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Short Communication

Y-Chromosomal Deletions − a Risk Factor for Male Infertility

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ABSTRACT

In this study, contribution of micro-deletion towards male infertility among Pakistani male individuals has been described. Our analyses involved evaluation of seven genetic markers spanning the AZFa, b and c region among infertile males and observed micro-deletions were compared with the control healthy fertile counterparts. Data revealed AZFa deletion in 2% infertile azoospermic and oligospermic males, while the AZFb and AZFc deletions were found in 3.8 and 5.8% infertile males, respectively. These deletions were found solely in non-obstructive azoospermic patients, which consisted of 17.6% of the total non-obstructive infertile males. Our data clearly demonstrate association of Y-chromosomal micro-deletions with male infertility.

Key Words: Male infertility; AZFa; AZFb; AZFc; Y chromosomal deletions

INTRODUCTION

Almost 10-15% couples have been reported to be childless worldwide. In 50% of these cases, different male related factors are the causative element. Infertility in such males could have been caused by different environmental or genetic causes. The most frequently documented genetic cause of male infertility is Y-chromosomal deletions, which result in impaired spermatogenesis (Mansour, 2004). The human Y chromosome contains approximately 60 Mega bases and encodes 80 different proteins, some of which are responsible for germ cell development and maintenance (spermatogenesis). Y-chromosomal genes involved in spermatogenesis mostly reside on the AZFa, b and c regions. In the AZFa region, USP9Y is an important functional gene involved in spermatogenesis. The main candidate gene in the AZFb region is the RBMY (RNA-binding motif) gene family, which has a restricted expression in the testis (Ferlin *et al*., 2003). The AZFc region also has a specific expression in the testis and therefore, has been shown to play a crucial role in male fertility. Deletions in the AZFc regions are responsible for 8% cases of male infertility (Raicu *et al*., 2003), which thus represents the most frequently deleted region in infertile men. Analysis of the micro-deletions in the azoospermia factor (AZF) region of the Y chromosome by PCR is an important screening tool in the work-up of infertile males opting for assisted reproductive techniques. In this study, 51 infertile males were studied from Pakistan to determine the contributions of the Y chromosomal deletions in this select group. These data were then compared to the normal fertile male to obtain the specificity of the observed deletions.

MATERIALS AND METHODS

In the present study, deletion analysis of the AZF a, b and c regions was carried out in 51 infertile males and 100 control males with known fertility. The infertile males had various types of infertility including non-obstructive azoospermia, oligospermia and infertility due to unknown reason. The Y chromosome micro-deletions were analyzed by PCR using primers that amplified 7 sequence tagged sites (STS) covering five genes of the AZF region (USP9Y, DBY, RBMY, CDY1 & DAZ). Out of these seven markers, five previously published Y STS were used to screen deletion of the AZFa, b and c regions. Two additional markers DBY in the AZFa region and DAZ in the AZFc region were also used so as to provide maximum coverage of the region (Table I). The STS were amplified in 3 sets of multiplex reactions; SRY 1532 was used in all the three reactions as an internal control to check for amplification failures.

RESULTS AND DISCUSSION

The present study was designed to elucidate the role of

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Y chromosomal micro-deletions in causing male infertility in Pakistani populations. For this purpose, 51 infertile males were compared with 100 fertile control males. The status of azoospermic infertile males included in this study was classified into non-obstructive azoospermia or obstructive azoospermia based upon their biopsy results, whereas classification into the oligospermia group was based upon microscopic examination of the ejaculate.

Y-chromosomal genes involved in spermatogenesis mostly reside in the AZFa, b and c regions. Therefore, different STS markers were studied in these regions. Results of the deletion analysis show that 2% (1/51) patients had the AZFa deletion. This patient was classified to have spermatogenic arrest at the primary stages of sperm production based upon his biopsy results. By mapping AZFa deletions in patients, Sargent *et al.* (1999) have shown that this deletion results in loss of the DFFRY and DBY genes and caused sertoli cell only (SCO) syndrome in such patients, while the patients who retained the DBY gene were oligospermic.

AZFb deletions have also been shown to be associated with spermatogenic failure (Brandell *et al*., 1998). In infertile males, deletion in this region have been found in 3.9% of the patients. All of these deletions were found in non-obstructive azoospermic patients and not in oligospermic patients. However, it must be pointed out that these markers were designed to detect the gross deletion of in the region (Raicu *et al*., 2003) but not the microdeletions.

In the Pakistani infertile males, we find the AZFc deletions in 5.8% (n=3/51) of the total infertile population (Table II). In order to ascertain the selectivity of these markers, 100 fertile males in control group were also analysed. None of these males had any deletion in the AZFa, b or c region, thus confirming the association of these deletions in a subset of the infertile males in Pakistan. Foresta *et al*. (2005) have also reported micro-deletions in 6% infertile men, which included deletions in the AZFc region only; whereas no deletions were found in the control subjects.

In the current study, three individuals with the micro-deletions make up 17.6% of the non-obstructive azoospermic group and they had various levels of deletion interval. The AZFc region was deleted in all the three individuals, while one patient had the AZFb deletion in addition to the deletion in the AZFc region and the third male had all the three regions deleted.

Kuroda-Kawaguchi *et al*. (2001) have also reported 12% deletion frequency in non-obstructive azoospermia and about 6% in severe oligospermia cases. Mohammed *et al*. (2007) while studying Kuwaiti infertile males found microdeletions in the AZFb and AZFc regions in only 2.6% (n=7/266), which is at a lower frequency than that in Pakistani patients. The frequency of these deletions reported in various studies ranged from 3-18% in non-obstructive azoospermia or oligospermia (Reijo *et al*., 1995; Simoni *et al*., 1998; Viswambharan *et al*., 2007). However, Foresta *et al.* (1997) found a very high percentage (55.5%) of Italian infertile males to carry these Y-chormosomal micro-deletions. Our results for non-obstructive azoospermia are in accordance with the reported results of between 13%–23% micro-deletions.

Table I. Product length and annealing temperature of the seven AZF a, b and c loci and the internal control

Y-Chromosome marker (STS)	Locus	Product length	Annealing Temp	Reference
AZFa prox-2	AZFa	220 bp	58°C	Raicu *et al.,* 2003
DBY	AZFa	303 bp	66°C	Genome database
SY127	AZFb	274 bp	58°C	Raicu *et al*., 2003
SY134	AZFb	301 bp	58°C	Raicu *et al*., 2003
SY254	AZFc	350 bp	58°C	Raicu *et al*., 2003
SY255	AZFc	126 bp	58°C	Raicu *et al.,* 2003
DAZ	AZFc	96 bp	58°C	Genome database
SY1532	SRY	167 bp	58°C	Raicu *et al*., 2003

Table II. Distribution of AZF a, b and c deletions in infertile and fertile males

Fertility Status	Subjects without deletions	Subjects with deletion(s)
Spermatogenic arrest	14	3 (1 with AZF a, b and c region deletion, 1 with AZF b and c region deletion and 1 with AZF c deletion)
Obstructive Azoospermia	16	0
Severe Oligospermia $<1\times10^6$ mL^{-1}	9	0
Oligospermia $<10\times10^6$ mL^{-1}	9	0
Fertile Control samples	115	0

A main candidate gene in the AZFc region is the CDY1 and the DAZ cluster. Multiple copies of the DAZ gene with >99% of sequence identity exist in the AZFc region. The presence of multiple copies is to create redundancy in this important gene in case mutation damages one of the genes. After analysis of the relationship between AZFc and male infertility, it is concluded that spermatogenesis is controlled by a number of genes, most of which are located on the Y chromosome, with many more on the autosomes or maybe even the X chromosome. This could be the reason that not all infertile males carry the micro-deletion that have been investigated in the present study and only a subset of them had these deletions. However, further work is needed to understand the complete spectrum of normal and pathogenic variations in Y-chromosomal structure before a complete understanding of the causes of infertility is understood. Such information could be used for genetic counseling and evidence based medical practice.

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