

AZFc Deletions in Idiopathic Infertile Males from South India

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KEY WORDS Infertile males; microdeletions; sequence tagged sites.

ABSTRACT The frequency of Y chromosome microdeletions was studied on idiopathic infertile males with normal karyotype. Genomic DNA was isolated from peripheral blood and PCR analysis was carried out with STS markers – SY 158 and SY 254 to detect the AZFc mutations. Out of 50 infertile males analyzed, four males exhibited AZFc deletions. The SY 158 deletions were observed in three males, viz., azoospermic, severe oligozoospermic and oligozoospermic males. In one azoospermic male SY 254 deletion was observed.

INTRODUCTION

Recent studies have established that Y-chromosome microdeletions are a common cause for male infertility (McElreavey et al. 1999). The reports on the frequency of microdeletions in infertile males indicated wide variation. While Foresta et al. (1998) have shown high frequency of Y chromosome microdeletions, some others (Vander ven et al. 1997; Selva et al. 1997) reported a low frequency. Molecular studies have suggested that azoospermia factor (AZF) present in the intervals 5 and 6 of the human Y chromosome plays an important role in the causation of infertility (Krausz and McElreavey 1999). It is further observed that AZF region on the Y chromosome is divided into three non-overlapping regions AZFa, AZFb and AZFc microdeletions were reported in all the three regions (Vogt et al. 1996).

Studies on the role of Y chromosome microdeletions in infertile males are available from developed countries such as USA, UK, France, Italy, etc., and so far no reports are published from India. Hence, an investigation was taken up to detect microdeletions in AZFc region, which is more susceptible for deletions among AZFa, AZFb and AZFc regions in idiopathic infertile males.

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MATERIALS AND METHODS

Couples with infertility problem who attended the Institute of Genetics and Hospital for Genetic diseases were evaluated. All the couples who had complaints of infertility (one year of unprotected intercourse not leading to conception) were investigated in both the partners to know the cause of infertility and only 50 males whose female partners were normal were selected for the study. The age of the infertile men ranged from 25 to 50 years.

Detailed information from male partners on their life style, habits (such as smoking, alcohol and drug use), exposure to physical or chemical agents during their occupation, surgical history and family history were recorded in a standard questionnaire. Physical examination like assessment of secondary sexual characteristics, inspection of penis and determination of testicular size by orchidometry was also carried out.

Cytogenetic investigation was carried out and the males with abnormal karyotypes were excluded from the study. Every man provided a semen sample after sexual abstinence for three days. These samples were analysed on the basis of the criteria of the World Health Organization (Pryor et al. 1997). Ten proven males who fathered at least one child were selected from the same age group as control subjects.

Y-Chromosome Microdeletions

Genomic DNA was extracted from peripheral blood using standard technique of Maniatis et al. (1982). Quantification of DNA was carried out using spectrophotometer. STS primers used for detection of AZFc deletions are given below.

Left	Right
SY 254 5'-GGG TGT	5'-GAC CGT ATC TAC
TAC CAG AAG GCA	CAA AGC TGC-3'
AA-3'	
SY 158 5'-CTC AGA	5'-ACA GTG GTT TGT
AGT CCT CCT AAT	AGC GGG TA-3'
AGT TCC-3'	

Polymerase chain reaction was performed with 200ng of DNA as template, 0.2mm dNTP's, 50p mole of each primer, 10X Taq buffer (10mM Tris (pH8.3), 50mMKCl, 1.5mMMgCl₂ and 0.01% gelatin), 1U Taq DNA polymerase in a total volume of 50 µl. PCR was carried out with 35 cycles at 94°C for 45 sec, 55°C for 30 sec and 72°C for 45 sec. The programmes were preceded by a 5-minute denaturing step at 95°C followed by a final extension step at 72°C for 5 minutes. Female genomic DNA was used as a negative control.

Electrophoresis of PCR amplified DNA products were carried out on 1.2% agarose gel. A patient sample was considered positive for the given STS when the PCR product of the expected size was present and was considered negative when the PCR product of the expected size was not obtained. PCR amplifications found to be negative for expected bands were repeated at least three times to confirm the deletion of a given marker.

RESULTS

The details of the type of infertility and the sperm concentrations are given in table 1. Out of 50 cases, 10 were azoospermic, 12 were severe oligozoospermic and 28 were oligozoospermic males. The table 2 shows the nature of deletion, type of infertility and semen analysis. Out of 50 cases in 4 (8.0%) infertile males AZFc microdeletions were observed. Two males were azoospermic, one male was severe oligozoospermic and one male was oligozoospermic male. One azoospermic male had a deletion in SY 254 and another azoospermic male had a deletion in SY 158 STS marker. SY 158 deletion was observed in one severe oligozoospermic male and one oligozoospermic male.

Figures 1 and 2 show deletions of SY 254 and SY 158 in the infertile males. The expected band of 380 bp for SY 254 was absent in one azoospermic male besides female control while

Table 1: Classification of infertile males with their sperm concentration

Number of cases	Sperm concentration 10 ⁶ /ml	Type of case
10	None	Azoospermia
12	<5	Severe oligozoospermia
28	>5-20	Oligozoospermia

it was observed in infertile males and positive control (Fig.1). The expected band of 231 bp was absent in one azoospermic case, one severe oligozoospermic case, one oligozoospermic case and female control while it was present in positive control and other infertile males (Fig.2).

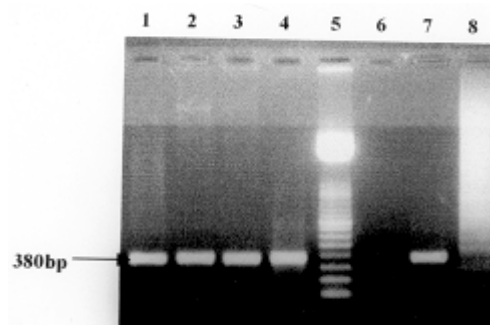


Fig. 1. PCR analysis on DNA of infertile males using STS (SY 254)

Lane 1 : Positive control
Lanes 2-4 : Oligozoospermia males
Lane 5 : Mol. wt marker (100 bp DNA ladder)
Lane 6 : Azoospermia (with deletion)
Lane 7 : Azoospermia
Lane 8 : Negative control

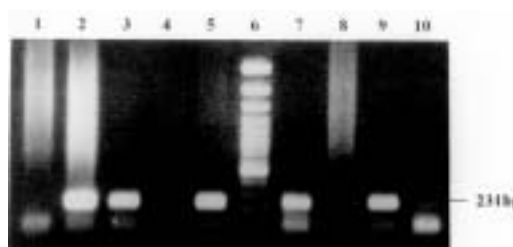


Fig. 2. PCR analysis on DNA of infertile males using STS (SY 158)

Lane 1 : Negative control
Lane 2 : Positive control
Lanes 3 & 5 : Azoospermia
Lane 4 : Azoospermia (with deletion)
Lane 6 : Mol. wt marker (100 bp DNA ladder)
Lanes 7, 9 & 10 : Severe oligozoospermia males
Lane 8 : Oligozoospermia (with deletion)

DISCUSSION

There is substantial evidence for the involvement of Y-chromosome in sexual development and spermatogenesis (Kleiman et al. 2001). The Y chromosome, which is divided

Table 2: Classification of infertile males with their sperm concentration

Type of case	AZFc deletion		Semen analysis		
	SY 254	SY 158	Actively Motile %	Sluggishly motile %	Non motile %
Azoospermia	-	+	-	-	-
Azoospermia	+	-	-	-	-
Severe oligozoospermia	+	-	10	20	70
Oligozoospermia	+	-	30	10	60

into seven regions, consists of atleast 15 gene families that are involved in the control of spermatogenesis. Any change or mutation in these genes disrupts spermatogenesis process causing infertility (Song et al. 2000).

Recent reports have indicated that the deletions in AZFc region are common when compared to the deletions in AZFa and AZFb regions (Sawai et al. 2002). In the present study, 4/50 (8.0%) infertile males showed deletions in AZFc region. Our results are in agreement with that of Lin et al. (2000) who also detected 8.5% AZFc deletions in 94 infertile males studied in Taiwan. Ferlin et al. (1999) from Italy observed 6.5% infertile males with AZFc deletions. Kim et al. (1999) reported a high frequency of infertile males with AZFc deletions from China. However, Brandell et al. (1998) and Krausz et al. (1999) reported low frequency (2.5% and 0.74%) of infertile males with AZFc deletions from USA and France, respectively.

Screening for AZF regions is advisable in men with severe oligozoospermia and azoospermia with spermatozoa in the testis requiring Intra Cytoplasmic Sperm Injection (Yao et al. 2001) because Y-chromosomal inheritance is always of paternal origin and hence men with somatic Y deletions may likely to have sons with somatic Y defects after treatment with assisted reproduction techniques.

Great care must be taken in genetic counselling of the infertile males, as it raises the possibility of genetic transmission of infertility from father to son. Y microdeletion screening provides a useful diagnostic tool in identifying patients and gives precious information for a more appropriate clinical management of both infertile male and his future male child.

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