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Submicroscopic deletions in the Y chromosome of infertile men

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Recent investigations have suggested a high prevalence of Y chromosome submicroscopic deletions in men with severely impaired spermatogenesis. We evaluated the frequency of Y chromosome deletions in 160 infertile men using a series of 36 sequence-tagged-sites, emphasizing intervals 5 and 6 of the long arm of the Y chromosome. Peripheral leukocyte DNA was extracted and amplified with two parallel techniques to minimize potential overestimation of the frequency of deletions. The presence of deletions was evaluated relative to patient's sperm concentration, testis volume, and hormonal parameters. Men with sperm concentration <5 $\times$ 10^6/ml had a 7% prevalence of submicroscopic Y chromosome deletions. Deletions were detected in 7% of azoospermic men, 10% of men with <1 $\times$ 10^6 spermatozoa/ml, and 8% of men with >1 $\times$ 10^6 but < 5 $\times$ 10^6 spermatozoa/ml. Other clinical parameters did not identify men with Y chromosome deletions prior to polymerase chain reaction (PCR)-based testing for the presence of sequence-tagged-sites. Two distinct regions of Y chromosome deletions were detected, ~3.6 Mb and 1.4 Mb in length respectively. These deleted regions are present in AZFb and AZFc respectively. No deletions were detected in AZFa. The loss of these two distinct areas is supported by the finding of highly repetitive sequences along the Y chromosome, predisposing to deletion of specific intervals on the Y chromosome during meiosis. Men with severe male infertility are at high risk for Y chromosome deletions. Testing of men for these genetic abnormalities is indicated prior to treatment with assisted reproduction.

Key words: genetics/male infertility/microdeletion/spermatogenesis/Y chromosome

Introduction

Despite recent exciting advances in the treatment of infertility, the aetiology of male factor infertility has often been poorly understood. It has previously been suggested that the Y chromosome contains at least one gene necessary for testicular differentiation [the testis determining factor (TDF) or sex determining region of the Y chromosome (SRY)] (Bertha *et al.*, 1990). Recent information has also supported the importance of the Y chromosome in control of spermatogenesis.

Tiepolo and Zuffardi (1976) reported on the cytogenetic analysis of 1160 infertile men and found that 0.5% had macroscopic deletions of the distal long arm of the Y chromosome (Yq). They proposed the presence of an azoospermic factor (AZF) in the distal euchromatic region of Yq. The limited resolution afforded by karyotypic analysis was significantly improved with the advent of polymerase chain reaction (PCR) and the construction of Y chromosome maps. Chandley and Cooke (1994) studied 50 azoospermic and oligozoospermic men and found deletions in interval 6 of Yq for four men (8%). They characterized a family of genes from this deleted region with RNA-binding protein homology and testis-specific expression that were termed Y chromosome RNA recognition motifs [YRRM(RBM); Ma *et al.*, 1993]. However, YRRM(RBM) is a redundant gene, with multiple copies found along the long arm of the Y chromosome, and at least one YRRM(RBM) sequence variant (a polymorphism) has been found in a fertile man (Kobayashi *et al.*, 1994).

Reijo *et al.* (1995) studied 89 azoospermic men using a PCR-based analysis with 84 sequence-tagged sites on the euchromatic region of the Y chromosome. They found that 12 (13%) of these men had overlapping deletions on Yq. Exon-trapping analysis of this deleted region revealed a novel multi-copy gene referred to as 'deleted in azoospermia' (DAZ). There was no relationship between the deletion size and spermatogenic development on testis biopsy. Subsequent analysis of severely oligozoospermic men revealed that DAZ deletions may be present in men with sufficient spermatogenesis to have spermatozoa in the ejaculate (Reijo *et al.*, 1996).

Vogt *et al.* (1996) have also studied 370 men with idiopathic azoospermia or severe oligozoospermia for submicroscopic deletions of intervals 5 and 6 on Yq. They identified 12 men with interstitial deletions in this multi-centre, collaborative study. Based on their characterization of patients by testicular histology, Vogt *et al.* (1994) suggested that the specific deleted region on Yq (AZFa, AZFb or AZFc) may result in different sperm production defects. Whereas men with AZFc deletions may have spermatozoa in the ejaculate, AZFa deleted patients will more commonly have germinal cell aplasia. However, all studies reported to date have been able to characterize only limited numbers of patients with Y chromosome submicroscopic deletions. Therefore, further evaluation of greater numbers of infertile men for submicroscopic Y chromosome deletions is clearly needed to elucidate the relationship between Y-based genetic defects and abnormalities of spermatogenesis.

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Using the technique of multiplex PCR, we screened the entire euchromatic region of the long arm of the Y chromosome in 160 men with male factor infertility, including men with non-obstructive azoospermia. This study was designed to investigate the regions and frequency of deletions, and to correlate genetic findings with the patients' clinical data to delineate further the phenotypic characteristics of men with submicroscopic deletions in Y. Unlike past generations, men with submicroscopic Y deletions and severe male factor infertility can now father children (Kent-First *et al.*, 1996). The likelihood that these genetic defects will be passed on to the male children of affected men after treatment of these patients with assisted reproduction (Mulhall *et al.*, 1997; Schlegel and Girardi, 1997), including the use of testicular sperm extraction (Devroey *et al.*, 1995; Kahraman *et al.*, 1996; Schlegel *et al.*, 1997), mandates the need to understand the clinical effects of these submicroscopic Y chromosome deletions.

Table I. Multiplex PCR analysis scheme used for detection of submicroscopic Y chromosome deletions, adapted from Henegariu *et al.* (1993)

Multiplex mix	Sequence-tagged site	Concentration in mix (μM)	Expected PCR product length (bp)
I	sY84	0.12	326
	sY134	0.3	301
	sY117	0.08	262
	sY102	0.07	218
	sY151	0.24	183
	sY94	t0.2	150
	sY88	0.4	123
II	sY143	0.2	311
	sY157	0.1	285
	sY158	0.2	231
	sY81	0.24	209
	sY182	0.36	125
	sY147	0.36	100
III	sY86	0.4	320
	sY105	0.08	301
	sY82	0.12	264
	Y6 HP35pr	0.2	226
	Y6 PHc54pr	0.16	166
	sY153	0.24	139
	sY97	0.24	104
IV	sY14	0.22	472
	sY254	0.2	350
	sY95	0.16	303
	sY127	0.3	274
	sY109	0.16	233
	sY149	0.24	132
V	pY6BaH34pr	0.08	910
	Fr15-Iipr	0.24	313
	Y6HP52pr	0.56	258
	Y6HP35pr	0.14	226
	Y6D14pr	0.24	134

Materials and methods

Patient selection and clinical evaluation

Screening of the Y chromosome was performed for 160 consecutive patients who were referred to our Centre for evaluation of male factor infertility and consented to genetic testing. Patients were excluded if clinical evidence of obstructive azoospermia or known cytogenetic defects were present. Charts were reviewed for semen analysis, hormone profile, and testis biopsy results. Testicular volume was evaluated by one examiner using an orchidometer. After obtaining informed consent, blood samples were drawn from patients and maintained at 4°C until genomic DNA was extracted from peripheral leukocytes. Control DNA was extracted from men with a documented history of fertility and a normal genitourinary examination who were having blood drawn for routine evaluation of other medical conditions. DNA extracted from fertile females was used as a negative control. Whenever available, the father or brother of men detected to have deletions was evaluated to determine the de-novo nature of the deleted region.

Multiplex PCR Y chromosome analysis

Genomic DNA was extracted from whole blood using two different methods. Using a Stratagene DNA Extraction kit (Stratagene, La Jolla, CA, USA; catalogue #200600), 5 ml of whole blood was processed using digestion of cellular proteins and subsequent salting out of the proteins with sodium chloride, and precipitation of DNA with ethanol. Parallel isolation of purified genomic DNA was performed by lysis of red blood cells, followed by lysis of white cells and their nuclei. Cellular proteins were removed by salt precipitation, and genomic DNA was precipitated with isopropanol using a Genomic DNA Purification Kit (Promega, Madison, WI, USA; catalogue #A1120). Repeat analyses of each patient with deletions was carried out using DNA extracted with the two different isolation techniques.

A series of 35 sequence-tagged sites (STS) on Yq were used for detection of submicroscopic deletions. In addition, testing for the Yp was performed using sY14, an STS located within the gene SRY (Vollrath *et al.*, 1992). Previously published primer sequences were used for STS on Yq (Henegariu *et al.*, 1993; Reijo *et al.*, 1995, 1996). Primers were produced as dried oligonucleotides on an Applied Biosystem Model 380B automated DNA synthesizer. The rapid molecular screening technique known as multiplex PCR, as described by Henegariu *et al.* (1993), was used initially to evaluate these STS. Specific primers used for each multiplex mix and the expected PCR product length are listed in Table I. Individual PCR reactions were routinely performed for four STS: sY160, sY255, sY277, and sY283. Whenever failure of amplification was detected for a primer pair, subsequent PCR analysis using single primer pairs was performed and repeated, for a total of three evaluations with appropriate positive and negative controls to confirm the absence of each STS.

Genomic DNA (0.15 μg) was added to a mixture of 20 mM Tris–HCl (pH 8.3), 100 mM KCl, 3.0 mM $MgCl_2$, 200 μM of each dNTP, 5% DMSO, 0.07–0.56 μM of each primer pair (as indicated in Table I), 2 IU AmpliTaq DNA polymerase (Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA), and adjusted to a final volume of 25 μl. Amplifications were carried out on a Perkin-Elmer Thermocycler with the following programme: initial denaturation at 94°C for 4 min and a subsequent series of 45 cycles of 54°C, 45 s (annealing), 65°C, 2 min (extension), 94°C for 30 s (denaturation). Final extension was carried out at 65°C for 5 min. All products of PCR amplification were subjected to electrophoresis on 4% agarose gel (NuSieve 3:1; FMC BioProducts, Rockland, ME, USA), stained with ethidium bromide and visualized by exposure to ultraviolet light.

For each man with Y chromosome deletions, a blood sample was also requested from his father or a fertile brother and analysed for Y deletions.

Results

A total of 160 men was screened for submicroscopic Y chromosome deletions. All 160 men had semen analysis

Table II. Frequency of Yq deletions in infertile men, classified by sperm concentration

No. of patients	Classification	Sperm concentration (/ml)	Deletion frequency (%)
108	Azoospermic	None	7/106 (7)
20	Virtually azoospermic	$0–1 \times 10^6$	2/20 (10)
12	Severely oligozoospermic	$>1–5 \times 10^6$	1/12 (8)
10	Moderately oligozoospermic	$>5–20 \times 10^6$	0/10 (0)
10	Normozoospermic	$>20 \times 10^6$	0/10 (0)

information available. When multiple semen analyses were recorded, sperm concentration was calculated as a single average value of all available analyses. A total of 108 men were azoospermic (no spermatozoa in the ejaculate), 42 men were oligozoospermic (greater than zero, but $<20\times10^6$ spermatozoa/ml), and 10 men were normozoospermic ($>20\times10^6$ spermatozoa/ml). Subclassification for oligozoospermia is listed in Table II. The average testis volume for evaluated patients was 14 ml (normal >15 ml; range 2–25 ml). Average serum hormone levels for evaluated patients were follicle stimulating hormone (FSH) 13 mIU/ml (normal 2–10 mIU/ml; range 1.4–32), luteinizing hormone (LH) 4 mIU/ml (normal 2–9 mIU/ml; range 1.6–18 mIU/ml), and testosterone 471 ng/dl (normal 300–1000 ng/dl; range 150–913 ng/dl).

Deletions in the Y chromosome were detected and confirmed with PCR-based analysis for 10 patients. The deletions are schematically depicted in Figure 1. Eight patients had interstitial deletions that could not be detected with classical cytogenetic analysis. For two patients, subsequent karyotype analysis was abnormal, reflecting the terminal deletion of the Y chromosome for SMG 99, and the lack of detectable Y chromosome sequences with standard karyotype evaluation (46, XX[Sry+]) for SMG 135. Subsequent PCR analysis has revealed the presence of Sry for SMG 135 with a short segment of Yp. Additional molecular analysis of this deletion is underway to characterize further the exact length of the presumably translocated Y chromosome.

The eight patients with interstitial deletions had a distinct pattern of Y chromosome regions deleted. Three patients with interstitial deletions (SMG 32, 78, and 126) had lesions involving an approximately 3.6 megabase area spanning STS Y6D14pr–sY143 (intervals 5L–6B; AZFb region), inclusively. An additional four men with interstitial deletions were detected where the deleted region spanned STS sY153–sY158 (intervals 6C–6F), inclusively, which includes the DAZ gene cluster (AZFc; Figure 1). One patient (SMG 62) had a deletion extending over both of the above-mentioned regions Y6D14pr–sY158 (intervals 5L–6F) (Figure 2).

For all ten patients with Yq deletions, the presence of STS between sY143 and sY153, a region spanning approximately 1 megabase, were tested. In this region, the presence of GY26, OX2, GY49, OX8, GY48, sY272, sY144, sY145, sY146, sY148, sY152, and sY220 were evaluated using PCR analysis with previously published primer sequences and reaction conditions (Jones *et al.*, 1994; Reijo *et al.*, 1995). All eight patients with interstitial deletions had GY48, sY272, sY144

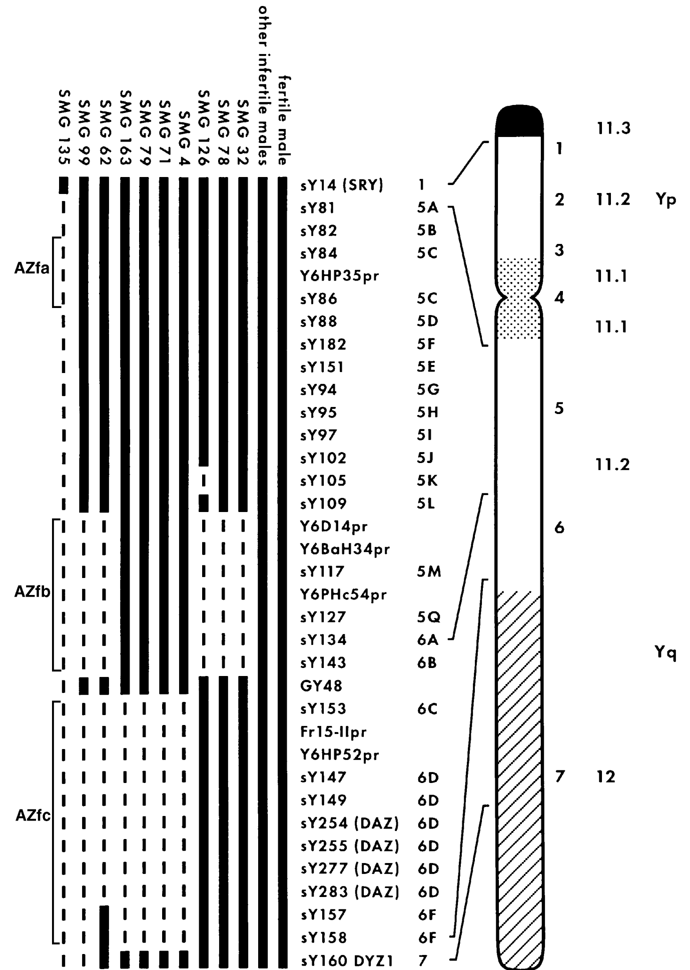

Figure 1. Schematic depiction of Y chromosome microdeletions found for 10 men evaluated in this study, compared to men with normal fertility and other infertile men. The positions of sequence-tagged sites for AZFa, AZFb and AZFc are shown.

and sY145 detected, indicating that the regions of deletion did not overlap. The presence of these STS in the middle of the interstitial deletion for patient SMG 62 suggests that the long interstitial deletion detected on the Y chromosome of this patient actually reflects two separate deletions, with a preserved segment of the Y chromosome between the deletions. The amplification of GY48 and OX8 in patient SMG 99 suggests that this patient had a terminal and interstitial deletion, not initially recognized with multiplex analysis alone. The failure to detect interval STS for patient SMG 135 and female controls suggests that the detection of GY48, sY272, sY144 and sY145 does not reflect artefactual amplification of these STS for patient SMG 62, but supports the apparent termination point for deletions near this region in patients with adjacent interstitial deletions.

No deletions were detected using blood obtained from the six fertile control patients, men of known fertility. No Y chromosome STS were detected in two control female blood samples.

Clinical data were available for all patients with detectable deletions. All were either azoospermic or severely oligozoospermic. Of the three patients with spermatozoa in the ejaculate,

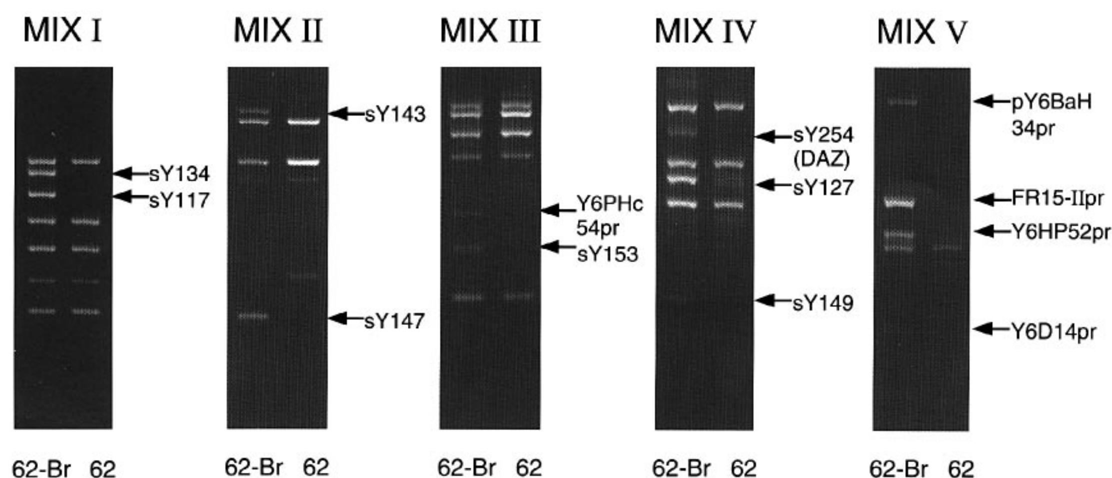

Figure 2. Multiplex PCR results for a representative patient with a deletion (SMG 62) and his normal, fertile brother (62-Br) are demonstrated.

Table III. Summary of clinical data for 10 patients with submicroscopic Y chromosome deletions detected by PCR-based analysis

Patient	Age	Sperm concentration	Testis volume (in ml: L/R)	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/dl)	Karyotype	Testis biopsy results
SMG 4	38	5×10^6	14/14	6	3	317	46, XY	Not biopsied
SMG 32	27	Azoospermic	6/6	28	5	–	–	Not biopsied
SMG 62	39	Azoospermic	17/17	22	7	592	46, XY	Sertoli cell only
SMG 71	34	Azoospermic	15/15	10	5	368	46, XY	Sertoli cell only
SMG 78	27	Azoospermic	20/20	4	4	827	46, XY	Maturation arrest (spermatocytes)[c]
SMG 79	26	0.5×10^6	10/10	18	7	244	46, XY	Sertoli cell only (85%) MA[a](5–10%), HS[b](5–10%)
SMG 99	30	Azoospermic	20/20	17	5	408	46,Xdel(Y)(q11.2)	Maturation arrest (spermatocytes)[c]
SMG 126	36	Azoospermic	18/20	8	5	564	46, XY	Maturation arrest (spermatids)[c]
SMG 135	29	$<0.001 \times 10^6$	8/8	20	18	276	46, XX[d]	Sertoli cell only
SMG 163	29	Azoospermic	12/14	4	3	345	46, XY	Maturation arrest (spermatocytes)[c]

[a]MA: maturation arrest.
[b]HS: hypospermatogenesis.
[c]Maturation arrest (spermatocytes/spermatids): maturation arrest where the most developed spermatogenic cells present were spermatocytes/spermatids.
[d]Subsequent PCR analysis has detected the Sry region in patient SMG 135.
FSH = follicle stimulating hormone; LH = luteinizing hormone; T = testosterone.

all three had deletions in the more distal region of Yq, which includes deletion of the DAZ gene. The rate of detection of submicroscopic deletions was similar for the 108 azoospermic, 20 virtually azoospermic and 12 severely oligozoospermic men tested and ranged from 7 to 10%. These results are summarized in Tables II and III.

Five of the 10 patients with Yq deletions had normal volume (⩾15 ml) testes. Five of the patients had elevated (⩾11 mIU/ml) FSH levels; however, the mean FSH level among deleted patients did not differ significantly from the mean FSH of 13 mIU/ml for all patients evaluated. LH levels were within the normal range for eight of ten deleted patients and the mean LH level of 8 mIU/ml was similar to the overall LH levels for the entire pool of evaluated patients (4 mIU/ml). Seven of the nine evaluable patients had testosterone levels within the normal range, while two patients had low testosterone levels (244 and 276 ng/dl). Karyotype analyses were performed in nine of the 10 patients with Y deletions and were normal for seven.

Testis biopsy information was available for eight of the 10 patients with deletions. Results demonstrated predominantly Sertoli-cell-only pattern in four with focal maturation arrest at the early spermatocyte level, and maturation arrest at the late primary spermatocyte level in three with maturation arrest at the early round spermatid stage for one. One patient with spermatozoa in his ejaculate had a small component of hypospermatogenesis in the biopsy, despite a predominant pattern of Sertoli-cell-only tubules (SMG 79). However, emphasis on results of a single diagnostic testis biopsy should be minimized for these patients. Testicular sperm extraction results have shown that focal areas of spermatogenesis are frequently found in the testes of men with non-obstructive azoospermia (Devroey *et al.*, 1995; Kahraman *et al.*, 1996; Schlegel *et al.*, 1997; Tournaye *et al.*, 1997)

Two azoospermic patients have undergone attempted testicular sperm retrieval with intracytoplasmic sperm injection (ICSI). Neither patient SMG 126, nor patient SMG 62 had retrievable spermatozoa. Round spermatids were isolated for patient SMG 126. Neither of the patients with unsuccessful attempts at sperm retrieval had deletions only in the more distal, DAZ gene cluster (AZFc), region of Yq. One patient (SMG 79) has been followed sequentially during infertility evaluation and treatment over 30 months. During that period of time, his ejaculated sperm concentration has decreased from an average of 0.5×10^6 spermatozoa/ml to azoospermic, without external evidence of gonadotoxins or other definable factors that are likely to affect sperm production. His relatively young age (27 years) may support the hypothesis that older azoospermic men with Yq deletions could have had spermatozoa in their ejaculate earlier in life and subsequently are at risk of becoming azoospermic.

To establish whether these deletions represent de-novo deletions, brothers and fathers were tested when possible. Two fertile brothers and the father of patient SMG 32, a brother of SMG 62, and the father of SMG 126 were all tested for Y chromosome deletions. To date, no deletions have been detected in the male family members of the patients, supporting the finding of de-novo deletions.

Discussion

Many recent studies have supported the concept of a genetic basis for male infertility. A gene responsible for normal spermatogenesis, and referred to as 'the azoospermic factor', has been proposed to exist on interval 6 of the long arm of the Y chromosome. We have screened a population of infertile men and, when possible, their relatives to (i) determine the frequency of deletions in the Y chromosome, (ii) help to map the gene or genes responsible for spermatogenesis, and (iii) establish that these are de-novo deletions.

Prior studies have suggested that 3–18% of men with non-obstructive azoospermia or severe oligozoospermia may have deletions of the Y chromosome present (Chandley *et al.*, 1994; Kobayashi *et al.*, 1994; Reijo *et al.*, 1995, 1996; Kent-First *et al.*, 1996; Najmabadi *et al.*, 1996; Vogt *et al.*, 1996; Mulhall *et al.*, 1997; Pryor *et al.*, 1997). Differences in the frequency of detection of anomalies may reflect exclusion of patients with known, non-genetic aetiologies of poor sperm production (e.g. gonadotoxic chemotherapy) or differences in the technique for PCR-based amplification of Y chromosome sequence-tagged sites. We offered genetic analysis to all men presenting for evaluation and treatment of severe male infertility. Therefore, the results presented in this study appear to reflect best the frequency of Yq deletions that could be detectable in patients undergoing ICSI with ejaculated spermatozoa or sperm cells retrieved from the testis. One study with high rates of detection of Yq deletions involved many men with only single sequence-tagged sites deleted (Najmabadi *et al.*, 1996) which could represent technical problems or polymorphisms. Unless deletion of adjacent STS is confirmed, it is possible that the 'deleted' individuals had inefficient amplification of sequence-tagged sites that were actually present in the man evaluated, since most studies have found that infertile men with Yq deletions will typically have deletions of at least $0.5–1\times10^6$ base pairs of DNA in length (Reijo *et al.*, 1995; Vogt *et al.*, 1996). Of note, a recent article by Pryor *et al.* (1997) has found a similar frequency of 'ultra-short' deletions (those involving three or fewer sequential STS) in infertile men (7/196; 3.5%) and fertile or obstructed (6/204; 2.9%) men. These findings suggest that many ultra-short deletions of the Y chromosome represent a polymorphism, a normal genetic variant, rather than the cause of infertility. Certainly, a very short deletion of a critical gene on the Y chromosome could theoretically be the cause of spermatogenic dysfunction, but this appears to be unusual in the population of men with Y chromosome deletions.

Our study has further documented two regions that are commonly deleted in men with severely impaired spermatogenesis. The more distal region overlaps the area described by Reijo *et al.* (1995, 1996), that includes the DAZ gene in interval 6E, which encodes for a novel RNA binding protein. This distal deleted region is ~1.4 Mb in length, based on published maps of the Y chromosome (Affara *et al.*, 1996). Of the four patients in this study in whom we detected deletions restricted to the DAZ gene region, two (50%) had spermatozoa in their ejaculate. Therefore, deletions of the DAZ gene cluster are not sufficient to cause azoospermia. Of note, all four patients with deletions of only the 'DAZ region' (AZFc) had very similar lengths of the Y chromosome deleted, extending from interval 6C–6F.

The detection of a second distinct region of deletions in patients SMG 32, 78, and 126 suggests that structural factors may predispose to the deletion of specific areas of the Y chromosome. This region of ~3.6 megabases correlates roughly with the area described as 'AZFb' by Vogt *et al.* On our chromosome map in Figure 1, these regions appear to overlap, but at least one STS, GY48, is present between 'AZFb' and the 'DAZ region'. We evaluated for the presence of the GY48 STS in each of the patients who had localized deletions of the Y chromosome and found that all seven of the patients had GY48 present. Analysis of DNA from SMG 62 also detected the GY48 STS, suggesting that this patient actually had two separate, non-overlapping deletions. The absence of GY48 for SMG 135 as well as female DNA supports GY48 as a reliable marker for this region of the Y chromosome (Jones *et al.*, 1994). We have also localized adjacent markers to GY48 in all patients with deletions except patient SMG 135. Several markers, such as sY 272, are repetitive along the Y chromosome and therefore are not shown in Figure 1. However, sY272 is localized both adjacent to GY48 and in the DAZ cluster region deleted for four patients (SMG 4, 71, 79, 163) supporting the preservation of the region near GY48 despite adjacent deletions. Similar results were obtained for the adjacent STS, sY144 and sY145 (Vollrath *et al.*, 1992).

Y chromosome interstitial deletions may occur during meiosis, or with defective DNA packaging in spermiogenesis. The pattern of deletions on the Y chromosome that we have observed appears to be uniquely reproducible among different patients. The Y chromosome is known to contain a high frequency of repetitive elements clustered in short interspersed

tandem repeats and as long interspersed tandem repeats (Ohno and Tetsuya, 1991; Graves, 1995) with significant resultant Y chromosome instability (Kobayashi *et al.*, 1994). Taken together, these findings suggest that intra-Y chromosome or inter-Y chromosome pairing (sister chromatid exchange) may occur during meiosis with subsequent deletions of specific Y chromosome regions, such as AZFb or the DAZ gene cluster region (AZFc), flanked by highly repetitive regions, including that near the GY48 site. The consistent loss of a segment of the Y chromosome containing a gene or genes important for spermatogenesis may then occur.

Loss of a distinct region of the Y chromosome could occur at times other than early meiosis if a regular organization pattern of this chromosome was present. An alternative mechanism of Y deletions could relate to the specific patterns of DNA condensation and packing that occur during spermiogenesis. Ward (1993) has indicated that the DNA is packaged in a regular series of loop domains in the sperm nucleus. Human spermatozoal loop domains, formed during replacement of histones by protamines during spermatogenesis, have been suggested to be ~27 kilobase pairs in size (Barone *et al.*, 1994). It has been suggested that this configuration will allow specific regions of DNA, referred to as replicons, to be most accessible for transcription. Although the loop domains may vary in size for different regions of DNA, it is unlikely that an entire loop domain would be anywhere near the 1.4–3.6 megabase estimated size of Y chromosome submicroscopic deletions. Therefore, it seems unlikely that the deletions occur during spermiogenesis, rather they probably occur by sister chromatid exchange as suggested by Kent-First (1996).

We have not performed statistical analysis to compare the frequency of these documented submicroscopic but molecularly sizeable deletions. Data presented by Kent-First *et al.* (1996) in a survey of 700 fertile men with a wide variety of ethnic backgrounds, indicate that Y chromosome deletions of more than one or two STS are rarely found in fertile men. Our study was designed to evaluate the frequency of deletions in men with severely impaired spermatogenesis and emphasize the value of genetic testing prior to treatment with assisted reproduction.

The data presented here suggest that de-novo deletions of regions of the Y chromosome occur frequently in men with severe male factor infertility. The amount of chromosome deleted does not appear to affect the severity of the spermatogenic defect. Furthermore, it is not possible to detect which men with testicular failure will have these deletions based on clinical parameters such as testis volume, testosterone, FSH or age. Since Y chromosomal inheritance is always of paternal origin, men with somatic partial Y chromosome deletions are likely to have boys with similar genetic defects after treatment with assisted reproduction. Kent-First *et al.* (1996), in a limited study, have already found that 9% of ICSI-derived sons had Y chromosome deletions. These findings support the recommendation of Y chromosome deletion analysis for all men with severe oligozoospermia or non-obstructive azoospermia prior to treatment with assisted reproduction.

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