc region. The *RBM* gene is expressed specifically in the testis (Ma *et al.*, 1993) and belongs to a large family of genes spread over the Y chromosome (Schempp *et al.*, 1995). The *DAZ* gene is also expressed specifically in the testis and bears an RNA recognition motif (Reijo *et al.*, 1995). An autosomal homologue to *DAZ* (*DAZH-DAZLA*) was identified and mapped in human to chromosome 3 (Saxena *et al.*, 1996). This gene is expressed in both gonads, even if at a lower extent in the ovary. The definite proof of the involvement of these genes in spermatogenesis would be the identification of a point mutation in one of them, leading to oligozoospermia or azoospermia. However, the large number of *RBM*-like genes and the several copies of the *DAZ* gene makes the identification of such point mutations in non-deleted oligozoospermic and azoospermic men particularly difficult.

Until now, screening for Y chromosome microdeletions in men with oligozoospermia and azoospermia has been performed by analysing the presence of Y chromosome-specific STS markers isolated from lymphocytes. This test is performed on infertile men seeking assistance via assisted reproductive techniques. As blood cells and gametes are developed from different embryonic tissues (mesoderm and endoderm respectively), they may carry different genetic lesions. However, an extensive screening study on Y chromosome microdeletions in lymphocyte DNA and testis tissue DNA in the same patients has, to the best of our knowledge, not been published. In this paper we report the molecular and cytogenetic study of infertile men. The presence of Y chromosome microdeletions was studied in lymphocyte- and testicular tissue-derived DNA, while karyotype analysis was also carried out.

Materials and methods

Patients

The Y chromosomes of 133 males referred to the Institute for the Study of Fertility and who consented to genetic testing, were screened. In addition, 32 fertile males and 14 fertile females were tested. Men with clinical evidence of obstructive azoospermia, or with a history of mumps or injury, were excluded. Of the 133 patients, 105 were azoospermic and 28 oligozoospermic. Charts were reviewed for semen analysis, hormone profile, and histology of the testis biopsy performed during testicular sperm extraction.

Sperm concentration was measured after liquefaction using the Makler chamber. Hormone concentrations were measured by radio-immunoassay. After obtaining informed consent, blood samples were withdrawn and DNA was extracted from lymphocytes. Control DNA was prepared from men with proven fertility, while negative control DNA was extracted from females. DNA was also isolated from testicular biopsies in which no sperm cells were found.

DNA isolation and multiplex PCR Y chromosome analysis

Genomic DNA was extracted from isolated lymphocyte nuclei digested with proteinase K, and subsequent proteins were salted out with sodium chloride, followed by precipitation of DNA with ethanol. Recently, the Master Pure^{TM} Genomic DNA Purification kit (Cat No. MG71100; Epicentre Technologies, Madison, WI, USA) was used for lymphocyte DNA isolation. The DNA from testis was isolated after washing the mashed tissue twice with saline, as described for lymphocyte DNA.

Eighteen STS on Yq spread over intervals 5 and 6 were used to identify submicroscopic deletions. In addition, two STS located within the *SRY* gene were used as internal controls testing for the presence of the Y chromosome. A multiplex PCR technique in 25 µl on a PTC-200 thermal cycler (MJ Research, Inc., Watertown, MA, USA) was used to evaluate the STS. The primer mixes used in the multiplex PCR were: mix A (SRY 37, sY143, sY158, sY153); mix B (DAZ, sY14, sY134); mix C (sY14, sY84, sY127, sY81); mix D (sY254, sY136, sY255); mix E (sY86, sY160, sY121) and mix F (sY108, sY105, sY87, sY97). Whenever failure of amplification was detected, two additional PCR (in the multiplex PCR mix and PCR of the STS alone) were performed to confirm the absence of the unamplified STS. The STS primers have been previously published (except the DAZ primers) by Vollrath *et al*. (1992) and Reijo *et al*. (1995). The DAZ primers used were: left primer ggAAgCTgCTTTggTAgATAC; right primer TAggTTTCAgTgTTTggATTCCg. The DAZ PCR product is 1.3 kb.

Cytogenetic evaluation

Chromosome analysis was performed on peripheral lymphocytes with G-banding staining. On an average, 20–30 metaphases were analysed per male. In cases of complex structural chromosome aberrations, additional analysis by fluorescence in-situ hybridization (FISH) was performed. Patients who were diagnosed as having a chromosomal abnormality underwent genetic counselling.

Clinical data of men with and without microdeletions were calculated as mean ± SE.

Results

Y chromosome microdeletion screening

All 133 infertile and 32 fertile males were tested for the presence of microdeletions in AZF regions. Microdeletions were found in eight infertile patients (Figure 1); in seven

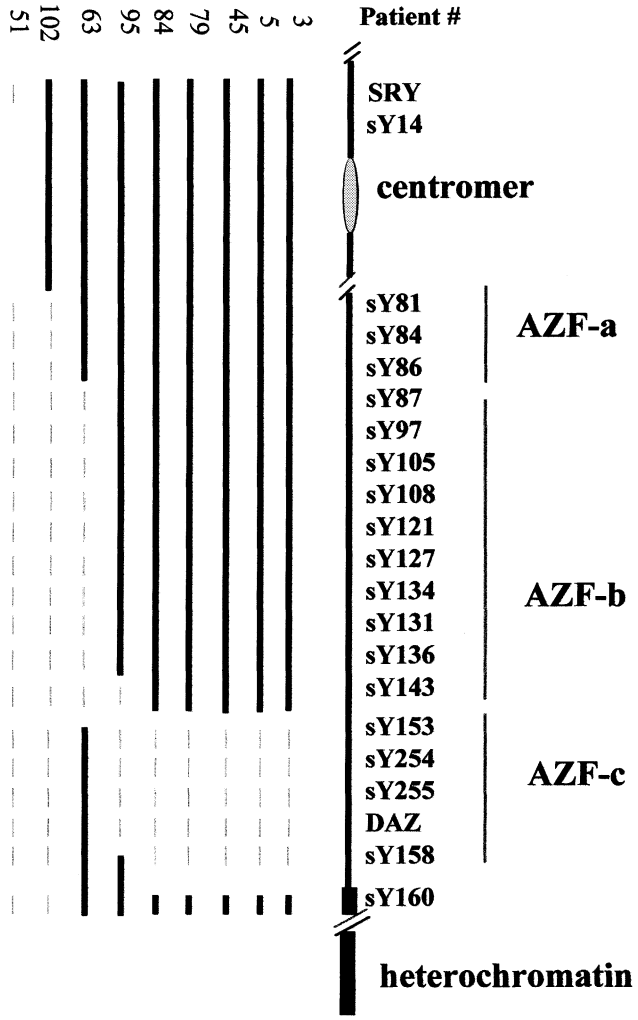

Figure 1. Schematic depiction of Y chromosome deletions found in nine patients, compared with a normal Y chromosome. Relative position of sequence tagged sites (STS) tested at AZFa, b and c regions are included. Each line exemplifies a patient. Dark areas denote STS detected; light areas denote STS deleted.

cases, the deletions included the *DAZ* gene, while in five patients the extent of the deletion was similar. Patient no. 45 with AZFc deletion agreed to screening of his father and four brothers, but no microdeletions were found. An azoospermic male (patient no. 63) had deletion in the AZFb region, including the RBM cluster. A large deletion, including intervals 5 and 6 and heterochromatin, was detected in patient no. 102 by the PCR test, and further confirmed by FISH. His father had a normal PCR test for all the STS checked. In addition, an XX male (patient no. 51) was detected by PCR and found to be negative to all the STS located in the Y chromosome, including SRY. Obviously, the XX male was positive for one STS located on the X chromosome (*dystrophyne* gene) and two STS located at the autosomal chromosome 7 (*SMN* gene). These findings were confirmed by the karyotype result. No microdeletions were detected in 32 fertile men and no Y chromosome STS were detected in 14 women.

Microdeletion screening in testicular tissue

The DNA isolated from testicular tissue of 26 azoospermic patients whose biopsy samples contained no spermatozoa, was

Table I. Frequency of genetic defects among infertile men classified by sperm concentration

Classification	Sperm concentration ($\times 10^6$/ml)	No. of patients tested	Defect	No. of patients
Azoospermia	0	105	XXY	4
			XX	1
			46,XY, t(X;4) (q13;p16)	1
			46,XY, t(Y,4) (q11;q12)	1
			46,XY, inv(9) (p11;q13)	1
			46,XY, r(18)	1
			46XdelY(q11.2–11.3) (AZFa–c del)	1
			AZFc del	5
			AZFb del	1
Virtual azoospermia	<1	13	None	
Severe/moderate oligozoospermia	1–5/5–30	15	46,XY, t(15;Y) (q15;q12)	1
			46,XY, inv(9) (p11;q13)	1
			AZFc del	1
Fertile	>30	32	None	0

Table II. Findings of andrological history and examination

Classification	No. of men	Cryptorchidism	Varicocele	After orchidectomy	Hypogonadotrophic hypogonadism
Azoospermia	105	7	5	4	3
Oligozoospermia	28	0	1	0	1

tested for the presence of microdeletions. In 22 patients, deletions were not detected in either their lymphocyte DNA, or in testicular-derived DNA. In four patients who had microdeletions in their lymphocyte DNA (patient nos 45, 63, 84, 102) the same deletions were observed in the testicular tissue.

Cytogenetic evaluation

Of the 133 infertile patients, 72 underwent karyotype analysis, with constitutional chromosomal aberrations being mainly identified among azoospermic men. In nine of 12 patients with large aberrations, one of the sex chromosomes was involved (Table I). Among the 72 patients, four (5%) were 47,XXY (Klinefelter syndrome), while in two of the three patients who had a translocation, the distal long arm of the Y chromosome was involved. An inversion of chromosome 9 (usually identified as a normal variant in the population) was observed in two men, one of whom was azoospermic and one who had a sperm concentration of 0.5×10^6/ml.

Clinical findings

Physical and andrological examination revealed clinical findings in 19 of 105 azoospermic men and in two of 28 oligozoospermic men examined (Table II). None of those with clinical findings had a karyotype aberration or a microdeletion. No significant differences were detected in the hormonal profile of azoospermic or oligozoospermic patients, with or without microdeletions (Table III).

Clinical data of patients with Y microdeletions are summarized in Table IV. Six of the eight patients with a Y microdeletion had normal testicular volume (⩾15 ml). Five patients had elevated follicle stimulating hormone (FSH) concentrations (⩾10 IU/l) and one patient (no. 45) had a low testosterone concentration (<4 ng/ml). Testicular histology was available in six of the eight patients with microdeletions. Arrest of spermatogenesis at different stages was observed in four patients. Patient no. 102 had a large deletion that included the entire AZF region, and the testicular histology was of Sertoli cell-only. In patient no. 84, who had a deletion in AZFc, a few spermatozoa were found in the testicular biopsy. Azoospermic factor-c microdeletion was also identified in a severely oligozoospermic male (patient no. 79).

Table III. Hormonal data of patients studied

Classification	No. of men	FSH (IU/l)	LH (IU/l)	Testosterone (ng/ml)
Azoospermia	103	20.6 ±1.64	7.2 ± 0.5	10.0 ± 1.2
with microdeletion	7	19.0 ± 3.2	7.5 ± 1.4	7.2 ± 2.7
without microdeletion	96	20.8 ± 1.7	7.2 ± 0.5	10.2 ± 1.3
Oligozoospermia	28	9.3 ± 1.5	5.3 ± 0.4	9.0 ± 1.9
with microdeletion	1	5.3	4.2	8.8
without microdeletion	27	9.5 ± 1.5	5.3 ± 0.4	9.0 ± 2.0

Values are mean ± SE.
FSH = follicular stimulating hormone; LH = luteinizing hormone.

Discussion

Recent studies have established the concept of a genetic basis for male infertility. This study summarizes the microdeletions and cytogenetic screening of azoospermic and oligozoospermic males who applied to our clinic. Relatives were evaluated where possible to detect whether the microdeletions were inherited or acquired. An additional objective was to compare lymphocyte and testicular DNA for genetic evaluation outcome.

Table IV. Summary of molecular and clinical data for patients with detected Y chromosome microdeletions

Patient no.	Age (years)	Sperm concn ($\times 10^6$/ml)	Testis volume (ml)		FSH (IU/l)	LH (IU/l)	Testosterone (ng/ml)	Karyotype	Deleted region	Clinical or histopathological findings[a]
			R	L						
3	27	0	25	25	18.0	5.7	5.1	ND	AZFc	Round spermatid
5	27	0	30	25	3.0	3.2	ND	ND	AZFc	Spermatogonia
45[b]	36	0	15	15	36.0	5.8	2.2	46,XY	AZFc	Spermatocyte
63[b]	34	0	15	20	19.0	10.0	4.8	46,XY	AZFb	Spermatocyte
79	52	0.3	15	12	5.4	4.2	8.8	ND	AZFc	OTA
84[b]	28	0	15	15	20.4	7.5	9.2	46,XY	AZFc	Few spermatozoa
95	31	0	22	25	26.9	11.4	22.2	46,XY	AZFc	ND
102[b]	31	0	12	15	9.6[a]	2.0	4.3	46X, delY (q11.2–11.3)	AZFa–c	Sertoli cell-only

[a]The most matured cell observed in the biopsies
[b]Patients tested for microdeletions in lymphocytes and testes.
FSH = follicular stimulating hormone; LH = luteinizing hormone; ND = not determined; OTA = oligoasthenoteratozoospermia.

Recent studies detected Y chromosome microdeletions at various prevalences (3–18%) among men with non-obstructive azoospermia or severe oligozoospermia (Vogt *et al.*, 1992; Kobayashi *et al.*, 1994; Reijo *et al.*, 1995, 1996; Najmabadi *et al.*, 1996; Girardi *et al.*, 1997; Pryor *et al.*, 1997). In our patient population, Y-microdeletions were detected in 6.7% (7/105) of azoospermic and 3.6% (1/28) of oligozoospermic males. The most frequently deleted region was AZFc, including the candidate gene for azoospermia, *DAZ*.

It was noted that aberrant crossover events led to microdeletions in chromosomes that recombined (Wyandt *et al.*, 1982). However, for genes outside the pseudo-autosomal pairing region (PAR), recombination events between areas of homologous or similar sequence repeats (e.g. Alu repeats) on the X and Y chromosomes could also give rise to deletions or duplication events (Yen *et al.*, 1990). Furthermore, it is possible that microdeletions may be caused by aberrant or unbalanced sister chromatid exchanges (SCE). The instability of the Y chromosome may be partially related to the high frequency of repetitive elements (SINES, LINES) (Graves, 1995). Evidence of this instability in the Y chromosome was provided by two patients in our study in whom de-novo microdeletions were detected, and also by others (Kobayashi *et al.*, 1994; Stuppia *et al.*, 1996).

Men with AZFc microdeletions had variable histopathological findings. Similar findings were reported by others (Reijo *et al.*, 1996; Girardi *et al.*, 1997). A male (patient no. 102) of short stature (1.60 m) had a large microdeletion that included the proximal Yq region. This region was reported to include stature determination, in addition to genes involved in spermatogenesis (Alvesalo and de la Chapelle, 1981). Sertoli cell-only was found in histology of his testis. Spermatocyte arrest was observed in a male patient (no. 63) with a microdeletion in AZFb. Our results partially concur with the concept proposed by Vogt *et al*. (1996) that deletions in the AZFa and AZFb are associated with impairment of spermatogenesis being worse when compared with that of the AZFc region, but additional infertile patients should be screened to establish this observation.

Deletions in the AZFc region are usually expanded between sY153 and sY158 and always include the *DAZ* gene, as exemplified by our results and those of others (Qureshi *et al.*, 1996; Reijo *et al.*, 1996; Girardi *et al.*, 1997; Pryor *et al.*, 1997). Hence, it could have been sufficient to check one STS in the *DAZ* gene to detect deletions in this region. In practice, it is highly recommended to confirm the deletion by checking adjacent STS, since the deleted STS could represent polymorphism or a technical problem, such as inefficient amplification of the STS.

Detection of microdeletions in azoospermic and oligozoospermic men, but not in their fertile fathers, suggested that the event occurred *de novo* in infertile patients (Ma *et al.*, 1992; Reijo *et al.*, 1995, 1996). We determined de-novo microdeletions in two patients, one with a deletion in AZFc, and another with a large deletion covering the entire AZF region. None of the four brothers of the AZFc-deleted patient, or the fathers of both patients had detectable deletions.

Following analyses of infertile fathers and their male babies derived by intracytoplasmic sperm injection (ICSI), Kent-First *et al.* (1996) suggested that mosaicism for AZF microdeletions may be a factor in male-related infertility and that somatic cell lineages may not always be a direct reflection of the germ cell lineage. The possible origin of Y chromosome deletions has recently been discussed (Edwards and Bishop, 1977). Our study performed on testicular biopsies of 26 patients has confirmed that the PCR results obtained from lymphocyte-derived DNA analysis reflects the situation in testes. Among the four men who had microdeletions, a similar extent of deletion was detected in both tissues. Moreover, of those who were found to carry a normal AZF region in lymphocyte DNA, their testicular DNA analysis also appeared to be normal. However, to detect low-level mosaicism, single-cell analysis of spermatogenic cells is required (Edwards and Bishop, 1997).

Chromosomal aberrations have been shown to be frequent among infertile patients: 13.7–15.4% among azoospermic men, compared with 1.76–4.6% in oligozoospermic men with sperm counts of $1–20\times10^6$/ml (reviewed by Van Assche *et al.*, 1996). Chromosomal abnormalities among male patients undergoing the ICSI procedure and in a large cohort of 1210 infertile men have recently been reported to be 3.8% and 3.6%, respectively (Pandiyan and Jequier, 1996; van der Ven *et al.*, 1997). The frequency of chromosomal aberrations in our patient population

was found to be 16.6%. This included two patients with pericentric inversion of chromosome 9 which is usually considered as a normal polymorphism (Court-Brown *et al.*, 1965). The relatively high frequency of chromosomal abnormalities found in this study may be the reflection of patient selection (84% of the infertile patients tested for karyotype were azoospermic) and group size.

The important role of the sex chromosomes in infertility is reflected in the chromosomal aberrations found: one of the sex chromosomes was involved in nine of 12 cases identified. Moreover, on two of the three translocations identified, the distal long arm of the Y chromosome was involved. Van Assche *et al.* (1996) indicated that in the azoospermic group, sex chromosome abnormalities predominated (12.6%), whereas in the oligozoospermic group, autosome anomalies were the most frequent (3%). The high percentage of gonosome anomalies detected among azoospermic men was attributed to the Klinefelter syndrome (47,XXY), which was found to be the leading chromosome aberration among infertile men. In a large number of patients comprising 10 728 karyotyped men, 3.5% were found to carry 47,XXY (Van Assche *et al.*, 1996). In our group of infertile men, 5.5% were 47,XXY.

The 46,XX males lacking the sex-determining Y gene (*SRY*) were detected by molecular analysis and reconfirmed by cytogenetic analysis. The presence of testicular tissue in such individuals is postulated to be the result of an activating mutation in a gene that functions in the cascade triggered by *SRY* (Ferguson-Smith *et al*., 1990). Three patients were found to have reciprocal translocations between a sex chromosome and an autosomal one that usually lead to infertility.

Unfortunately, the andrological evaluation in this study failed to define a subgroup of infertile men at risk of having microdeletions. No abnormal andrological findings (cryptorchidism, varicocele, hypogonadotrophic hypogonadism or following orchidectomy) were identified in patients with microdeletions. Kremer *et al.* (1997) arrived at a similar conclusion with a group of men who were mainly oligozoospermic: microdeletions were found in those with a lower concentration of FSH and lower frequency of abnormal findings during andrological history taking, or examination. In our study, neither testis volume nor testosterone, FSH and luteinizing hormone concentrations could predict the severity of the spermatogenesis defect, or the presence of a genetic defect.

The data presented here suggest that de-novo deletions at the AZF regions and chromosomal aberrations occur frequently in infertile men, a genetic defect being identified in 19 of 133 infertile patients. Microdeletions identified in the Y chromosome demonstrate a genetic basis to infertility, but are not entirely related to the severity of the spermatogenetic defect. Our findings strongly support the recommendation of screening for genetic defects among infertile patients before enrolment in the ICSI–in-vitro fertilization programme, and to provide them with the best counselling available.

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References

Alvesalo, L. and de la Chapelle, A. (1981) Tooth sizes in two males with deletions of the long arm of the Y chromosome. *Ann. Hum. Genet.*, **54**, 49–54.

Andersson, M., Page, D.C., Pettay, D. *et al.* (1988) Y: autosome translocations and mosaicism in the aetiology of 45,X maleness: assignment of fertility factor to distal Yq11. *Hum. Genet.*, **79**, 2–7.

Bardoni, B., Zuffardi, O., Guioli, S. *et al.* (1991) A deletion map of the human Yq11 region: implications for the evolution of the Y chromosome and tentative mapping of a locus involved in spermatogenesis. *Genomics*, **11**, 443–451.

Court-Brown, W.M., Jacobs, P. and Brunton, M. (1965) Chromosome studies on randomly chosen men and women. *Lancet*, **ii**, 561–562.

De Kretser, D.M. (1997) Male infertility. *Lancet*, **349**, 787–790.

Edwards, R.G. and Bishop, C.E. (1997). On the origin and frequency of the Y chromosome deletions responsible for severe male infertility. *Mol. Hum. Reprod.*, **3**, 549–554.

Ferguson-Smith, M., Cooke, A., Affara, N. *et al.* (1990) Genotype-phenotype correlations in XX males and the bearing on current theories of sex determination. *Hum. Genet.*, **84**, 198–202.

Foote, S., Vollrath, D., Hilton, A. *et al. (*1992) The human Y chromosome: overlapping DNA clones spanning the euchromatic region. *Science*, **254**, 60–66.

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

Graves, J.M. (1995) The origin and function of the mammalian Y chromosome and Y-borne genes – an evolving understanding. *BioEssays*, **17**, 311–320.

Jaffe, T. and Oates, R.D. (1994) Genetic abnormalities and reproductive failure. *Urol. Clin. North Am.*, **21**, 389–408.

Kent-First, M.G., Kol, S., Muallem, A. *et al.* (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**, 943–950.

Kobayashi, K., Mizun, K., Hida, A. *et al.* (1994) PCR analysis of the Y chromosome long arm in azoospermic patients: evidence for a second locus required for spermatogenesis. *Hum. Mol. Genet.*, **3**, 1965–1967.

Kremer, J.A.M., Tuerlings, J.H.A.M., Meuleman, E.J.H. *et al*. (1997) Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic. *Hum. Reprod.*, **12**, 687–691.

Ma, K., Sharkey, A., Kirsch, S. *et al.* (1992) Towards the molecular localization of the AZF locus: mapping of the microdeletions in azoospermic men within 14 subinterval 6 of the human Y chromosome. *Hum. Mol. Genet.*, **1**, 29–33.

Ma, K., Inglis, J.D., Sharkery A. *et al.* (1993) A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell*, **75**, 1287–1295.

Najmabadi, H., Huang, V., Yen, P. *et al.* (1996) Substantial prevalence of microdeletions of the chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using sequence-tagged site-based mapping strategy. *J. Clin. Endocrinol. Metab.*, **81**, 1347–1352.

Pandiyan, N. and Jequier, A.M. (1996) Mitotic chromosomal anomalies among 1210 infertile men. *Hum. Reprod.*, **11**, 2604–2608.

Pryor, J.L., Kent-First, M., Muallem, A. *et al.* (1997) Microdeletions in the Y chromosome of infertile men. *N. Engl. J. Med.*, **336**, 534–539.

Qureshi, S.J., Ross, A.R., Ma, K. *et al.* (1996) Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically determined spermatogenic failure in men. *Mol. Hum. Reprod.*, **2**, 775–779.

Reijo, R., Lee, T.Y., Alagappan, R. *et al.* (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet.*, **10**, 383–392.

Reijo, R., Raaji, K.A., Pascuale, P. *et al.* (1996) Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet*, **347**, 1290–1293.

Saxena, R., Brown, L.G., Hawkins, T. *et al.* (1996) The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. *Nature Genet.*, **14**, 292–299.

Schempp, W., Binkele, A., Arnemann, J. *et al.* (1995) Comparative mapping of YRRM- and TSPY- related cosmids in man and hominoid apes. *Chrom. Res.*, **3**, 227–234.

Stuppia, L., Calabrese, G., Franchi, P.G. *et al.* (1996) Widening of a Y-chromosome interval-6 deletion transmitted from a father to his infertile son accounts for an oligozoospermia critical region distal to the RBM1 and DAZ genes. *Am. J. Hum. Genet.*, **59**, 1393–1395.

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

Van Assche, E., Bonduelle, M. and Tournaye, H. (1996) Cytogenetics of infertile men. *Hum. Reprod.*, **11** (suppl.4), 1–24.

van der Ven, K., Montag, M., Peschka, B. *et al.* (1997) Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. *Mol. Hum. Reprod.*, **3**, 699–704.

Vogt, P., Chandley, A.C., Hargreave, T.B. *et al.* (1992) Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of AZF, a human spermatogenesis gene. *Hum. Genet.*, **89**, 491–496.

Vogt, P.H., Edelmann, A., Kirsch, S. *et al.* (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum. Mol. Genet.*, **5**, 933–943.

Vollrath, D., Foote, S., Hilton, A. *et al*. (1992) The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science*, **258**, 52–59.

Wyandt, H., Kasprzak, A., Lamb, K. *et al*. (1982) Human chromosome 2 rodring mosaicism: probable origin by prezygotic breakage and intrachromosomal exchange. *Cytogenet. Cell Genet.*, **33**, 222–231.

Yen, P.H., Xiao-Miao, L., Siao-Ping, T. *et al.* (1990) Frequent deletions of the human X chromosome distal short arm result from recombination between low copy repetitive elements. *Cell*, **61**, 603–610.

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