

## MICRODELETIONS IN THE Y CHROMOSOME OF INFERTILE MEN

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## ABSTRACT

**Background** Some infertile men with azoospermia or severe oligospermia have small deletions in regions of the Y chromosome. However, the frequency of such microdeletions among men with infertility in general is unknown. We sought to determine the prevalence of Y-chromosome microdeletions among infertile men and to correlate the clinical presentation of the men with specific deletions.

**Methods** We studied 200 consecutive infertile men. Each man was evaluated comprehensively for known causes of infertility, and Y-chromosome microdeletions were studied with use of the polymerase chain reaction to amplify specific regions of the chromosome. The Y chromosomes of 200 normal men were also analyzed.

**Results** Fourteen infertile men (7 percent) and four normal men (2 percent) had microdeletions of the Y chromosome. Nine of the infertile men had azoospermia or severe oligospermia (sperm concentration, <5 million per milliliter), four had oligospermia (sperm concentration, 5 million to <20 million per milliliter), and one had normospermia (sperm concentration, ≥20 million per milliliter). The size and location of the deletions varied and did not correlate with the severity of spermatogenic failure. The fathers of six infertile men with microdeletions were studied; two had the same deletions as their sons, and four had no deletions.

**Conclusions** A small proportion of men with infertility have Y-chromosome microdeletions, but the size and position of the deletions correlate poorly with the severity of spermatogenic failure, and a deletion does not preclude the presence of viable sperm and possible conception. (N Engl J Med 1997;336:534-9.)

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INFERTILITY affects about 15 percent of all couples attempting pregnancy,<sup>1</sup> with the man responsible in approximately half the cases. It is best defined as the inability to conceive after one year of unprotected intercourse, and thus the definition includes men with subfertility. Proposed causes of infertility in men include varicocele, obstruction of the spermatic ducts, agglutination of sperm, high semen viscosity, necrospermia, low volume of ejaculate, ejaculatory dysfunction, and high sperm density; when no cause is known, the man is described as having idiopathic infertility.<sup>2</sup> Many of these diagnostic categories are descriptive; for example, a diagnosis of necrospermia does not provide any information about why the sperm are dead. Conse-

quently, the estimated proportion of men with idiopathic infertility is as high as 66 percent, depending on the definition of "idiopathic."<sup>3,4</sup>

Cytogenetic analysis of men with Y-chromosome translocations has revealed a region on the long (q) arm of the Y chromosome that is required for spermatogenesis.<sup>5,6</sup> This region includes the azoospermia factor (AZF) locus, which contains a gene or genes that are required for normal spermatogenesis. The AZF locus has been mapped to deletion interval 6, a region in band q11.23 of the Y chromosome that contains 5 million base pairs.<sup>7,9</sup> Three genes — two members of the *YRRM* (Y-specific gene with RNA recognition motif) gene family (*YRRM1* and *YRRM2*) and the *DAZ* (deleted in azoospermia) gene — have been cloned from this region, whereas *DAZ* and one or more members of the *YRRM* family have been found to be absent in some men with azoospermia or severe oligospermia.<sup>10-12</sup>

To date, only men with azoospermia or severe oligospermia have been studied for Y-chromosome deletions.<sup>10-16</sup> These analyses, which have been limited to the distal euchromatin of the q arm, have revealed deletions in 10 to 15 percent of the men studied. The frequency of Y-chromosome microdeletions in infertile men with less severe abnormalities of the sperm concentration is unknown. Furthermore, how other regions of the Y chromosome are involved in infertility has not been studied. We sought to determine the frequency of the Y-chromosome microdeletions in a large group of infertile men and to determine the phenotypes associated with specific deletions.

## METHODS

## Study Subjects

We studied 200 consecutive men presenting with infertility at the urology clinic of the University of Minnesota (Minneapolis) or Reproductive Health Associates (St. Paul, Minn.) and 200 normal men. All men who were referred for evaluations of infertility and met the definition of infertility (one year of unprotected intercourse not leading to conception) were enrolled in the study, regardless of the fertility status of their partners. The infertile men ranged in age from 24 to 52 years (mean, 34).

The men completed detailed questionnaires on their medical

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and surgical history, lifestyle habits (such as smoking, alcohol use, and drug use), exposure to gonadotoxins (such as drugs used in cancer chemotherapy, and solvents), sexual history, and family history. They then underwent a physical examination that included an assessment of secondary sexual characteristics, an inspection of the penis, a determination of testicular size by orchidometry, an evaluation of the vas deferens and epididymis, and a rectal examination to evaluate the prostate. Each man provided a minimum of two semen specimens, each after sexual abstinence for two to five days. These specimens were evaluated on the basis of the criteria of the World Health Organization, except for sperm morphology, which was assessed by the strict criteria of Kruger et al.<sup>17</sup> (with normal morphology defined as the presence of more than 14 percent sperm of normal shape), and the results were averaged.

On the basis of their mean sperm concentrations, the men were categorized as having azoospermia, severe oligospermia (<5 million sperm per milliliter), oligospermia (5 million to <20 million sperm per milliliter), or normospermia ( $\geq 20$  million sperm per milliliter). Testicular biopsies were performed in most infertile men with azoospermia. The presence of antisperm antibodies was determined by the direct immunobead test in semen samples in which sperm agglutination or decreased sperm motility was seen and in samples from men with abnormal postcoital tests or idiopathic infertility. In addition, blood samples were obtained for DNA extraction and for the measurement of serum testosterone, prolactin, and follicle-stimulating hormone (FSH) by radioimmunoassay.

After the evaluation, each man was given one of the following diagnoses: varicocele, antisperm antibodies, ejaculatory dysfunction (such as those associated with spinal cord injury or diabetes mellitus in which there was anejaculation or retrograde ejaculation), endocrinopathy (hypogonadotropic hypogonadism or hyperprolactinemia), obstruction of the spermatic duct (azoospermia in the presence of normal results on testicular biopsy), dysfunction induced by gonadotoxins (cancer chemotherapy), and infection (urethritis or prostatitis). If a man could not be assigned to one of these diagnostic categories, he was classified as having idiopathic infertility, regardless of the results of the semen analysis.

We also obtained blood samples from the fathers of six infertile men who were found to have Y-chromosome microdeletions. DNA from 200 normal men was provided (Promega, Madison, Wis.) from a serum bank of men proved fertile by paternity testing. The study design was approved by the institutional review board of the University of Minnesota, and all the participants gave informed written consent.

#### Screening for Y-Linked Sequence-Tagged Sites

Genomic DNA was prepared from peripheral-blood lymphocytes (Wizard Genomic DNA Purification Kit, Promega) and amplified in multiplex polymerase chain reactions (PCRs) containing 5 to 8 primer pairs. Each primer pair amplifies a specific region of the Y chromosome (a sequence-tagged site). The reaction products were separated on 3 percent agarose gels (Metaphor, FMC Bio-products, Rockland, Me.) and visualized with ethidium bromide. The men were screened for 85 sequence-tagged sites specific to the Y chromosome (Fig. 1). These sites were derived from the maps of Vollrath et al.,<sup>18</sup> Affara et al.,<sup>19</sup> and Kent-First et al. (unpublished data). The primers used for *YRRMI* were as described by Ma et al.<sup>10</sup> and corrected by Kobayashi et al.<sup>20</sup>

An example of the multiplex PCR analysis of DNA from an infertile man with a Y-chromosome deletion and a normal man is shown in Figure 2, where the absence of an amplified DNA fragment indicates a deletion of that portion of the Y chromosome. Figure 2 shows the loss of 23 sequence-tagged sites in all. For each blood sample, the entire DNA analysis was repeated at least three times. If the analysis of the first blood sample revealed a deletion of one or more sites, a second sample was obtained and the analysis was repeated. In each case, the analysis of the second sample confirmed the initial result. All the PCR analyses were done

without knowledge of the man's clinical diagnosis or the results of the semen analysis. Every analysis contained a blood sample from a normal man, and samples from a normal woman were assayed intermittently. When sufficient DNA was available, the PCR findings regarding microdeletions were confirmed by Southern blot hybridization.<sup>21</sup>

#### Cytogenetic Analysis

For men with microdeletions in the Y chromosome, karyotyping was performed by standard techniques.<sup>22</sup>

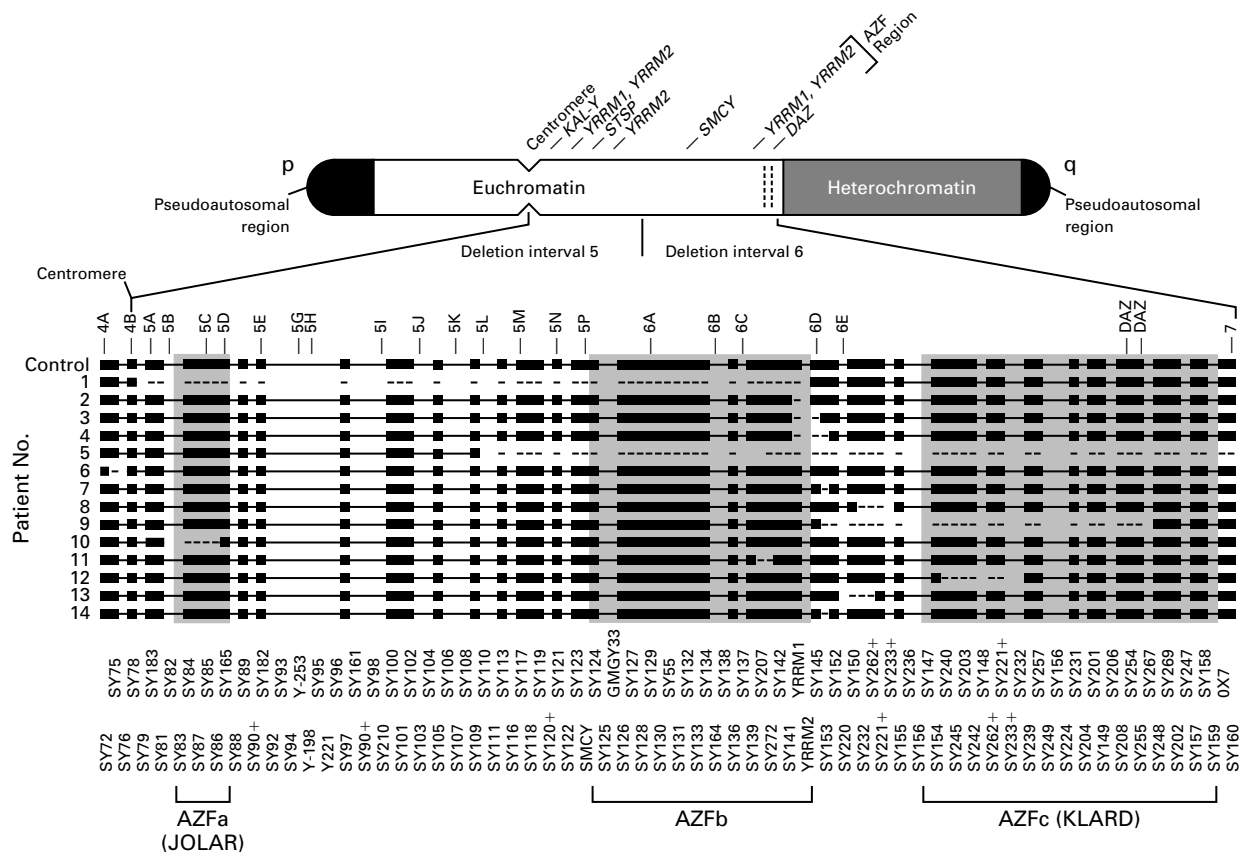
## RESULTS

Among the 200 men with infertility, the most common assigned diagnoses were idiopathic infertility (102 men, or 51 percent) and varicocele (71 men, or 36 percent) (Table 1). Twenty-six of the men with infertility (13 percent) had azoospermia, four of whom had evidence of spermatic-duct obstruction. Thirty men (15 percent) had severe oligospermia, 42 men (21 percent) had oligospermia, and the remaining 102 (51 percent) had normospermia.

Of the 200 infertile men, 14 (7 percent) were found to have Y-chromosome microdeletions (Fig. 1 and Table 2). Of these 14 men, 6 had azoospermia, 3 had severe oligospermia, 4 oligospermia, and 1 normospermia. Seventy-one percent of the men with deletions (10 of 14) had idiopathic infertility, as compared with 51 percent of the group as a whole (102 of 200). All the men with microdeletions had normal serum testosterone concentrations, and two had high serum FSH concentrations. Two men, Patients 2 and 11, had fathered children. Patient 2 fathered a child in 1995 at the age of 37 years by intrauterine insemination. Patient 11 fathered two children, one in 1987 at the age of 29 and one in 1991 at the age of 33.

Microdeletions of the Y chromosome were found in 4 of the 200 normal men (2 percent). Two normal men had deletions of site SY207, and two had deletions of the adjacent site SY272 (Fig. 1). The deletion in Patient 11, who had fathered two children, was limited to these two sites. The large deletions in Patients 1 and 5 also included these two sites. The microdeletions in the remaining 11 infertile men did not overlap with those in the normal men.

The deletions detected in 12 of the 14 infertile men either were completely within deletion interval 6 or included some portion of it (Fig. 1). However, one man (Patient 6) had a completely intact q arm, and a small deletion in the proximal portion of the p arm. A second man (Patient 10) had a deletion in the proximal portion of deletion interval 5. The deletions ranged greatly in size; Patient 5 was missing 53 of the sequence-tagged sites studied, whereas Patients 2, 6, 7, and 14 were missing only 1 site. The three men with the largest deletions (Patients 1, 5, and 9) all had azoospermia or severe oligospermia. The remaining men had small deletions, none of them in the region of the *DAZ* gene. Three men



**Figure 1.** Maps of the Y Chromosome in 14 Men with Infertility and Deletions in the Chromosome.

A diagram of the entire chromosome is shown at the top, with certain sequence-tagged sites indicated in deletion intervals 5 and 6 of the q arm. This section of the chromosome is enlarged below, with the results of the analyses of the 14 men with infertility and a normal control appearing as horizontal lines. Three regions (AZFa, or JOLAR; AZFb; and AZFc, or KLARD) are shaded on the map; the names of the sequence-tagged sites appear at the bottom of the figure. In each map, the bars represent sites found to be present, and the solid lines sites not studied but assumed to be present because they fall between sites confirmed as present. Dashes indicate sites found to be deleted. The total number of dashes shown for each patient corresponds to the number of deleted sequence-tagged sites given in Table 2.

The order of the sites was derived from the literature<sup>10,12,17</sup> and from the analysis of deletions in this study. Plus signs indicate sites, including those in *YRRM1* and *YRRM2*, whose exact order is not confirmed. Patients 5, 8, 9, 10, 13, and 14 underwent testicular biopsy. Additional information about the patients, including sperm counts, is given in Table 2. *KAL-Y* denotes Kallmann's syndrome locus, *STSP* steroid sulfatase pseudogene, and *SMCY* histocompatibility Y locus. Other abbreviations are spelled out in the text.

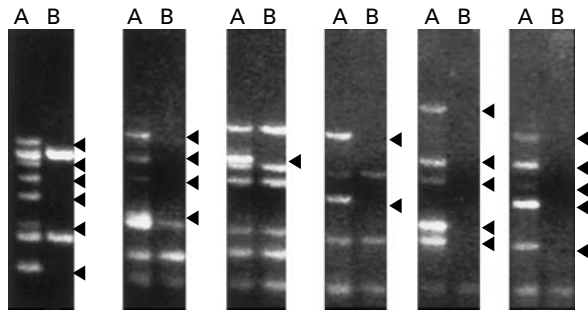
(Patients 2, 3, and 4) had deletions only in the region of *YRRM1* and *YRRM2*; two of these men had oligospermia, and one had severe oligospermia. Patients 7 and 14 had deletions of the same sites; the former had normospermia, and the latter had azoospermia. Overall, there was no correlation between the sperm concentration and the size or the location of the deletions.

Six men with azoospermia or severe oligospermia who had microdeletions underwent testicular biopsy (Table 2). Four of these men (Patients 5, 8, 9, and 10) had severe defects of spermatogenesis. Patient 8 had the same histologic features as Patient 9, but a much smaller deletion. Similarly, the biopsy specimens from Patients 5 and 10 revealed only Sertoli

cells and no germ cells, but the deletions in these patients differed substantially. Two of the four men with spermatic-duct obstruction and normal testicular-biopsy specimens had microdeletions.

Cytogenetic analysis revealed morphologically normal Y chromosomes in 11 of the 12 infertile men whose karyotypes were obtained. Only Patient 5 had an abnormal-appearing Y chromosome.

In the six fathers of infertile men whose Y chromosomes we studied, two (the fathers of Patients 9 and 11) had microdeletions identical to those of their sons, and four (the fathers of Patients 3, 8, 12, and 13) had no microdeletions. Paternity was established in these six fathers by DNA analysis of short tandem-repeat polymorphisms at nine loci.



**Figure 2.** Results of PCR Amplification of Six Sets of Sequence-Tagged Sites in Y-Chromosome DNA from a Normal Man (A) and a Man with Infertility (B).

The arrowheads indicate the Y-chromosome deletions found in the man with infertility (Patient 1). The conditions of thermocycling were as follows: 94°C for one minute, 61°C for one minute, and 72°C for one minute, for 35 cycles. The DNA products were separated by electrophoresis on a 3 percent Meta-phor agarose gel. The PCR analysis shown represents a sub-group of the sequence-tagged sites shown in Figure 1.

**DISCUSSION**

We found microdeletions in the Y chromosome in 7 percent of an unselected group of infertile men. Among the men with azoospermia or severe oligospermia, 16 percent (9 of 56) had deletions, a proportion consistent with those (10 to 15 percent) reported previously.<sup>11,12,14</sup> If we considered only the men with azoospermia, the frequency of deletions in-

creased to 23 percent. However, some men with Y-chromosome deletions had sperm concentrations of 5 million per milliliter or above. Thus, microdeletions are not necessarily associated with azoospermia or very low sperm concentrations. In addition, not all the men with deletions had idiopathic infertility. Specifically, four such men had other possible causes of their infertility: two had spermatic-duct obstruction, and two had varicoceles. Although varicoceles are associated with infertility, only one man in six with a varicocele presents with infertility.<sup>23</sup> Therefore, the primary cause of the infertility of the two men with varicoceles may well have been the microdeletion in the Y chromosome. Likewise, if reconstructive microsurgery did not restore fertility in the two men with spermatic-duct obstruction, the Y-chromosome deletion might explain their infertility.

It is possible to have a deletion in the Y chromosome and to father children. Two of the infertile men we studied fathered children at least once, and the fathers of two of the infertile men had microdeletions identical to those of their sons. As discussed below, the deletion in the father of Patient 11 may not have affected his fertility. However, the father of Patient 9 reportedly had low sperm concentrations, was able to father only one child, and then adopted other children, suggesting that in his case the deletion did affect fertility. Deletions in the fathers of men with infertility that are similar or identical to those in their sons have been reported previously.<sup>12,24</sup> Thus, an inherited deletion in the Y chromosome can cause subfertility.

**TABLE 1.** CHARACTERISTICS OF 200 MEN WITH INFERTILITY.\*

CAUSE OF INFERTILITY	NO. OF MEN (%)	AZOO-SPERMIA	SEVERE OLIGO-SPERMIA	OLIGO-SPERMIA	NORMO-SPERMIA	ATROPHIC TESTIS†		ELEVATED SERUM FSH‡	Y-CHROMOSOME DELETIONS
						RIGHT	LEFT		
no. of men									
Idiopathic infertility	102 (51)	20	19	14	49	28	32	25	10
Varicocele	71 (36)	0	11	22	38	11	16	6	2
Antisperm antibodies	7 (4)	0	0	0	7	1	0	0	0
Ejaculatory dysfunction	5 (2)	1	0	1	3	1	1	1	0
Endocrinopathy§	6 (3)	0	0	4	2	1	1	0	0
Obstruction	4 (2)	4	0	0	0	0	0	0	2
Gonadotoxin¶	4 (2)	1	0	1	2	1	1	3	0
Infection	1 (0.5)	0	0	0	1	0	0	0	0
Total	200 (100)	26	30	42	102	43	51	35	14

\*Severe oligospermia was defined as a sperm concentration of less than 5 million sperm per milliliter, oligospermia as a concentration of 5 million to less than 20 million sperm per milliliter, and normospermia as a concentration of 20 million sperm per milliliter or higher.

†An atrophic testis was defined as one with a volume of less than 20 ml, as measured with an orchidometer.

‡The normal range is 1 to 12 IU of follicle-stimulating hormone (FSH) per liter; the lower limit of sensitivity of the assay was 0.2 IU per liter.

§All the men with endocrinopathy had either hyperprolactinemia or hypogonadotropic hypogonadism.

¶All the men with exposure to gonadotoxins had received chemotherapy for cancer.

**TABLE 2.** FINDINGS IN MEN WITH INFERTILITY WHO HAD MICRODELETIONS IN THE Y CHROMOSOME.\*

PATIENT No.	CAUSE OF INFERTILITY	TESTIS†		SPERM CONCENTRATION ( $\times 10^{-6}/\text{ml}$ )	ASTHENO-SPERMIA‡	TERATO-SPERMIA§	SERUM FSH (IU/liter)¶	KARYOTYPE	PATHOLOGICAL FINDINGS ON TESTICULAR BIOPSY	No. OF DELETED SEQUENCE-TAGGED SITES
		RIGHT	LEFT							
1	Idiopathic	Atrophic	Atrophic	0	NA	NA	18.0	Not done	No biopsy	40
2	Idiopathic	Absent	Atrophic	14	Yes	Yes	9.0	Normal	No biopsy	1
3**	Idiopathic	Atrophic	Atrophic	1	Yes	No	20.0	Normal	No biopsy	2
4	Idiopathic	Atrophic	Atrophic	18	No	No	5.0	Normal	No biopsy	3
5	Idiopathic	Atrophic	Atrophic	0	NA	NA	12.0	idic(Y)(q12)	Sertoli cells only	53
6	Idiopathic	Normal	Atrophic	7	Yes	No	1.0	Normal	No biopsy	1
7	Idiopathic	Normal	Normal	89	Yes	Yes	7.1	Normal	No biopsy	1
8**	Idiopathic	Atrophic	Atrophic	0	NA	NA	9.0	Normal	Severe HSG or SGA	3
9††	Idiopathic	Normal	Normal	0.3	No	No	7.0	Normal	Severe HSG or SGA	22
10	Idiopathic	Normal	Normal	0	NA	NA	8.7	Not done	Sertoli cells only	4
11  ††	Varicocele	Normal	Normal	17	Yes	Yes	4.0	Normal	No biopsy	2
12**	Varicocele	Normal	Normal	1	Yes	Yes	5.3	Normal	No biopsy	6
13**	Obstruction	Normal	Normal	0	NA	NA	2.0	Normal	Normal	3
14	Obstruction	Normal	Normal	0	NA	NA	3.5	Normal	Normal	1

\*FSH denotes follicle-stimulating hormone, NA not applicable, HSG hypospermatogenesis, and SGA spermatogenic arrest.

†An atrophic testis was defined as one with a volume of less than 20 ml, as measured with an orchidometer.

‡Asthenospermia (decreased motility) was defined as overall sperm motility of less than 50 percent.

§Teratospermia (abnormal sperm morphology) was defined as present when the proportion of normal-shaped sperm was 14 percent or less.

¶The normal range is 1 to 12 IU of FSH per liter.

||This patient had fathered children.

\*\*This patient's father had no microdeletions of the Y chromosome.

††This patient's father had Y-chromosome microdeletions identical to those of the patient.

Some Y-chromosome microdeletions represent normal polymorphisms of the Y chromosome, as appeared to be true of the small deletions we found in four fertile men.<sup>24,25</sup> It is possible that in some men with infertility Y-chromosome microdeletions are fortuitous and unrelated to the men's infertility. This was likely to be the case in Patient 11, who had a small deletion in the same region as the deletions we found in the four fertile men. The absence of Y-chromosome microdeletions in the normal men that resembled the deletions in most of the infertile men suggests that the deletions in the latter contributed to their infertility.

No strict correlation between particular deletions and spermatogenesis was found in this study, but some trends were apparent. First, all the large deletions were associated with azoospermia, but small deletions were not necessarily associated with less severe defects in the sperm concentration. Second, deletions were found in several locations on the Y chromosome, with the majority in deletion interval 6. Recently, Reijo et al. described 12 men with azoospermia who had deletions in the Y chromosome (selected from 89 men with azoospermia), and they cloned the *DAZ* gene from deletion interval 6.<sup>11</sup> We found that many of the deletions in interval 6 that were associated with azoospermia were out-

side the region of the *DAZ* gene. This corroborates the recent observation by Najmabadi et al.<sup>12</sup> that not all deletions in men with azoospermia are in *DAZ*. In addition to the deletions in the q arm of the Y chromosome that we identified, a deletion confined to the proximal region of the p arm was found in one man. However, since his father was not studied, the importance of this deletion is questionable.

Ma et al.<sup>10</sup> and Henegariu et al.<sup>26</sup> have described Y-chromosome deletions in infertile men that define two regions associated with azoospermia: JOLAR (in the proximal q arm) and KLARD (in the distal q arm). More recently, Vogt et al.<sup>24</sup> described three men with azoospermia who had microdeletions occurring between the deletions in JOLAR and KLARD; they termed this intermediate region AZFb and designated the JOLAR region AZFa, and the KLARD region AZFe (Fig. 1). On the basis of the testicular histology in these men, the deletion of AZFa was associated with the presence only of Sertoli cells, the deletion of AZFb with the developmental arrest of germ cells at the pachytene stage, and the deletion of AZFc with the developmental arrest of germ cells at the spermatid stage. In our study, Patient 10 had an AZFa deletion, and his biopsy specimen revealed only Sertoli cells; Patient 8 had a deletion in AZFc and had spermatogenic ar-

rest. Patient 5 had an intact AZFa region, but his biopsy sample revealed only Sertoli cells. Patients 2, 4, and 7 had AZFc deletions but not azoospermia, and Patients 13 and 14 (with azoospermia) had deletions in AZFc although their biopsy specimens showed normal spermatogenesis. From our results it is impossible to attribute a given phenotype to a given deletion interval. Finally, 11 of the 12 men with Y-chromosome microdeletions whose karyotypes we determined were cytogenetically normal, showing that a PCR-based assay is needed to detect microdeletions in the Y chromosome.

The correlation between Y-chromosome deletions and infertility, and the relative absence of such deletions in fertile men, suggest a cause-and-effect relation between the deletions and infertility. As compared with other known causes of infertility, Y-chromosome deletions are relatively frequent (7 percent), and their frequency increases with the severity of the spermatogenic defect. However, Y-chromosome microdeletions cannot be predicted on the basis of clinical findings or even the results of semen analyses. The role of analyses of Y-chromosome microdeletions in evaluating men with infertility remains to be determined. With the advent of intracytoplasmic sperm injections, the potential for passing on these defects to offspring is real and should be considered when infertile couples are counseled about this procedure.

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