

## Y Chromosome Microdeletions in Idiopathic Infertile Men from West Azarbaijan

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### ABSTRACT

**Introduction:** Although assisted reproduction techniques are used extensively in Iran, screening for Y chromosome microdeletions before intracytoplasmic sperm injection is often undervalued. Our aim was to investigate Y chromosome microdeletions in men with idiopathic azoospermia or severe oligospermia.

**Materials and Methods:** In 99 selected patients with azoospermia or severe oligospermia and elevated levels of follicle-stimulating hormone and luteinizing hormone in combination with low serum testosterone levels, 20 pairs of sequence-tagged site-based primer sets specific for the Y microdeletion loci were analyzed. Primers were chosen to cover azoospermia factor (AZF) regions as well as deleted in azoospermia (DAZ) and the sex-determining region on Y chromosome (SRY) genes. Also, 100 healthy men served as a control group.

**Results:** Twenty-four patients (24.2%) had microdeletions in AZF genes, but no microdeletions were found in men in the control group. In 15 patients (62.5%), 1 deletion was found. Six patients (25%) had 2, and 3 (12.5%) had 3 deletions. The deletions mainly comprised the AZFc region (in 21 of 24 patients; 87.5%), which corresponds to the DAZ gene. Deletions in AZFb were found in 7 patients (29.2%), and 4 (16.7%) had deletions in the proximal part of AZF regions near SRY gene. No microdeletions were seen in the AZFa or SRY gene.

**Conclusion:** Our results emphasize that Y chromosome microdeletion analysis should be carried out in all patients with idiopathic azoospermia or severe oligospermia who are candidates for intracytoplasmic sperm injection.

**KEYWORDS:** oligospermia, microdeletion, infertility, Y chromosome

### Introduction

Infertility occurs in approximately 14% of couples, and abnormalities in the male partner are estimated to be present in up to 50% of cases.<sup>(1,2)</sup> Efforts to evaluate the causes of azoospermia have shown that regardless of traditionally recognizable causes (abnormal karyotype, obstruction, varicocele, hormonal

defect, etc), most cases (50% to 75%) are unexplained and are considered idiopathic.<sup>(3)</sup> The existence of an essential spermatogenesis factor called azoospermia factor (AZF) was suspected as early as 1976, from de novo Yq deletions in azoospermic patients.<sup>(4)</sup> Polymerase chain reaction (PCR) studies of sequence-tagged sites (STSs) distributed every 30 kb enabled detection of small deletions in the AZF region that had been undetectable with classical cytogenetic techniques.<sup>(5,6)</sup> This led to the identification of 3 loci in Yq11 carrying genes involved in the control of spermatogenesis, corresponding to 3 deleted regions: AZFa, AZFb, and AZFc.<sup>(7,8)</sup> Y

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chromosome microdeletions are the most frequently encountered genetic abnormality in male infertility. Up to 30% of men with idiopathic azoospermia have microdeletions of the Y chromosome,<sup>(3,9-13)</sup> and the incidence of Y chromosome microdeletions in infertile men varies between studies, from 1% to 55%.<sup>(14)</sup> The molecular diagnostics of the Y chromosome have been restricted mostly to selected patients presenting with either azoospermia or severe oligospermia (sperm concentration of less than  $5 \times 10^6/\text{mL}$ ), so that the majority of Y chromosome microdeletions have appeared in this group of infertile men.

Screening for Y chromosome microdeletions is recommended in patients with severely impaired spermatogenesis, in particular, before intracytoplasmic sperm injection (ICSI).<sup>(15)</sup> In the West Azarbaijan province of Iran, at least 5 infertility centers are involved in treating infertile couples, but none of them checks their male candidates for Y chromosome microdeletions. Our study aimed to determine the incidence of Y chromosome microdeletions in our patients in Urmia, the center of West Azarbaijan. The findings of this study may provide enough evidence for clinicians to decide whether or not to screen all idiopathic infertile men for Y chromosome microdeletions before attempting ICSI procedures.

## Materials and Methods

In a case-control study, 99 consecutive men examined in our infertility clinics from November 2001 to December 2003 were screened for Y chromosome microdeletions. The inclusion criteria were azoospermia or severe oligospermia (sperm concentration of less than  $5 \times 10^6/\text{mL}$ ), small testis volume, elevated serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), low serum testosterone level, and the 46,XY karyotype. Informed consent was obtained from each patient, according to protocols approved by the ethics review board of Urmia University of Medical Sciences.

Semen analysis was carried out using WHO criteria<sup>(16)</sup> with a Nikon phase contrast microscope (Nippon Kogaku, Tokyo, Japan). Serum hormone levels of FSH, LH, and testosterone were measured by solid-phase, two-site chemiluminescent enzyme immunoassay (Immulate, Diagnostic Products

Corporation, Los Angeles, Calif, USA). Normal reference ranges for men were FSH, less than 10 mIU/mL; LH, less than 10 mIU/mL, and testosterone, 270 ng/dL to 1070 ng/dL.

Cytogenetic analysis was carried out on peripheral lymphocytes to rule out cases of abnormal karyotypes. GTG-banding was performed according to standard procedures.

One hundred age-matched fertile men with a normal semen analysis without genital abnormalities, selected from couples who had been referred for tubectomy or vasectomy, served as controls. The PCR and cytogenetic analyses were carried out in controls, too.

**Polymerase chain reaction amplification of the three AZF loci.** Genomic DNA was obtained from peripheral leukocytes using the Nucleon Kit II (Scotlab, Wiesloch, Germany). A set of 20 Y-specific STSs spanning the euchromatic region of Yq from centromere to interval 7, with particular interest in interval 6 (the AZF region), was tested in each patient. To check the AZFa region, PCR amplifications were carried out to evaluate the sY81, sY83, and sY121 sites. Using the sY128, sY130, sY133, and sY143 sites, the AZFb region was checked. The AZFc region was screened using the sY147, sY149, sY242, sY231, sY254, sY255, sY182, and sY238 sites. In addition, sY202, sY158, and sY157 were included, corresponding to the downstream area of the DAZ (deleted in azoospermia) gene, as well as sY14 for the sex-determining region on the Y chromosome (SRY) gene and sY274 as the site next to the SRY region. As a negative control, every PCR reaction included 1 sample of female genomic DNA. A sample was considered negative if a product of the expected size was not obtained after 3 PCR attempts.

The PCR program was as follows: amplification of DNA by 35 cycles with 94°C for 50 seconds, 57°C for 30 seconds, and 72°C for 90 seconds; including an initial denaturation step at 94°C for 2 minutes, and a final extension step at 72°C for 10 minutes. The PCR products were separated on 1.5% agarose gels.

**Statistical analyses.** Frequencies of the Y chromosome microdeletions were compared between the patients with infertility and controls using the chi-square test. Values for *P* less than .05 were considered statistically significant.

## Results

Overall, of 99 infertile men, 39 (39.4%) had

**TABLE 1.** Mean serum concentrations of FSH, LH, and testosterone in men with severe oligospermia and azoospermia

	Mean (Range)		
	Men with oligospermia	Men with azoospermia	Infertile men
FSH (mIU/mL)	9.9 (2 to 28)	14.9 (6 to 28)	14.1 (2 to 44)
LH (mIU/mL)	5.2 (1 to 18)	7.6 (2 to 11)	6.3 (1 to 18)
Testosterone (ng/dL)	366 (135 to 565)	344 (223 to 482)	355 (135 to 565)

oligospermia and 60 (60.6%) had azoospermia. The paraclinical data of infertile patients are summarized in Table 1. Of 39 patients with oligospermia, 6 (15.4%) had deletions, and of 60 patients presenting with azoospermia, 18 (30%) had deletions in the AZF region of the Y chromosome. Five of the 6 patients with oligospermia and Y chromosome microdeletions had a sperm concentration of less than  $0.1 \times 10^6/\text{mL}$  with only a few immotile spermatozoa observed after centrifugation of the specimen. The sperm concentration of the sixth patient in this group was  $2.4 \times 10^6/\text{mL}$ .

In general, 24 infertile men (24.2%) showed microdeletions of the Y chromosome, while no microdeletions were detected in controls ( $P < .001$ ). Some of the patients' PCR products that were run on a 1.5% agarose gel are shown in Figure 1.

In 15 patients (62.5%), 1 deletion was found. Six patients (25%) had 2, and 3 (12.5%) had 3 deletions. The deletions mainly comprised the AZFc region (in 20 out of 24 patients; 83.4%), which corresponds to the DAZ gene. Deletions in AZFb were found in 7 patients (29.2%), and 4 (16.7%) had deletions proximal to the AZF regions near the SRY gene. No microdeletions in

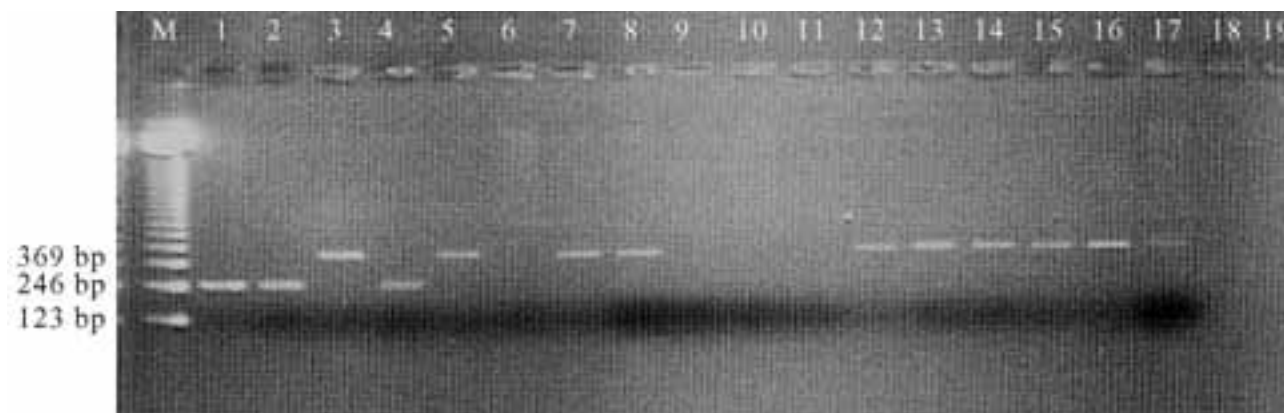
the AZFa or SRY genes were identified. A schematic diagram of the STS markers used in this study and the deleted markers are summarized in Figure 2.

Chromosome analyses of peripheral lymphocytes of all selected patients and controls were normal male karyotype (46,XY).

## Discussion

Interest in Y chromosomal deletion analysis in infertile men arises largely from the likelihood that Yq microdeletions will be transmitted by ICSI and cause the same infertility problem in male offspring. This transmission of microdeletions has been described previously,<sup>(17)</sup> an observation that underlines the necessity of proper genetic counseling in infertile men. Some authors have emphasized the importance of genetic counseling and testing for Y chromosome microdeletions in all ICSI candidates, irrespective of their sperm concentration.

Of 99 patients examined in our study, 24 had microdeletions in the AZF regions on the Y chromosome (24.2%), which is in agreement with previous studies. The Kurd and Azari ethnic groups are the majority in our region; thus, it can be inferred that Y chromosome microdeletion

**FIG. 1.** An example of deletions of the Y chromosome in the AZF region in men with oligospermia: PCR products of the AZF region from 19 tested patients, which were run on 1.5% agarose gel identified a deleted region in 6 patients.

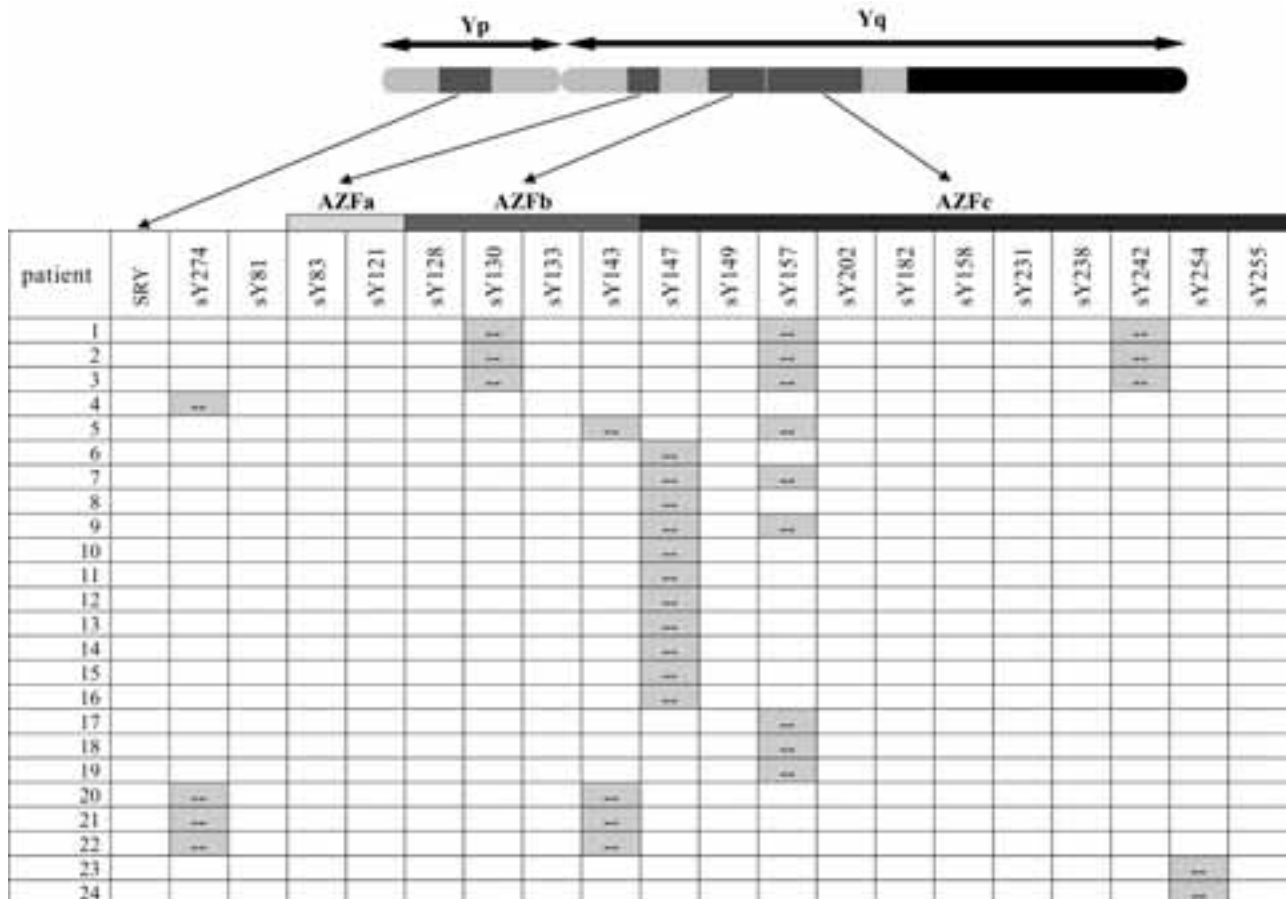


FIG. 2. Schematic diagram of a Y chromosome illustrating sequence-tagged sites, AZF regions, and the different deletion patterns of the patients in this study. A "--" denotes deletion of a specific sequence-tagged site.

may be relatively prevalent in West Azarbaijan.

The deletions mainly involved the AZFc region (in 87.5% of the patients), which corresponds with the DAZ gene, as well as deletion in the AZFb (in 29.2% of the patients). No microdeletions in the AZFa or the SRY gene have been identified. We found deletions in the proximal part of the AZF region near the SRY gene in 16.7% of the patients. The deletion of this region may affect SRY gene function, but to check this hypothesis, we must perform more experiments including functional studies. Despite of the report of Pryor and associates,<sup>(3)</sup> no microdeletions were found in healthy men of our control group. This variability in the detection of microdeletions between studies is probably explained by the different clinical selection criteria used by different research groups. Stringent selection of patients according to histologic, endocrinologic, and clinical criteria have been found to be associated with high deletion frequencies.<sup>(7,14,18)</sup> For instance, Foresta and colleagues have studied patients with idiopathic azoospermia and bilateral Sertoli-cell-only syndrome and found a

very high number of Yq11 microdeletions.<sup>(14)</sup> By contrast, less stringent criteria for selection has been associated with low deletion frequencies in studies on a large number of men with oligospermia.<sup>(19,20)</sup> Our study shows the influence of the selection criteria on the reported incidence of microdeletions; we had a high rate of Y chromosome deletion in our patients since we used strict patient selection criteria.

Microdeletion frequency in a sample of infertile men is not significantly related to the number of STS loci analyzed.<sup>(21)</sup> Kent-First and colleagues have analyzed a large number of STSs in different Y chromosome regions.<sup>(22)</sup> They have shown that each STS is statistically correlated with male infertility. It seems that patients' selection criteria have a much more profound effect on the rate of detection of microdeletions than do the numbers of STSs analyzed.

Of the 24 infertile men with microdeletions of the Y chromosome, 18 had azoospermia and 6 had severe oligospermia. In fact, most microdeletions have been found in men with azoospermia and severe oligospermia, because in

the majority of the studies published so far, the analyses were limited to patients with severe defects of spermatogenesis. Pryor and colleagues were the first to examine 200 consecutive patients who included 102 men with a normal sperm count. They found deletion frequencies of 23.1%, 9.7%, and 1% in infertile men with azoospermia, oligospermia, and normal sperm count, respectively. They concluded that the microdeletion in men with a normal sperm count probably indicated a polymorphism, because it comprised only one STS, which was also found in fertile men.<sup>(3)</sup>

Finally, all of the men with Y chromosome microdeletions in our study were cytogenetically normal, showing that PCR-based assay is needed to detect microdeletions in the Y chromosome.

### Conclusion

The correlation between Y chromosome microdeletions and infertility, and the relative absence of such deletions in fertile men, suggests a cause-and-effect relationship between the deletions and infertility. As compared with other known causes of infertility, Y chromosome microdeletions are relatively frequent, 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 ICSI, the potential for passing on these defects to offspring is serious and should be considered when infertile couples are counseled about this procedure.

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