Role of the AZFd locus in spermatogenesis

To determine the prevalence of Y-chromosome microdeletions among infertile men and to correlate the clinical presentation of the men with specific deletions, microdeletion analysis in 53 infertile men (30 nonobstructive azoospermic, 23 severely oligozoospermic patients), and 100 age-matched, fathered normospermic men who had fathered children was performed by the multiplex PCR with 18 different Y-chromosome–specific STS primer sets, spanning the AZFa, AZFb, AZFd, and AZFc regions. Detection of the same locus deletion of the AZFd region in three cases indicated the possible importance of the genes located in this region in spermatogenesis. (Fertil Steril® 2005;84:519–22. ©2005 by American Society for Reproductive Medicine.)

An association between male infertility and cytogenetically detectable terminal deletion of the long arm of the human Y chromosome, where the entire Yq heterochromatin had disappeared, was first proposed by Tiepolo and Zuffardi (1). They suggested the existence of an *azoospermia factor* (AZF) located within intervals five and six. Subsequently, several combined molecular studies have defined correlations between variable degrees of spermatogenic impairment and recurrent deleted nonoverlapping subregions, from proximal to distal AZF regions, including AZFa, AZFb, AZFc, and AZFd (the proximal AZFc), each of them bearing candidate genes or gene families, such as DFFRY, DBY, RBM, and DAZ, that are related to spermatogenesis (2–6).

The overall frequency of microdeletions has been estimated to be 12% in azoospermic men and 3.4% in oligozoospermic men (7). Although these subregion deletions appear to be involved in the disruption of spermatogenesis, the roles of genes located in these regions and genotype–phenotype correlations could not be cleared well. However, assisted reproductive technologies, such as testicular sperm extraction and intracytoplasmic sperm injection (ICSI), have been applied widely to overcome male infertility. It further has been shown that the Yq microdeletion specifically transmits from father to son in patients undergoing ICSI (8). Therefore, this evidence clearly reinforces the importance of this screening procedure in routine andrology practice, especially in ICSI (9, 10). Determination of the prevalence and type of the Y-chromosome microdeletions in infertile Turkish men and their clinical respects were addressed in this preliminary study. The microdeletion status of the putative AZF region, AZFd, also will be addressed in the study.

The study included 53 consecutive men (30 nonobstructive azoospermic, 23 oligozoospermic patients) aged 27 to 48 years (mean age: 32.3 years) presenting with idiopathic infertility at the urology clinic of Osmangazi University and 100 age-matched normospermic men who all had fathered children. Peripheral blood samples were drawn from patients and controls. At least two complete semen analyses were conducted using World Health Organization criteria. The patients were classified as azoospermic (no sperm in ejaculate) and severely oligozoospermic (sperm count of <5 million/mL). Of the 53 infertile men, 30 were nonobstructive azoospermic (NOA), and 23 were severely oligozoospermic (SO). All of the azoospermic men were confirmed as having nonobstructive status by testicular biopsy after a pellet test. Standard cytogenetic techniques of G banding were used to analyze karyotype, and the cases with abnormal karyotypes were excluded. Patients with hypogonadotrophic hypogonadism, obstructive azoospermia, a history of undescended testes, and varicocele were excluded from this study.

Genomic DNA was extracted from peripheral blood samples by using standard DNA extraction methods. In the microdeletion analysis, patients and controls were screened by using 18 different Y-chromosome–specific sequence-tagged site primer sets spanning the AZFa, AZFb, AZFd, and AZFc regions of the Yq chromosome: SY81 and SY182 in AZFa; SY121, SYPR3, SY124, SY127, SY128, and SY130 in AZFb; SY133, SY145, SY153, and SY152 in AZFd; and SY242, SY239, SY208, SY254, SY255, and SY157 in AZFc. All of the primers were nonpolymorphic short DNA fragments that were grouped into four sets for use in multiplex PCR. Each multiplex primer set contains a control primer pair that amplifies the X-linked SMCX locus.

Multiplex PCRs were performed in duplicate in 25-μL reaction volumes containing 200 ng of genomic DNA. The PCR mixture and reactions were performed according to the instructions of the Y-Chromosome Deletion Detection System (Promega MD 1101). A negative result was scored when the amplification product was not obtained after 2 PCR attempts.

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TABLE 1

Analysis of Y-chromosome deletions and STSs.

		AZFa		AZFb					
Semen analysis	Histology	DYS271 SY81	KAL-Y SY182	DYS212 SY121	SMCY SYPR3	DYS215 SY124	DYS218 SY127	DYS219 SY128	DYS221 SY130
SO	NA	+	+	+	+	+	+	+	+
AZO	MA	+	+	+	+	+	+	+	+
AZO	SCO	+	+	—	—	—	—	—	—
AZO	MA	+	+	+	+	+	+	—	+
AZO	MA	+	+	+	+	+	+	+	+
AZO	SCO	+	+	+	+	+	—	+	+
AZO	MA	+	+	+	+	+	+	+	+
SO	NA	+	+	+	+	+	—	+	+
SO	NA	+	+	+	+	+	+	+	—
AZO	MA	+	+	+	+	+	+	+	+
SO	NA	+	+	+	+	+	+	+	+
AZO	SCO	+	+	—	—	—	—	—	+

Note: SO = severe oligozoospermia; AZO = azoospermia; MA = maturation arrest; SCO = sertoli cell only syndrome; NA = not available; + = non-deleted region; — = deleted region.

Müslümanoğlu. AZF deletions of azoospermia and severe aligozoospermia. Fertil Steril 2005.

Among the 53 infertile men, a total of 12 cases (22.64%) were found to have deletions in the regions of AZFb, AZFd, and AZFc. No microdeletions were noted in controls. Of these 12 men, 8 had azoospermia, and 4 had severe oligozoospermia. No microdeletion was detected in the AZFa region. Large terminal deletions, involving the AZFb+AZFd+AZFc regions, were seen in three NOA cases, and testicular biopsies were diagnosed as SCO syndrome in two of them. Deletions of the AZFd+AZFc regions were detected in one azoospermic man with maturation-arrested testicular histology and in one severely oligozoospermic man who refused to allow a testicular biopsy. Of the NOA cases, three had only AZFb region deletions. Two of them were SO, but testicular biopsies of these cases could not be performed. The other deletion was detected in an azoospermic man with a testicular histology compatible with SCO syndrome. The microdeletion bounded into the AZFc region was seen in only one case with SO phenotype (Table 1).

It was interesting that the three azoospermic men with maturation-arrested testicular histology had the same single locus (SY152) microdeletion in the AZFd region. The single locus microdeletion was confirmed by the following two confirmation analyses.

This study identified the Y-chromosome microdeletion in 8 of the 30 azoospermic men (26.7%) and 4 of the 23 men with severe oligozoospermia (17.4%). The frequency of deletions was higher in those cases with azoospermia than in those of severe oligozoospermia. The overall frequency of microdeletions in infertile men was 22.64% (12/53). Because of the different selection criteria for infertile men, the deletion rates were wide ranging from 1% to 55.5% (11, 12). The frequencies of the present study were within range, and the results confirmed that Y-chromosome microdeletions are relatively frequent in the infertile men.

Though there have been numerous studies in which infertile men have been screened for Yq microdeletions, the number of STSs used in the screening protocols are vary. In this work, 18 STSs with positive and negative controls we used. Whereas the AZFa region was screened by two STSs, six, four, and six STSs were used in the screening of the AZFb, AZFd, and AZFc regions, respectively. Simoni et al. (7) stated that the analysis of two STS loci in each region is sufficient to determine whether any STS deletion is present in AZF regions with a high degree of accuracy. However, the preliminary findings of the present study clearly show that usage of a large number of STS markers for each region might be more informative while one is attempting to locate single-locus deletions. This approach might be important in determining the roles of loci in spermatogenesis.

In this work, the whole set of primers was repeated in simplex PCR when the multiplex PCR results indicated a deletion, and the same results were found in all cases with microdeletions. Because most of the deletions reported in the literature were involved in more than one STS locus, the analyses performed in the present study were valuable in the cases with one locus deletion (5). The presence of single-locus deletion in a nonobstructive azoospermic man