

# Male infertility: polymerase chain reaction-based deletion mapping of genes on the human chromosome

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## ABSTRACT

**Introduction:** Y chromosome microdeletions are common in about 10-15 percent of men with azoospermia or severe oligospermia. These microdeletions are too small to be detected by karyotyping. They can be easily identified using polymerase chain reaction (PCR). Most of the microdeletions that cause azoospermia or oligospermia occur in the non-overlapping regions of the long arm of the Y chromosome. These regions, also called azoospermia factor regions (AZF), are responsible for spermatogenesis. The loci are termed AZFa, AZFb and AZFc from proximal to distal Yq. Several genes located in AZF regions for spermatogenesis is viewed as "AZF candidate genes". This study aims at PCR-based rapid analysis of Y chromosome microdeletion, which is a cause for male infertility.

**Methods:** PCR amplification using Y-specific STS (sequence tagged sites) of AZF regions for AZFa: DBY and sY84, AZFb: RBMI and sY127, and AZFc: BPY2 and sY254, were conducted.

**Results:** Of the 30 infertile men, 17 were azoospermic and 13 were severely oligospermic. Severe oligospermia was diagnosed in those patients who produced only one-third the concentrations of the sperm of that found in fertile men. Four patients showed a deletion of one or more STS. Two patients had complete deletion of AZFc loci, three patients had complete deletion of AZFa loci and two patients had complete deletion of AZFb loci.

**Conclusion:** The frequency involving the microdeletion in the AZF region was found in four out of 30 azoospermic and severely oligospermic infertile men, i.e. 13.3 percent of the total deletions.

**Keywords:** azoospermia factor, gene mapping, male infertility; Y chromosome microdeletion

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## INTRODUCTION

Infertility affects about 15% of couples and in 40%-50% of cases, the male partner has quantitative or qualitative abnormalities of sperm production.<sup>(1)</sup> The identification and analysis of Y chromosome deletions is an important research tool for studying male infertility.<sup>(2)</sup> In each azoospermia factor (AZF) region of the AZFa, AZFb and AZFc, candidate genes have been proposed, i.e. DBY (dead box on the Y, exhibits transcripts unique to the testis), USP9Y (Ubiquitin-specific protease 9) in the AZFa region, RBMY (RNA-binding motif on Y) in the AZFb region, and DAZ, BPY2 and CDY in the AZFc region.

Deletion of the AZFa region (least common class of deletions) is most frequently associated with azoospermia manifested by the sertoli cell only syndrome (SCOS) and less often with oligospermia.<sup>(3)</sup> Deletion of the AZFb region is associated with azoospermia, oligospermia and normozoospermia. Deletion of AZFc interval is associated with azoospermia, severe to mild oligospermia, and the production of insufficient mature sperm to enable reproduction.<sup>(4)</sup> This study aims at polymerase chain reaction (PCR)-based rapid analysis of Y chromosome microdeletion, which is a cause for male infertility.

## METHODS

We studied 30 infertile men and 20 normal men. DNA was prepared from one drop of blood (Oncophyta DNA isolation kit, Madurai, India) and amplified in multiplex PCRs using two multiplex primer mixes with three primer sets in each mix specific for the AZFa, b and c regions (Table I). The sequence tagged sites (STS) and gene sequences for deletion analysis were taken from the early literature.<sup>(5-10)</sup> Each primer pair amplifies a specific region of the Y chromosome (i.e. an STS). Standard condition for the PCR amplification was five minutes initial activation at 94°C, a 40-cycle reaction with 94°C denaturation for 30 seconds, 55°C annealing

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**Table I. Primer sequence****I. Multiplex I primer mix:**

- 1) AZFa:DBY (164 bp)  
Forward: 5' – AGT TTA TTC TAA CCT AGG CAAACG – 3'  
Reverse: 5' – TCC AAC CAG GCC TGT AGT GAG GCC – 3'
- 2) AZFb:RBM1 (800 bp)  
Forward: 5' – CTTTGAAAA CAATTC CTTTTC C – 3'  
Reverse: 5' – TGC ACT TCA GAG ATA CGG – 3'
- 3) AZFc:BPY 2 (209 bp)  
Forward: 5' – CCC AGATTTTCA CAG GTG CT – 3'  
Reverse: 5' – CTC ATT TGT ATG CTG GGC CT – 3'

**II. Multiplex II primer mix:**

- 1) AZFa:sY84 (320 bp)  
Forward: 5' – AGAAGG GTCTGA AAG CAG GT – 3'  
Reverse: 5' – GCC TAC TAC CTG GAG GCTTC – 3'
- 2) AZFb:sY127 (274 bp)  
Forward: 5' – GCC TCA CAAACG AAAAGAAA – 3'  
Reverse: 5' – CTG CAG GCA GTAATAAGG GA – 3'
- 3) AZFc:sY254 (400 bp)  
Forward: 5' – GGG TGT TAC CAG AAG GCAAA – 3'  
Reverse: 5' – GAA CCGTAT CTA AAG AAG CAG – 3'

for 30 seconds, and 65°C extension for four minutes. A final extension was done at 65°C for four minutes. The reaction products were separated on 5% polyacrylamide gels (Sigma-Aldrich Corp, Bangalore, India) and visualised with ethidium bromide. If any band was absent, it was considered to be the deletion of that specific STS marker region.

**RESULTS**

Of the 30 infertile men, 17 were azoospermic and 13 were severely oligospermic. PCR microdeletion analysis was done in all 30 infertile patients. PCR amplification produced a band of expected size for all six loci investigated in normal fertile men. Four patients showed a deletion of one or more STS. Two patients had complete deletion of AZFc loci, three patients had complete deletion of AZFa loci, and two patients had complete deletion of AZFb loci.

**DISCUSSION**

Spermatogenesis is regulated by a number of genes on the Y chromosome and autosomes. Y chromosome deletions are emerging as a prevalent cause of male

factor infertility. The frequency of the Y chromosome deletion increases with the severity of the spermatogenic defect.<sup>(11)</sup> About 15% of azoospermic and 5%–10% of oligospermic men show Y chromosome deletions. However, these Y chromosome microdeletions cannot be predicted cytogenetically, on the basis of clinical finding, by semen analysis. Thus, PCR-based screening of AZF regions for microdeletions on Y chromosome are necessary. In the past, the diagnosis of a genetic aetiology had little clinical significance. Today, the assisted reproductive technology helps in overcoming this infertility problem, but there is still transmission of the genetic defects, like microdeletion, to their offsprings. Hence, this diagnosis will provide the information necessary to counsel these couples effectively, particularly with regard to the birth of an infertile male offspring, who may have the same or secondary, larger deletions with more severe testicular phenotype.

Recent studies have shown a marked variation in the deletion frequency. This is due to selection of different patient groups and use of different marker sets. The position of AZF deletion was correlated with the phase in which spermatogenesis was arrested.<sup>(8)</sup> Each AZF locus acts at a different phase of spermatogenesis, and deletion of each locus causes spermatogenic arrest at a particular stage. On the basis of testicular histology, the deletion of AZFa was associated with the complete absence of germ cells and the presence of Sertoli cells in the seminiferous tubules, characteristic of SCO syndrome and was associated with azoospermia. The deletion of AZFb was associated with the developmental arrest of germ cells at the pachytene stage and led to meiotic maturation arrest. The deletion of AZFc was associated with the developmental arrest of germ cells at the spermatid stage, but was also found to be associated with hypospermatogenesis or maturation arrest and was associated with low sperm counts. Thus, deletion of a particular AZF locus results in a characteristic phenotype, and genes at each locus act at a particular stage of germ cell differentiation.

The frequencies of deletions of Yq, reported in different studies, range between 3% and 18% of males with non-obstructive azoospermia or severe oligospermia.<sup>(7,12)</sup> In our screening of 30 infertile males, we found four patients carrying microdeletions corresponding to a frequency of 13.3% (four out of 30 patients had microdeletions). In comparison to the statistical values obtained from all the surveys reported to date,<sup>(12)</sup> some studies reported 13% of infertile microdeletions in the Y chromosome,<sup>(13,14)</sup> while others, less than 5%.<sup>(4,15)</sup> Another study showed an incidence of microdeletion between 5.1% and

9.6% in the infertile males.<sup>(16)</sup> However, 55.5% of infertile males with Y microdeletions were found in Italy.<sup>(17)</sup> Our results are in accordance with the reported results of between 13%–23%.<sup>(18)</sup>

Deletion of the AZFc region was detected in two patients, accounting for 50% (2/4) of total detection of azoospermic men. This gene was named DAZ (deleted in azoospermia), now renamed as DAZ gene family. This is in agreement with earlier studies, which showed that deletion in the AZFc region was high when compared with that in the AZFa and AZFb regions. Reduction in the copy number in the DAZ gene (AZFc) was reported to have an increased risk in subfertility and infertility.<sup>(19)</sup> In our study, 33.3% (3/9) of the total Y chromosome deletion was in the AZFa region. This result is similar to another report that 24.2% of total Y chromosome deletion was in the AZFa region.<sup>(20)</sup> Sun et al reported that point mutation in the genes present in the AZFa region can cause azoospermia.<sup>(21)</sup> This is in agreement with the present observation that the deletion in the AZFa region was very small, and that all individuals with deletion in the AZFa region were azoospermic. In the AZFb region, there was 22% (2/9) deletions from that of total deletions in azoospermic men. This study suggests that a few sets of the STS marker should be routinely used in the clinical laboratories to screen for the microdeletions before the employment of assisted reproductive techniques.

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