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The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers

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Microdeletions linked to deletion intervals 5 and 6 of the Y chromosome have been associated with male factor infertility. Members from at least two gene families lie in the region containing azoospermia factor (*AZF*), namely *YRRM* and *DAZ*. With the advent of intracytoplasmic sperm injection (ICSI), it is possible for men with severe male factor infertility to produce a child. The genetic consequences of such a procedure have been questioned. This report describes the first study of a population (32 couples) of infertile fathers and their sons born after ICSI. The objectives were firstly to determine the incidence and map location of Y chromosome microdeletions and to compare the frequencies with other population studies involving severe male factor infertility, and secondly to formulate a working hypothesis concerning developmental aetiology of Y chromosome microdeletions. The incidence of microdeletions in the ICSI population was shown to be 9.4% (within the range 9–18% reported for populations of severe male factor infertility patients). Microdeletions in two out of three affected father/son pairs mapped in the region between *AZFb* and *AZFc* and the third involved a large microdeletion in *AZFb* and *AZFc*. Of three affected father/son pairs, microdeletions were detected in the blood of one infertile propositus father and three babies. Assuming that the gonomes of the ICSI-derived babies are direct reflections of those of their fathers' germ lines, it is possible that two of three infertile fathers were mosaic for intact Y and microdeleted Y chromosomes. In such cases, the developmental aetiology of the microdeletion may be due to a de-novo microdeletion arising as a post-zygotic mitotic error in the infertile propositus father, thus producing a mosaic individual who may or may not transmit the deletion to his ICSI-derived sons depending on the extent of primordial germ cell mosaicism. In one of three affected fathers, the microdeletion detected in his blood was also detected in his ICSI-derived son. In this case the de-novo event giving rise to the microdeletion may have occurred due to a post- (or pre-) meiotic error in the germ line of this father's normally fertile father (i.e. the ICSI-derived baby's grandfather).

Key words: aetiology/intracytoplasmic sperm injection/Y-chromosome microdeletions

Introduction

Recently, there have been several studies devoted to defining key regions on the Y chromosome which are suspected to contain genes which have important roles in spermatogenesis (Ma *et al.*, 1993; Nagafuchi *et al.*, 1993; Henegariu *et al.*, 1994; Kobayashi *et al.*, 1994; Kent-First *et al.*, 1995; Reijo *et al.*, 1995; Najmabadi *et al.*, 1996; Pryor *et al.*, 1996; Qureshi *et al.*, 1996; Vogt *et al.*, 1996). The general regions which are on the long arm of the Y chromosome relevant to infertility are found in deletion intervals 5 and 6 (Figure 1) (Kent-First *et al.*, 1995; Reijo *et al.*, 1995; Affara *et al.*, 1996). Early cytogenetic and deletion analysis of infertile patients with aberrant Y chromosomes suggested that gene(s) termed azoospermia factor (*AZF*) reside in this region (Tiepolo and Zuffardi, 1976; Vergnaud *et al.*, 1986; Ma *et al.*, 1992). To date, two candidates for *AZF* have been described, namely, the *YRRM*(*RBM*) (Ma *et al.*, 1993) and *DAZ* (Reijo *et al.*, 1995) gene families; however, neither of the gene families has been completely characterized. There have been numerous clinical studies in which DNA derived from the blood of azoospermic or severely oligozoospermic males has been screened using Y-linked sequence tagged sites (STS) (Kent-First, 1996; Najmabadi *et al.*, 1996; Pryor *et al.*, 1996; Qureshi *et al.*, 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996). These studies demonstrate that the frequency of microdeletions is in the range 9–30% depending on patient selection criteria. Generally, deletions have occurred in at least one of three regions, provisionally termed *AZFa* (Jolar), *AZFb*, and *AZFc* (Klard) which are detailed in Figure 1 (Affara *et al.*, 1996; Vogt *et al.*, 1996). It has been suggested that deletions occurring in *AZFa* result in Type I Sertoli cell-only (SCO) (no spermatogonia present), deletions in *AZFb* result in patients with spermatogenic arrest, and deletions in *AZFc* result in patients with Type II SCO (some spermatogonia present with limited spermatogenesis) (Vogt *et al.*, 1996). However, in other similar studies, these associations could not be made (Reijo *et al.*, 1995; Pryor *et al.*, 1996; Qureshi *et al.*, 1996). Deletions may be small, involving only a single STS, or large, involving numerous STS and a substantial part of the chromosome. Larger deletions

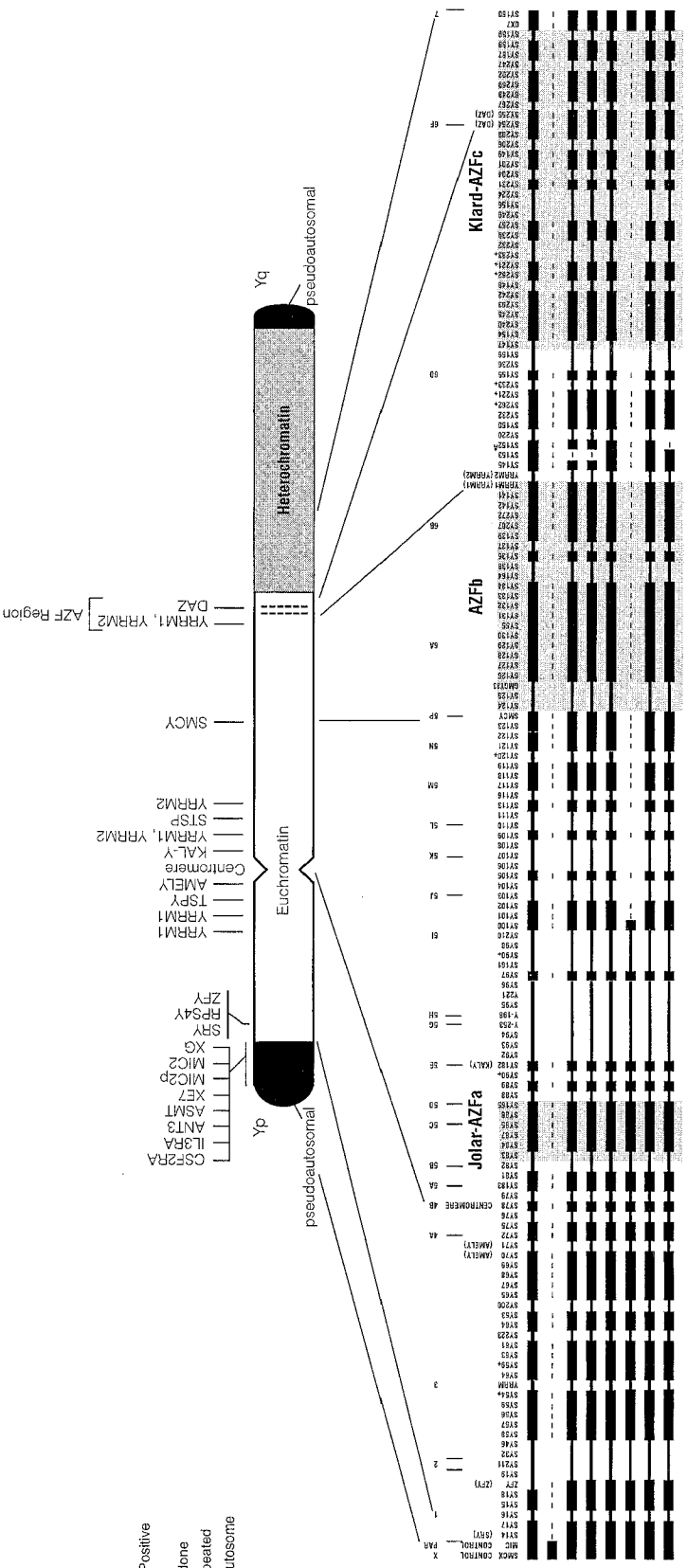

Figure 1. Y chromosome maps of microdeletions detected in blood from father/son pairs ICSI-F10/ICSI-B10, ICSI-F20/ICSI-B20 and ICSI-F31/ICSI-B31 are mapped relative to a normal (control) male (C-M) and female (C-F). *SMCX* and *MIC* [X-chromosome and pseudoautosomal pairing region (PAR)-linked respectively] are positive controls for the polymerase chain reaction (PCR). Sequence-tagged sites (STS) which were screened and found to be present are depicted as a solid, thick black line. STS which were screened and found to be absent are depicted by dashes (–). STS which were not used in the study, but which are assumed to be positive since they map between two confirmed positive STS, are depicted as a solid thin black line. The order of STS is derived from Affara *et al.* (1996), Vogt *et al.* (1996), Reijo *et al.* (1996), Najmabadi *et al.* (1996) and Vollrath *et al.* (1992), as well as from deletions in our own patients. The exact order of some STS, including *YRRM1* and *YRRM2* is not confirmed and some STS are repeated (denoted by +). Sperm counts are given beside the maps for each infertile F_1 generation father. The *AZFa* (Jolar), *AZFb* and *AZFc* (Klard) regions are highlighted in grey. Lines are drawn from the deletion map to the corresponding location on the Y-chromosome ideogram.

almost always result in azoospermia or severe oligozoospermia with partial or total spermatogenic arrest and in some cases, only Sertoli cells are present in the testis. Smaller deletions which involve *YRRM1(RBM1)*, the *DAZ* region, or the region between the two, may also result in an equally severe phenotype. However, Pryor *et al.* (1996) demonstrated that men with mild oligozoospermia and normozoospermia (normal sperm counts) but abnormal morphological parameters in spermatozoa can have microdeletions in either *AZF*a, *AZF*b or *AZF*c. Certainly the frequency of microdeletions seems to be greater in *AZFb* which is distal to the centromere, as well as the region between *AZFb* and *AZFc* (Kent-First *et al.*, 1995; Kent-First, 1996; Najmabadi *et al.*, 1996; Qureshi *et al.*, 1996).

As a control for insignificant STS polymorphism, several studies have involved the screening of DNA from leukocytes of the men who fathered the infertile males manifesting microdeletions (Ma *et al.*, 1992, 1993; Kobayashi *et al.*, 1994; Qureshi *et al.*, 1996; Reijo *et al.*, 1996; Vogt *et al.*, 1996). With four exceptions, none of more than 50 fathers of men carrying microdeletions and described in these studies had microdeletions, suggesting that the microdeletions occurred as de novo meiotic or mitotic events. The microdeletions detected in one of the four exceptions was shown to be due to an apparently insignificant STS polymorphism (Pryor *et al.*, 1994), however, in at least one of the four exceptions, the microdeletion found in the fertile father of the infertile propositus was smaller than that found in his son (Komaki *et al.*, 1995). Furthermore, in one of the four exceptions the father of the propositus also suffered from severe oligozoospermia and infertility, but eventually fathered a son who similarly suffered from severe oligozoospermia and infertility (Pryor *et al.*, 1996). Reijo *et al.* (1996) recently reported the occurrence of a microdeletion in DNA derived from the spermatozoa of a severely oligozoospermic male who also exhibited the same deletion in DNA derived from peripheral blood leukocytes, thus providing evidence that these mesodermal derivatives can be reflective of the germ cell lineage.

With the advent of intracytoplasmic sperm injection (ICSI), new hope for fertility has been given to couples suffering from even the most severe forms of male factor infertility (Van Steirteghem *et al.*, 1993). Success rates in most centres are greater than those for in-vitro fertilization (IVF) (Silber *et al.*, 1995). Concerns have been expressed regarding the potential for adverse genetic consequences in the children derived from ICSI (de Kretser 1995; Jonson *et al.*, 1996; Moog *et al.*, 1996; Morris and Gleicher, 1996; Peschka *et al.*, 1996; Selva *et al.*, 1996). Furthermore, in human and non-human species, infertility is frequently associated with other physiological abnormalities resulting from genetic or cytogenetic perturbation (Eldridge, 1985; Halnan, 1989; Meschede *et al.*, 1996). For example, Salo *et al.* (1995) reported facial dysmorphism and developmental anomalies in adult males with large, q-arm deletions of the Y chromosome. The oldest ICSI-derived children were produced in 1993, and thus far there have been no reports of dramatic increases in phenotypic anomalies in this population. However, several groups have reported an increased incidence of sex chromosome abnormalities as well as cystic fibrosis mutations (associated with congenital absence of vas deferens) among either infertile men who seek ICSI or the products of ICSI (Cummins and Jaquier, 1995; Hoegerman *et al.*, 1995; Liebaers *et al.*, 1995; Martin-du-Pan *et al.*, 1995; Moosani *et al.*, 1995).

Here we report the first genetic screen for microdeletions on the Y chromosome in a population of males manifesting severe male factor infertility and their ICSI-derived sons. We also report evidence that mosaicism 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.

Materials and methods

Patient evaluation

A total of 32 unselected couples, who were being treated using ICSI for male factor infertility, consented to participate in this study. Each couple produced at least one male baby from ICSI after an average of 7.8 years of infertility and an average of 2.8 (range: 0–13) previous attempts at IVF or ICSI. All patients gave complete medical histories and underwent complete physical examinations. A minimum of two semen analyses were obtained on each adult male patient. All semen parameters were evaluated using World Health Organization criteria. Sperm morphology was assessed by Kruger's strict criteria (Kruger, *et al.*, 1988). For categorization, severe oligozoospermia was defined as $>0-<500\ 000$ spermatozoa/ml, oligozoospermia as $\geqslant 500\ 000$ to $<20\times10^5$ spermatozoa/ml and normozoospermia as concentrations $\geqslant 20\times10^6$ spermatozoa/ml. Blood was drawn for DNA extraction and serum hormone measurement. In the cases of azoospermic and one severely oligozoospermic patient, testicular biopsies were obtained. At the completion of the evaluation, data for each patient were placed in the appropriate diagnostic categories shown in Table I. The female partner underwent standard ovarian stimulation and oocyte collection regimes followed by ICSI (Palermo *et al.*, 1992). Upon the birth of the ICSI-derived infants, gestation and birth statistics were noted and are shown in Table II. Blood (1.5 ml) was obtained from each male infant for assessment of Y chromosome deletions.

Fertile control population

Blood was drawn from 200 men who had fathered at least one child by natural conception. DNA was extracted from all blood samples using WIZARD Genomic DNA Purification System (Promega, Corp., Madison, WI, USA). Paternity was confirmed by DNA typing of every father and child using short tandem repeat (STR) polymorphism analysis of at least six loci in both the control and experimental populations (™Geneprint STR System, Promega Corp.). Two STR multiplex systems were used, one containing F13AO1, FESFPS, and vWA and one containing CSF1PO, TPOX, and THO1. Amplified STR were visualized via silver staining of denatured polyacrylamide gels. Paternity was similarly confirmed in father/offspring pairs from the experimental population.

Y-linked STS screening protocol

Patients were screened for the presence of 85 selected Y chromosome-specific STS using the Y deletion detection system (Promega Corp.) in combination with individually amplified STS (Kent-First *et al.*, 1995) and two X-linked loci as positive controls. The distribution of the STS tested in our patient population was as follows: 21 p-arm STS, 16 STS between the centromere and *SMCY*, 21 STS between *SMCY* and *YRRM1*, and 27 STS between *YRRM1* through the *DAZ* region, as summarized in Figure 1. STS were derived from the STS maps of Vollrath *et al.* (1992), Chalmers *et al.* (1995), Reijo *et al.* (1995) and Affara *et al.* (1996). *YRRM1* primers were as described in Ma *et al.* (1993), and corrected in Kobayashi *et al.* (1994). STS

Table I. Characteristics of patient populations

No. of fathers	Sperm concentrations*				No. of male progeny	No. of Y deletions detected in blood	
	A	SO	O	N**		Father	Child***
32	3	11	8	10	35	1	3

*Sperm concentration categories are: A = azoospermia; SO = severe oligozoospermia (>0 to < 5×10^6 spermatozoa/ml); O = oligospermia (≥5 to 20×10^6 spermatozoa/ml); N = normozoospermia (≥20×10^6 spermatozoa/ml).
**These patients had other sperm anomalies such as teratozoospermia and/or asthenozoospermia.
***Deletion detected in ICSI-derived F_2 generation sons which we assume to be direct reflections of the germ lines of their infertile F_1 generation fathers.

Table II. Findings of father/infant pairs involving microdeletions

Family	Male	Offspring	Years infertile	Semen analysis: No. spermatozoa (10^6/ml)	Semen analysis: Sperm motility (%)	Semen analysis: Sperm morphology (% abnormal)	Testicular histology	Deletion: Father Mesoderm	Deletion: Father Germ line	Deletion: Child Mesoderm	Deletion: Child Germ line	Karyotype
1	ICSI-F10	Son: ICSI-B10	6	6	25	100	NA	Yes	P	Yes	P	46XY
2	ICSI-F20	Son: ICSI-B20	7	47	50	90	NA	NDT	P	Yes	P	46XY
3	ICSI-F31	Son: ICSI-B31	8	0.6	16	74	Ltd.Sp.	NDT	P	Yes	P	46XY

Abbreviations: NA = not available; Ltd.Sp. = limited spermatogenesis, few spermatids/spermatocytes; NDT = not detected, P = predicted.

map order was derived as a consensus among Affara *et al.* (1996), Reijo *et al.* (1995), Najmabadi *et al.* (1996), Qureshi *et al.* (1996), and Vogt *et al.* (1996). DNA (100–250 ng) was amplified in a 40 µl reaction volume consisting of 1.87 mM $MgCl_2$, 2.5 mM each dNTP, polymerase chain reaction (PCR) reaction buffer (Promega Corp.), and 1.5 IU *Taq* DNA polymerase (Amplitaq; Perkin Elmer-Cetus, Emeryville, CA). Oligonucleotide primers were selected or designed to permit identical conditions for all PCR reactions. Thermocycling conditions consisted of an initial 2 min denaturation at 96°C followed by 35 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min. To determine if a normal Y chromosome bearing clonal line was present in cases where microdeletions were detected, the PCR protocol described was repeated using 50 cycles. Amplified products were separated on 3% Metaphor (FMC Bioproducts, Rockland, ME, USA) agarose gels and visualized by staining with ethidium bromide. Two different multiplexed amplifications (I, II) of two father/son pairs from families 1 and 2 are shown in Figure 2. Each individual result (negative or positive) was repeated a minimum of three times before scoring. All samples were analysed in a blinded fashion, without knowledge of the patient's name, clinical diagnosis or semen analysis results. Every PCR experiment contained at least two male, female, and water controls (positive, negative, and blank respectively).

Verification of deleted STS by Southern blot hybridization

Due to limited template availability, deletions detected by PCR were confirmed by Southern blot analysis in patient ICSI-F10 and a normal control male only. For Southern analysis, 5 µg of DNA was digested with EcoRI, electrophoresed on a 1% agarose gel, transferred to a nylon membrane and hybridized overnight at 65°C with random primed, [^{32}P]-labelled DNA probe. The probe for this hybridization consisted of the gel purified PCR STS product (SY153) amplified from normal male genomic DNA.

Results

ICSI procedure

Of the 32 parental couples, 21 each produced a single male baby, and three parental couples produced dizygotic male twins, seven couples produced male and female twins and one couple produced a set of trizygotic triplets (two males, one female). A total of 36 male babies were tested. Cumulatively, the 32 couples had suffered infertility for an average of 7 years and had undergone an average of 2.8 previous IVF trials. An average of 15.8 oocytes were recovered per couple per cycle. The average fertilization and cleavage rates among the patient population were 54.8 and 94.7% respectively. An average of 3.7 embryos were transferred per couple and an average of 2.8 embryos per couple were frozen for use in future cycles. Clinical details of the three families in which Y chromosome microdeletions were detected are shown in Table II.

Of the 44 babies produced (male and female), four (9.1%) had problems that required intensive care. Average birth weight was 2891 g (range 1100–4475). Though the mean birth weight was no different from that expected in births resulting from natural conception, the rate of neonatal intensive care was higher due to the large number of births resulting in twins or triplets.

Histology

Histological results on the three azoospermic patients revealed obstruction of the testis in two cases and spermatogenic arrest in the third. None of these three patients or their ICSI-derived babies exhibited microdeletions. Severely oligozoospermic propositus ICSI-F31 exhibited spermatogenic arrest with relatively few spermatids and spermatocytes present (Table II).

Microdeletions

Out of 32 father/baby pairs, Y chromosome microdeletions were detected in three sets (9.4%) (Table I). Using a two-tailed Fisher's exact test (Woolson, 1987), the observed incidence in the experimental population is significantly different from the normal control population in which no deletions were detected in 200 fertile men ($P = 0.003$) and within the range (9–18%)

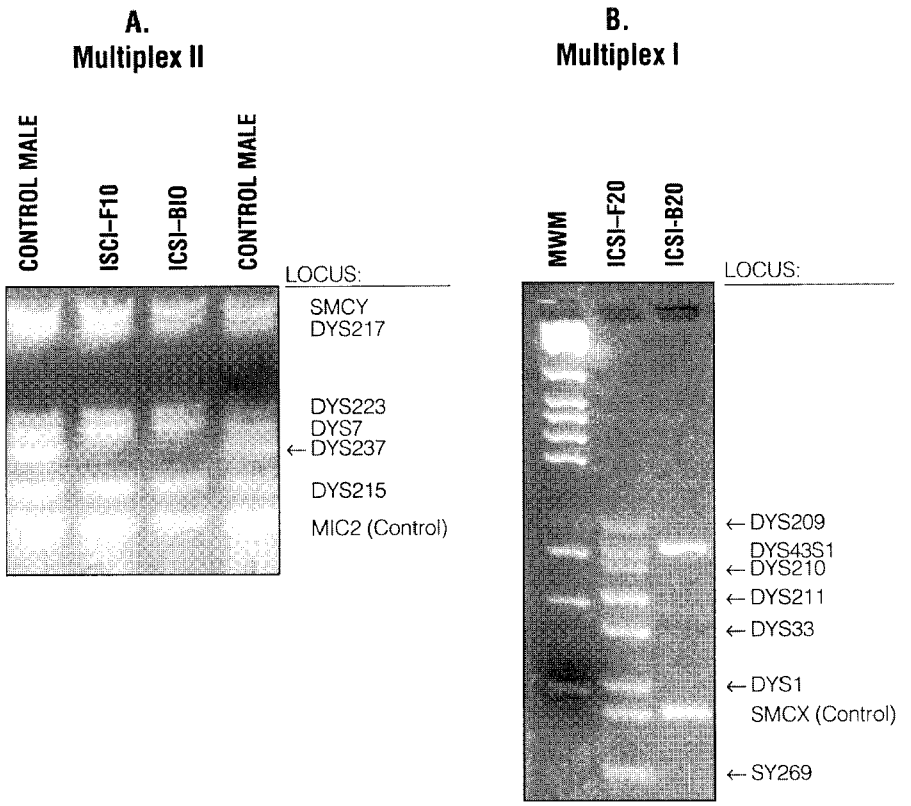

Figure 2. The gel on the left shows the amplified product of DNA derived from a normal control male, ICSI-F10 and ICSI-B10 using multiplex II (a 7-plex which includes *MIC* as a positive control) from the Y deletion detection system. One STS (SY153) which corresponds to locus DYS237 is deleted from both of the patient samples and is indicated with an arrow. The gel on the right shows the amplified product of DNA derived from the blood of infertile patient ICSI-F20 and his ICSI derived son, ICSI-B20, using multiplex I (an 8-plex which includes *SMCX* as a positive control) from the same Y deletion detection system described above. Lane 1 of this gel shows the control molecular weight marker (MWM). Six of the deletions detected in ICSI-B20 are indicated with an arrow.

of deletion incidence reported in comparable populations of azoospermic or severely oligozoospermic infertility patients (Bardoni *et al.*, 1991; Vogt *et al.*, 1992; Kobayashi *et al.*, 1994; Pryor *et al.*, 1996; Qureshi *et al.*, 1996; Reijo *et al.*, 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996). Within the three affected father/baby pairs, a small microdeletion distal to *YRRM1(RBM1)(AZFb)* and excluding *DAZ(AZFc)* was detected in the leukocytes of one of the adult male fathers (ICSI-F10) and his ICSI-derived baby (ICSI-B10). Southern blotted DNA from ICSI-F10 which was hybridized with labelled probe SY153 and compared with normal male DNA revealed a deleted fragement of ~2.5 kb. Though microdeletions were not detected in blood from the other two fathers (ICSI-F20 and ICSI-F31), their respective ICSI-derived sons (ICSI-B20 and ICSI-B31) displayed microdeletions. The microdeletion detected in ICSI-B31 was in close map proximity to the microdeletion detected in ICSI-F10, distal to *YRRM1(RBM1)* and excluded the *DAZ* locus whereas the deletion detected in ICSI-B20 was a large interstitial microdeletion involving many consecutive STS in deletion intervals 5 and 6. A microdeletion map indicating deleted STS relative to the intact Y chromosome is given in Figure 1. Microdeletions found in patients ICSI-B31, ICSI-F10 and ICSI-B10 occurred between *AZFb* and AZFc, whereas the microdeletion in patient ICSI-B20 was large and involved *AZFb* and *AZFc*. Paternity was confirmed by DNA typing in every father/baby pair tested. No deletions were found in the normal control population consisting of 200 fertile males which was screened in parallel with the experimental population.

Discussion

The studies which have reported intact Y chromosomes in fertile fathers of infertile sons have concluded that the mutation event causing the microdeletion occurred *de novo* and was therefore the likely cause of the infertility observed in the propositus (Pryor *et al.*, 1996; Reijo *et al.*, 1996; Vogt *et al.*, 1996). However, when the same microdeletion was detected in DNA from the blood and spermatozoa of an infertile severely oligozoospermic propositus, Reijo *et al.* (1996) concluded that limited spermatogenesis is compatible with a microdeletion involving AZF and could be transmitted to his offspring should fertilization occur. Unfortunately, any attempts to associate specific phenotypes with given deletion events become problematic because of large variations in clinical diagnosis of sperm counts and other measurable parameters. If a given deletion event is considered in terms of developmental timing and the generation in which it originates, perhaps our understanding of the effect of a microdeletion in a given infertile propositus and the potential ramifications of the given deletion on the offspring of an infertile propositus will improve. Peripheral blood leukocytes provide a convenient source of

DNA; however, blood is late differentiating mesoderm in origin and may not provide an accurate or complete depiction of other tissue types (ectoderm and endoderm) including the germ cell lineage. Furthermore, in a given somatic cell lineage such as leukocytes, when an intact Y chromosome undergoes a mutation event leading to a microdeletion, cells containing the intact Y chromosome should continue mitosis, in parallel with cells containing a microdeleted Y, thus producing a mosaic condition. In the case of mosaicism, it is possible that a microdeletion will be missed using PCR-based STS analysis alone.

Assuming that the progeny derived from ICSI (referred to as the F_2 generation) are direct reflections of the germ cell lineages of their infertile propositus fathers (referred to as the F_1 generation), our dataset suggests that the microdeletions detected in the ICSI-F20/ICSI-B20 and ICSI-F31/ICSI-B31 father/baby pairs share similar developmental aetiologies which are different from the probable developmental aetiology in the ICSI-F10/ICSI-B10 father/baby pair. In the ICSI-F20/ICSI-B20 and ICSI-F31/ICSI-B31 father/baby pairs, a microdeletion was not detected in DNA from the blood of either father, although microdeletions were detected in blood from both their children. The probability that post-zygotic mitotic errors occurred in these ICSI-derived babies which were unrelated to their fathers' infertility is possible, but low because in this scenario, two cell lines would be present, namely, the cell line carrying the intact Y chromosome and the mutated cell line carrying the deleted Y. A PCR-based STS analysis assay would amplify the DNA derived from the non-mutated cell line, and thereby should mask underlying deletions. Therefore, a more likely developmental aetiology of the microdeletions observed in the ICSI-F20/ICSI-B20 and ICSI-F31/ICSI-B31 father/baby pairs is that a post-zygotic mitotic error occurred in the F_1 cells destined to develop as the germ cell lineage and/or possibly in F_2 cells which contributed to the soma. Amplification of the DNA from single cells similarly using Y-linked STS could provide unequivocal proof of this hypothesis. In any case, if ICSI-F20 and ICSI-F31 are mosaics for a de-novo mutation, one could speculate that the mutation event leading to severe oligozoospermia in ICSI-F31 occurred earlier in fetal development than the mutation event proposed for infertile propositus ICSI-F20, who had normal sperm numbers but very abnormal sperm morphology and a history of >7 years of infertility. Although both babies, ICSI-B20 and ICSI-B31, apparently inherited microdeletions from their fathers, the case may exist in which the germ cell lineages of their fathers could also give rise to spermatozoa carrying intact Y chromosomes. In this scenario it could be possible for men such as these to father sons possessing an intact Y. Only single sperm PCR will confirm this hypothesis.

A second developmental aetiology reminiscent of the one described by Reijo *et al.* (1996) is suggested by the microdeletion shared in the ICSI-F10/ICSI-B10 father/baby pair. In this case the F_2 generation (baby ICSI-B10) apparently inherited his microdeletion from his infertile propositus father. In this case, the same microdeletion was also detected in blood leukocytes from ICSI-F10, similar to patient WHT2712 described in Reijo *et al.* (1996), but the microdeletions and specific phenotypes observed in these two patients differed. The severely oligozoospermic patient WHT2712 (40 000–90 000 spermatozoa/ml) carried a large interstitial deletion which involved most of *AZFc* including *DAZ*, whereas oligozoospermic ($5–6\times10^6$ spermatozoa/ml) ICSI-F10 carried a small interstitial microdeletion in the region between *AZFb* and *AZFc*. The fertile parental generation (the father of ICSI-F10) was not screened in this pedigree and therefore the possibility that the microdeletion observed in ICSI-F10 and ICSI-B10 arose, not as a de-novo mutation, but as an insignificant polymorphism, cannot be excluded. Likewise, the possibility of ICSI-F10 inheriting a microdeletion which underwent a pre- or post-meiotic expansion in the germ line of the fertile parental generation cannot be excluded (Medvedev, 1981; Komaki *et al.*, 1995). A more likely explanation of developmental aetiology is that the de-novo mutation event which ultimately led to the infertile F_1 generation propositus ICSI-F10 must have occurred as a pre- or post-meiotic error in the germ cell lineage of his normally fertile father. Similar cases of germ line mosaicism resulting from either pre-meiotic or post-meiotic errors are well documented (Palmiter *et al.*, 1984; Jansen *et al.*, 1994; Saito *et al.*, 1995). Furthermore, a population of 200 fertile men were screened for all STS used in this study, including SY153 (deleted in the ICSI-F10/ICSI-B10 pair) and SY152 (deleted in ICSI-B31 and hypothetically in the germ line of ICSI-F31). No STS were found to be deleted in this fertile, ethnically diverse control population (consisting of American and European Caucasian, Afro-American and Asian individuals), and in a second population of 700 similarly fertile control males, only two STS, namely SY272 and SY207, were found to be deleted in two and three individuals respectively (A.Muallem *et al.*, unpublished). Therefore, using the two-tailed Fisher's exact test (Woolson, 1987), the probability that the deletion of these two STS in the experimental population is different from the control population is significant ($P = 0.021$). In a similar and separate control population of 700 fertile males, no microdeletions of SY152 and SY153 were detected (A.Muallem *et al.*, unpublished). Small microdeletions involving SY152 and/or SY153 have been reported in patients with azoospermia (Najmabadi *et al.*, 1996; Pryor *et al.*, 1996; Stuppia *et al.*, 1996), severe oligozoospermia (Kent-First, 1996; Qureshi *et al.*, 1996) and infertility associated with normal sperm count asthenozoospermia and teratozoospermia (Pryor *et al.*, 1996).

A frequent cause of microdeletions in chromosomes that recombine is aberrant crossover events (Wyandt *et al.*, 1982). However, for genes outside the pseudoautosomal pairing region (PAR), recombination events between areas of homologous or similar sequence repeats (for example Alu repeats) on the X and Y chromosomes could also give rise to deletion or duplication events (Yen *et al.*, 1990). Furthermore, it is possible that the aetiology of microdeletions may be due to aberrant or unbalanced sister chromatid exchange (SCE) occurring due to an apparent instability of the Y chromosome (McClintock, 1933). The instability of the Y chromosome may be due in part to the high frequency of repetitive elements clustered along the length of it in the form of short interspersed tandem repeats (SINES) and long interspersed tandem repeats (LINES)

(Lewin, 1990; Ohno and Tetsuya, 1991; Graves, 1995). Evidence of this instability is provided by Kobayashi *et al.* (1994) in which a fertile parental generation father transmitted a microdeletion to his infertile F_1 generation son. In this case, the microdeletion observed in the infertile son was larger than that observed in the father (Komaki *et al.*, 1995).

One infertile propositus (ICSI-F20) manifested a normal sperm count with very abnormal sperm morphology (90% abnormal) whereas infertile propositus ICSI-F31 manifested severe oligozoospermia. The deletions which we postulate to occur at least in their germ lines and possibly in the precursor primordial germ cells (PGC) are quite different in size in the two cases. Father (ICSI-F20) manifesting a milder phenotype apparently carried a large interstitial deletion involving *AZFb*, AZFc and all of *DAZ* (Figure 1) whereas father (ICSI-F31) with a seemingly more severe phenotype had a single STS deletion comparable to that found in oligozoospermic propositus ICSI-F10 (in the region between *AZFb* and *AZFc*. These results further emphasize the importance of the *AZF* region for fertility generally, and perhaps suggest the presence of another spermatogenesis-related gene or gene copy residing between *DAZ* and *YRRM1*.

Though potential ramifications of Y-linked microdeletions associated with male infertility in an ICSI population have been speculated, the data presented here represent the first genetic screening of a population of ICSI-derived children and their fathers. The results of this study suggest it may be possible to predict the potential of inheriting Y-linked microdeletions, and likewise the ramifications of such inheritance, thus allowing for preimplantation genetic diagnosis of microdeletions in cases in which mosaicism is suspected in the infertile F_1 generation father.

The frequency of Y microdeletions in this population of infertile ICSI father/son pairs is 9.4% and within the incident range of 9–18% reported in other azoospermic and severely oligozoospermic populations (Kent-First, 1996). Only time will tell if, in the process of assisting infertile couples to achieve conception, we are also assisting in the production of a population of males who, having inherited microdeletions from their fathers, will also be faced with infertility when they are adults. Certainly perturbations of fertility-related genes are not life threatening and as reproductive technologies such as ICSI improve, the chances for achieving a pregnancy through artificial means will increase. In any case, the prior knowledge that natural conception could be problematic and that assisted reproduction techniques will be necessary could circumvent years of frustrated searching for answers by the infertile couple.

Though it is likely that the *DAZ* and *YRRM(RBM)* gene families both have roles in the complicated process of spermatogenesis, microdeletions occurring in infertile patients between *AZFb* and *AZFc* suggest that other yet uncharacterized spermatogenesis-related genes or gene copies may exist. Mutation events leading to Y-linked microdeletions may occur as pre- or post-meiotic de-novo germ line mutations in a fertile male. If a son is produced he may inherit the microdeleted Y chromosome and exhibit infertility. Such an individual who does not possess an intact Y chromosome in his soma or in his germ cell lineage may resort to ICSI, and produce sons who also inherit the infertility associated microdeletion as well as all generations to follow. Mutation events leading to microdeletions may also occur as post-zygotic de-novo events in the infertile propositus although this individual will most likely be mosaic for an intact Y and a Y with microdeletions. In the case of mosaicism, microdeletions may not be detected in the blood of the infertile propositus, but the occurrence of the microdeletion in the PGC could lead to germ line mosaicism such that if fertilization is achieved and a son is produced he may inherit the infertility-associated microdeletion. Unequivocal proof of mosaicism associated with Y chromosome microdeletions must be established by assessing the genotype of single spermatozoon in fertile patients and patients who carry Y-chromosome microdeletions. However, it may be possible for an infertile propositus who carries the Y-linked microdeletion in only a fraction of his spermatogonia to father children with an intact Y depending on the ratio of intact Y to mutant Y chromosomes in the germ line. In this scenario, specific microdeletions detected in the soma of the infertile propositus may be screened for by preimplantation genetic diagnosis of embryos resulting from ICSI, thereby permitting the replacement of unaffected male embryo(s).

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