

A Journey on Y Chromosomal Genes and Male Infertility

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KEYWORDS Y chromosome, AZF region, Spermatogenesis, Male Infertility, Yq microdeletions, Sertoli cell only syndrome.

ABSTRACT In the course of evolution Y chromosome has acquired an important role in sex determination owing to the differentiation of the *SRY* gene from its X homologue. Apart from the functionally specialized *SRY* gene, the Y chromosome harbors several genes responsible for normal fertility. Three different spermatogenic loci namely AZFa, AZFb and AZFc located in the long arm of Y chromosome (Yq) has the vital role in regulating normal spermatogenesis. A microdeletion occurring in any of these regions is attributed to spermatogenic failure leading to infertility in men. Genetic cause of male infertility is found to be 10-15% and the outcome is diverse ranging from no germ cells (Sertoli Cell Only syndrome) to hypospermatogenesis. Genes arrayed in the AZFc region have testis specific expression and deletion of the AZFc region is most common among the Y micro-deletions in men with azoospermia condition. Among the candidate genes of the AZFc region the deletion involving *DAZ* is considered to be the frequent cause leading to azoospermia. The mechanism of micro-deletion is found to be the same in case of AZFa and AZFc region. Among these two loci homologous recombination of flanking, identical sequences leads to micro-deletion. But in case of AZFb region the proximal and distal breakpoints does not exhibit sequence homology although interspersed repeated sequences exist in proximity to the break points.

INTRODUCTION

Y chromosome has occupied a unique status in the human genome owing to its size, organization and function. The haploid Y chromosome stands apart from the rest primarily due to three reasons, first one being, recombination confined to the extremities (5%) and the remaining region (95%) accounting for non recombining Male Specific region of the Y chromosome (MSY) (Skaletsky et al. 2003), second, for harboring genes exclusively for the expression of male characters (Huntington and Willard 2003) and finally due to gene conversion process occurring in the palindromic blocks, that rectifies mutations (Rozen et al. 2003).

X and Y chromosomes have evolved from a pair of identical autosome around 300 million years ago from reptiles before the rise of mammals (Graves and Foster 1994). Differentiation of the *SRY* gene from its X homologue *SOX3* is considered as the key event that led to the rise of sex chromosomes in mammals (Stevanovic et al. 1993; Foster and Graves 1994). During the course of evolution Y chromosome gradually acquired the genes for spermatogenesis (Marshall 2000). The recombination event between the X and Y chromosome began to cease gradually due to block wise mutations followed

by large scale inversions in the Y chromosome (Lahn and Page 1999; Lahn et al. 2001). Very less change occurred in the X chromosome owing to 50% chance of recombination with another X chromosome (Rao 2003). The cessation of recombination led to the Y chromosome losing the key ability of homology based repair. This has led to the progressive degeneration of Y chromosome and as a consequence Y chromosome has occupied 'one of the smallest human chromosome' status (Ali and Hasnain 2001).

Y Chromosome- Cytogenetic Partitions

Figure 1a shows the cytogenetic partitions of Y chromosome which consists of a short and long arm designated as Yp and Yq respectively. The euchromatic short arm is designated as Yp11 whereas long arm Yq is cytogenetically divided into a euchromatic proximal region Yq11 and heterochromatic distal region Yq12. The Yq11 band is subdivided into sub-bands Yq11.1, 11.21, 11.22, and 11.23 respectively (Foresta et al. 2001).

Y chromosome Deletion Mapping

With the advent of Y specific probes began the process of construction of deletion interval maps specific for the Y chromosome (Fig. 1b). Accordingly the Y was delineated into seven major intervals. The interval 1-4 harbors the Yp region and the centromere, distal to proximal.

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The interval 5-6 harbors the Yq region-Yq 11.21, Yq 11.22 and Yq11.23, proximal to distal. (Vergnaud et al. 1986).

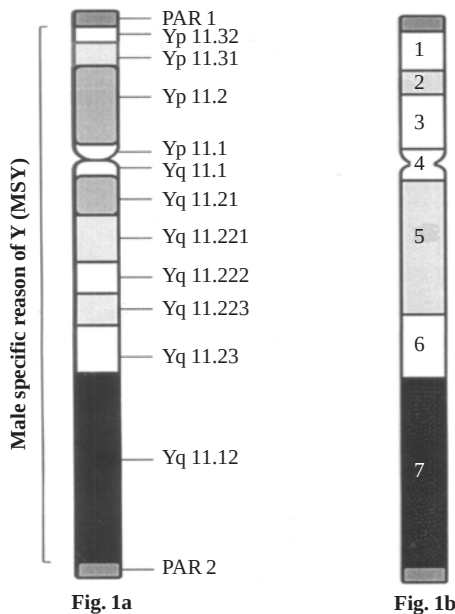


Fig. 1a. Cytogenetic partitions of Y chromosome
Fig. 1b. Seven interval map of Y chromosome

The heterochromatic region is confined to interval 7 (Foresta et al. 2001). Deletion interval 5 stretches approximately from Yq11.21 through the middle part of Yq11.22, and deletion interval 6 stretches from the middle part of Yq11.22 to Yq11.23. The resolution of the deletion map was enhanced by employing sequence tagged sites (STS) and led to the construction of a 43 interval map (Vollrath et al. 1992). Further enhancement was done by employing genomic clone subtraction followed by sequencing to identify more STS. Radiation hybrid mapping was employed to order the STS obtained after PCR tests of STS content (Tilford et al. 2001).

Male Specific Region of Y Chromosome

Recombination is restricted to the extremities known as pseudoautosomal region (PAR) and accounts for only 5% of the total chromosome (Foresta et al. 2001). The region encompassed by PARs does not involve in meiotic recombination and is called as the Non-Recom-

binning region of the Y chromosome (NRY) or recently as Male Specific region of Y chromosome (MSY), which accounts for 95% of the Y chromosome length (Skaletsky et al. 2003). The male specific region of Y harbors both euchromatic and heterochromatic sequences. Apart from the centromeric region, major part of the heterochromatic sequences is confined to the distal Yq12. Centromere sequences roughly accounts about 1Mb while the distal Yq12 heterochromatin region is of 40Mb (Skaletsky et al. 2003).

The euchromatic MSY harbors three classes of sequences namely X transposed, X degenerate and ampliconic sequences. The X transposed sequences are the ones that has been acquired by transposition from the X chromosome about 3-4 million years ago during the emergence of human lineage (Schwartz et al. 1998). The X degenerate sequences represent the ancestral sequences of the autosomes from which the X and Y chromosomes co-evolved. The ampliconic sequences are massive repeat units exhibiting 99.9% sequence identity. The amplicons exist as seven blocks and nine families of protein coding genes are arrayed in these segments (Kawaguchi et al. 2001; Skaletsky et al. 2003). The region harbors eight massive palindromes with highly symmetrical arms. Six of the palindromes carry recognized testis specific protein coding genes whose near identical gene copies exist in the opposite arms. Among the nine multicopy protein coding gene families in the MSY, eight have members in the palindromes and interestingly six families are located exclusively on the palindromes (Skaletsky et al. 2003). The testis specific genes are present as two copies facing each other as mirror images in the palindromes. This unique organization enables to rectify the mutation occurring in one copy of the gene by means of molecular mechanisms between the two arms of the palindromes (Rao et al. 2003; Rozen et al. 2003). The X transposed sequences harbors two genes while the X degenerate sequences harbors single copy gene or pseudogene homologs of 27 X linked genes (Skaletsky et al. 2003).

Combined together the Y chromosome approximately has 78 protein coding genes of which 60 belong to nine different MSY gene families and the remaining 18 genes are present in single copy (Skaletsky et al. 2003).

Y CHROMOSOME AND MALE INFERTILITY

Male infertility was first related to Y chromosomal deletions by Tiepolo and Zuffardi (1976) after a massive screening of 1,170 subfertile males. By means of karyotyping they were able to identify microscopic deletions among 6 azoospermic individuals. The deletions were confined to the distal region of Yq11 and with this born the hypothesis of genes located at the distal Yq region that regulate the process of spermatogenesis. This spermatogenic locus was named 'Azoospermia factor' (*AZF*), as the first six men observed with terminal deletions in Yq were with azoospermic condition. Subsequent cytogenetic studies and molecular level studies carried out proved the fact out of question (Foresta et al. 1997; Reijo et al. 1995; Vogt et al. 1992).

Vogt et al (1996) took the study to the next level by screening 370 men with idiopathic azoospermia or severe oligozoospermia for deletion of 76 DNA loci in Yq11. This was in order to check for multiple spermatogenic loci. The study involved diverse departments of ten Universities of Germany and one from Brazil. Microdeletions were observed among 12 men and they were mapped to 3 different sub regions in Yq11 based on the pattern of deleted DNA

loci obtained. In order to confirm the existence of three different loci, histological analysis of testis was carried out. The analysis revealed three different phases of spermatogenic disruption corresponding to deletion in three different regions of Yq11. The three different spermatogenic loci was designated as *AZF*a (proximal), *AZF*b (middle) and *AZF*c (distal) (Figure 2).

The *AZF*a loci is located at the proximal portion of the deletion interval 5 (subinterval 5C), the *AZF*b region spans from the distal portion of the deletion interval 5 to the proximal end of deletion interval 6 (subinterval 5M to 6B) (Vogt et al. 1997) and the *AZF*c region is located at the distal part of deletion interval 6 (subinterval 6C to 6F) (Foresta et al. 2001). The *AZF*b and *AZF*c are overlapping regions, yet the classical *AZF* regions are still used to describe deletions (Sadeghi-Nejad and Farrokhi 2007). Apart from this a fourth loci designated as *AZF*d is proposed to exist between *AZF*b and *AZF*c (Kent-First et al.1999) Contradictory reports prevails regarding this loci (Simoni et al. 2004).

Studies indicate that 10-15% of men with idiopathic azoospermia and 5-10% of men with oligozoospermia have Yq microdeletion (Krausz and McElreavey 1999). Foresta et al. (2001) showed the prevalence of Y chromosome microdeletions to be 4% in oligozoospermic patients, 14% in idiopathic severely oligozoosper-

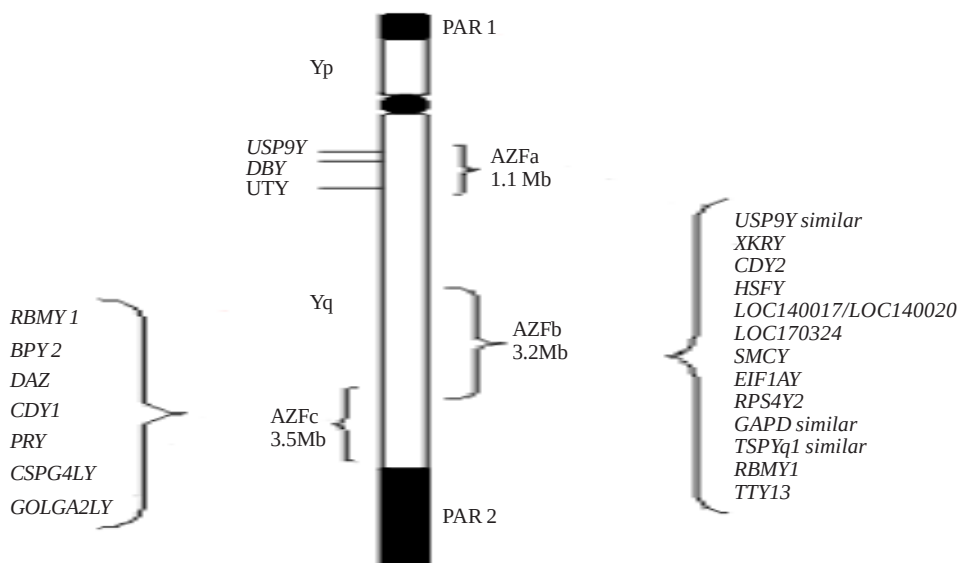


Fig. 2. Schematic representation of the human Y chromosome showing the three *AZF* regions and genes harbored by them.

mic men, 11% in azoospermic men and 18% in idiopathic azoospermic men. Upon screening and analysis among 260 infertile patients it was further shown that the frequency of deletions involving candidate gene *DAZ* of the AZFc region was high (42.5%) followed by candidate gene *RBMV* of the AZFb region (15%). Proximal deletions involving the overlapping AZFa region was found to be 12.5%, out of which a deletion involving candidate gene *DFFRY* (*USP9Y*) was found only in one patient (Ferlin et al. 1999). The high incidence of AZFc microdeletions has been reported by several workers (Oliva et al. 1998; Ferlin et al. 2007; Foresta et al. 1999). Infertile men who have varicocele condition (pathological dilation of small veins draining the scrotum) could also have Yq deletions that could lead to bilateral testicular damage wherein the actual cause need not be varicocele itself (Moro et al. 2000). Yq microdeletions can also result in severe bilateral testicular damage which may be phenotypically expressed by cryptorchidism (Foresta et al. 1999).

AZFa

AZFa portion is located in the proximal Yq 11.21 region which comes in the deletion interval 5 (subinterval 5C). The region spans about 1.1Mb (Wimmer et al. 2002). AZFa lies in that region of the Y chromosome which harbors single copy genes having homologue in the X chromosome (Pryor et al. 1997; Qureshi et al. 1996). The residing genes are found to be necessary for normal spermatogenesis (Vogt et al. 1992; Reijo et al. 1995). The candidate genes found in this region are *USP9Y*, *DBY*, and *UTY* (Table 1).

Candidate Genes of AZFa Region

USP9Y – Ubiquitin Specific Protease 9, Y Linked

USP9Y shares homology with Drosophila Fat Facets gene and was earlier known as *DF-FRY*

(Drosophila Fat Facets Related Y) (Jones et al. 1996). Related gene is also found in X chromosome and is named as *DFFRX*. It encodes a protein that is similar to ubiquitin-specific proteases which is involved in regulating protein degradation. It differs from the other candidate genes of the AZFb and AZFc region, in not coding for RNA binding protein and ubiquitous expression (Brown et al. 1998).

Molecular analysis in four infertile patients revealed the deletion of *USP9Y* and an anonymous expressed sequence tag-AZFaT1 from the AZFa region among all of them (Sargent et al. 1999). A *de novo* point mutation was also identified in the *USP9Y* gene in one patient with non-obstructive azoospermia. The vital role of *USP9Y* in regulating normal spermatogenesis was confirmed by the observation that the point mutation was absent in the patients fertile brother (Sun et al. 1999). Deletion analysis on 173 infertile men also revealed deletion of *USP9Y* gene in one patient and *USP9Y-DBY* deletion in another patient (Foresta et al. 2000).

However these observations do not serve to conclude *USP9Y* as the primary candidate gene, as a recent study reports normal spermatogenesis in an individual who has complete deletion of *USP9Y* from the AZFa region (Luddi et al. 2009). The results obtained by Foresta and co workers (2000), also demonstrated deletion in *USP9Y* was insignificant when compared to the deletion observed in *DBY* gene among 6 infertile men in the same study, when compared to one in *USP9Y*. Thus *USP9Y* cannot be considered as a major candidate gene of the AZFa region involved in normal spermatogenesis. It might exert its effect in combination with other genes of the region. Earlier Krausz and co workers (2006) reported two different deletions in the *USP9Y* gene transmitted through natural conception in two unrelated families. They concluded that gene *USP9Y* might play a role in improving the efficiency rather than controlling an essential function and also the encoded protein is not required for the sperm maturation or for the acquisition of the sperm fertilizing ability.

Table 1: List of AZFa genes demonstrated to encode proteins.

Gene symbol	Gene name	No. of copies in AZFa	Expression in tissue	X homologue	Encoding protein
<i>USP9Y</i>	Ubiquitin specific protease 9,	1	Ubiquitous	<i>USP9X</i>	Ubiquitin-specific Protease
<i>DBY</i>	Y-Dead Box Polypeptide3,	1	Ubiquitous	<i>DBX</i>	Dead box protein
<i>UTY</i>	YUbiquitously transcribed tetratricopeptide repeat gene	1	Ubiquitous	<i>UTX</i>	Tetratricopeptide repeat protein

Comparative mapping studies revealed the presence of made other gene namely *DBY* and *UTY* in the AZFa region having a possible role for normal spermatogonial development in both mice and humans. Both the genes have their homologue in the X chromosome (Mazeyrat et al. 1998).

DBY- Dead Box Polypeptide3, Y Linked

This gene encodes a DEAD box protein which is a RNA helicase involved in alteration of RNA secondary structure. The gene has a homologue on the X chromosome i.e. *DBX*. Deletion analysis on four infertile individuals revealed that three of them had deletions of *DBY* gene (Sargent et al. 1999). Similar analysis among 173 male infertile patients revealed deletion of *DBY* gene among 6 infertile patients (Foresta et al. 2000). The study also demonstrated the high prevalence of deletions of *DBY* when compared to *USP9Y* and *UTY*. It was also possible for them to show the association of *DBY* deletion in both azoospermic (3/6) and oligospermic (3/6) condition.

UTY-Ubiquitously Transcribed Tetra-ricopeptide Repeat Gene, Y Linked

UTY encodes a protein which is rich in tetra-ricopeptide repeats which might be involved in protein- protein interactions. The gene was mapped to the 5C interval corresponding to the AZFa region (Greenfield et al. 1998). Deletion analysis revealed that only one male infertile patient had deletion of *UTY* but that was in association with *DBY*. None of the patients did have *UTY* alone deleted (Foresta et al. 2000).

AZFb

The AZFb region is located approximately between the subintervals 5M to 6B (Vogt et al. 1997). The region harbors about 3.2Mb sequences and encompasses several genes essential for normal spermatogenesis (Ferlin et al. 2003). Table 2 shows the list of genes in the AZFb region which are as follows *USP9Y* similar, *XKRY*, *CDY2*, *HSFY*, *LOC140017/LOC140020*, *LOC170324*, *SMCY*, *EIF1AY*, *RPS4Y2*, *GAPD* similar, *TSPYq1* similar, *RBMY1* and *TTY13* (Ferlin et al. 2003). The authors have enlisted only the genes confirmed and annotated in Genbank. The incidence of

AZFb deletions are more in comparison to the AZFa region but however represent only a small fraction of the genetic etiology leading to infertility in men (Brandell et al. 1998; Kim et al. 1999; Martinez et al. 2000).

Table 2: List of AZFb gene families demonstrated to encode proteins.

<i>Gene symbol</i>	<i>Gene name</i>	<i>No. of copies in AZFb</i>	<i>Expression in tissue</i>
<i>RBMY1A1</i>	RNA binding motif Y chromosome 1, Subtype A1	1	Testis
<i>XKRY</i>	X kell blood group precursor Related protein, Y	2	Testis
<i>CDY2</i>	Chromodomain protein, Y, 2	2	Testis
<i>HSFY</i>	Heat shock transcription factor2, Y	2	Unknown
<i>SMCY</i>	Smcy homologue, Y	1	Testis
<i>EIF1AY</i>	Eukaryotic translation initiation Factor 1A, Y	1	Ubiquitous and testis specific transcripts
<i>RPS4Y2</i>	Ribosomal protein S4, Y linked	1	Testis
<i>TTY13</i>	Testis transcript Y 13	1	Unknown
<i>USP9Y similar</i>	Similar to Ubiquitin specific protease 9, Y chromosome.	2	Testis
<i>LOC140017/LOC140020</i>	-	2	Unknown
<i>LOC170324</i>	-	2	Unknown
<i>GAPD similar</i>	Similar to glyceraldehyde 3 phosphate dehydrogenase	1	Unknown
<i>TSPYq1 similar</i>	Testis specific protein, Y encoded q1	1	Unknown

Candidate Genes of AZFb Region

RBMY1- RNA Binding Motif Y Chromosome 1

This gene encodes a protein containing an RNA-binding motif in the N-terminus and a C terminal auxillary domain. The protein product is predicted to have a possible role in RNA processing or translational control during early spermatogenesis (Ma et al. 1993). The fact is supported by work on mouse homolog *Rbm* which is germ cell specific in expression (Elliott et al. 1996; Elliott et al. 1997), and mutation of *Rbm* leads to sperm abnormalities (Mahadevaiah et al. 1998). Abnormal sperms are produced in

transgenic mice ($XY^{d1}Sry$) with Y^{d1} deletion that removes most of the multicopy *Rbmy* gene cluster. The same was confirmed by staining testis sections with polyclonal anti-*Rbmy* antibody which showed decreased *RBMY* protein in the spermatids of the transgenic males compared to control males. Also augmenting the *RBMY* expression specifically on the spermatids of the $XY^{d1}Sry$ males failed to reduce the frequency of abnormal sperms (Szot et al. 2003)

RBMY1 is found in multiple copies across both arms of the Y chromosome, though most of them are pseudogenes (Prosser et al. 1996; Huynh et al. 2002). RT-PCR analysis confirmed the presence of at least 6 families of *RBM* genes (*RBM1-RBMVI*) which makes an approximate total of 30 *RBM* genes on both arms of the Y chromosome. Out of these only *RBM1* genes were found to actively transcribe and encode functional protein (Ma et al. 1993; Chai et al. 1997). The functional copies are located between distal interval 5 and proximal interval 6, which is coincident with the *AZFb* locus (Elliott et al. 1997). A total of 6 *RBM1* functional genes exist (Skaletsky et al. 2003). Deletion of this gene was reported in two oligospermic patients with no previous history of mutation (Ma et al. 1993). In another study, analysis of 153 Japanese men for 23 loci on Y chromosome including 15 loci within interval 6, a total of four deletions were found for *RBMY* gene (Nakahori et al. 1996). Initially *RBM1* was considered as the candidate gene of *AZFb* region, but several works later revealed *AZFb* deletion phenotype correlation to deletions outside the *RBMY1* gene.

Other Candidate Genes of *AZFb* Region

Through STS mapping analysis among 700 infertile patients, Ferlin and co workers (2003) were able to show deletions involving regions outside the *RBMY1* boundary, among four infertile patients. Five single copy genes (*LOC170324*, *SMCY*, *EIF1AY*, *RPS4Y2*, and *GAPD*-similar), and two duplicated genes (*HSFY* and *LOC14007/140020*) were found to be deleted among these infertile patients. Thus *RBMY1* cannot be considered as the sole candidate gene of *AZFb* region. The observations indicate that other genes also have a major role in regulation of spermatogenesis. The genes *XKRY*, *CDY2*, *SMCY*, *EIF1AY*, and *RBMY1A1* have been previously described while the rest are novel genes whose functions are yet to be elucidated. Dele-

tion of these genes may deregulate the *RBMY1* expression leading to spermatogenic disruption (Ferlin et al. 2003).

AZFc

The *AZFc* region is located at the distal end of interval 6 between subintervals 6C to 6F (Foresta et al. 2001). *AZFc* region resides among 3 large palindromic sequences which in turn are derived from 6 distinct families of amplicons (turquoise, gray, yellow, green, blue and red) (Kawaguchi et al. 2001). The members of each amplicon family are identical with sequence similarity of 99.98%. These amplicons are arranged so as to form 6 large inverted repeats and 3 large direct repeats. Out of the inverted repeats three are palindromes. The region spans 3.5Mb (within the 4.5 Mb palindromic complexes) and harbors seven gene families all of which are considered to be essential for normal spermatogenesis (Ferlin et al. 2003). These genes have testis specific expression which reflects the functional specialization of the palindromic complexes. Table 3 shows the list of *AZFc* gene families. Apart from the genes which are found to encode proteins, two gene families *CSPG4LY* and *GOLGA2LY* have been demonstrated to have significant predicted open reading frame. Along with this four families of genes were also found to generate noncoding transcripts (Kawaguchi et al. 2001).

Kawaguchi and co workers (2001) observed a striking similarity of deletion pattern of Yq deletions on the *AZF* region among 48 infertile men who were azoospermic or severely oligospermic. Deletion of the *AZFc* region is most common among the Y microdeletions in men with azoospermia condition (Reijo et al. 1995; Simoni et al. 1997; Kawaguchi et al. 2001). Pre-valence is about 12% in cases of non obstructive azoospermia and 6% in cases of severe oligospermia. Deletions removing any of the seven gene families (Table 3) located exclusively in the 3.5 Mb *AZFc* regions is considered to be the main cause for spermatogenic disruption (Kawaguchi et al. 2001).

Candidate Genes of *AZFc* Region

***DAZ*- Deleted in Azoospermia**

The most important candidate gene family in *AZFc* is Deleted in Azoospermia (*DAZ*), a

Table 3: List of AZC gene families demonstrated to encode proteins.

<i>Gene symbol</i>	<i>Gene name</i>	<i>No. of copies in AZFc</i>	<i>Expression in tissue</i>	<i>Encoding protein</i>
<i>CDY1</i>	Chromodomain Y	2	Testis	Chromodomain protein
<i>DAZ (DAZ1- DAZ4)</i>	Deleted in azoospermia, Isoforms 1-4	4	Testis	RNA binding protein
<i>BPY2</i>	Basic protein Y 2	3	Testis	Variably charged protein
<i>PRY</i>	PTP-BL related Y	2	Testis	Protein tyrosine phosphatase Non receptor type 13.
<i>RBM1</i>	RNA binding motifY isoform 1	4	Testis	RNA binding motif protein

multicopy gene family encoding a RNA binding protein, localized in the innermost layer of male germ cell epithelium and in the tails of spermatozoa (Habermann et al. 1998). The gene was first characterized by Reijo and co workers in 1995. Initially *DAZ* was considered to be present in single copy but later it was found that *DAZ* belongs to multicopy gene family (Saxena et al. 1996). Later four *DAZ* genes were reported by Saxena and co workers (2000), which were arrayed in 2 clusters *DAZ1*, *DAZ2* and *DAZ3*, *DAZ4*. Yen and co workers (1997) identified that there is polymorphism within the population in the number and sequence of *DAZ* repeats. The 2 clusters harbor variable number of copies of *DAZ* genes (Fernandes et al. 2006; Glaser et al. 1998; Lin et al. 2005; Saxena et al. 2000). Among them *DAZ1*, *DAZ2* and *DAZ3* are functional clusters.

In a screening for Y microdeletions among 89 confirmed azoospermic patients *DAZ* was found to be deleted among all the 12 infertile men who had distal Yq regions deleted (Reijo et al. 1995). Partial deletion of *DAZ* gene cluster was also found to cause hypospermatogenesis (Witzl et al. 2005). Ferlin and co workers (1999) screened 180 patients affected by idiopathic sertoli cell only syndrome and 50 patients with known cause of testicular alteration, 30 patients with obstructive Azoospermia and 100 normal fertile men, for deletion of candidate genes *DAZ*, *DDFY* (*UPS9Y*) and *RBM* of all the three AZF regions. They identified deletions among 40 infertile men (19 from group1 and 21 from group2). The high incidence of *DAZ* gene deletion was confirmed as 17 out of 40 patients (42.5%) had deletion of *DAZ*. But notably among the 17 individuals only in 5 cases deletion was confined exclusively to the *DAZ* region, while in remaining cases the deletion also removed the genes of flanking regions. This

suggests that the genes other than *DAZ* in the AZFc loci are also vital for spermatogenesis (Foresta et al. 1997; Stuppia et al. 1998). This involves the genes *BPY2*, *PRY*, *CDY1* and *TTY2* (Lahn and Page 1997; Kawaguchi et al. 2001; Yen et al. 1997). *CDY1* gene is expressed exclusively in the testis and is involved in replacement of histones during spermatogenesis (Vogt et al. 2005). Two *CDY1* genes map in the AZF region, one within the *DAZ* cluster and other at the distal end (Yen et al. 1998). Removal of *DAZ* also removes one copy of *CDY1* strongly suggesting that the gene could play a role in spermatogenesis. Restriction mapping has also identified three *PRY* and *TTY2* genes proximal to the AZFc region. Deletions specifically removing these genes have to be identified to confirm these novel genes as possible AZFc candidate genes (Huynh et al. 2002).

A patient affected with severe oligospermia was found to have retained only one copy of *DAZ* (Moro et al. 2000). A 1.6Mb deletion polymorphism of the AZFc region turns out to be the most frequent Y chromosome deletion (Repping et al. 2003). The deletion was named as gr/gr (green-red/green-red) deletion and removes two copies, *DAZ1* and *DAZ2* of the *DAZ* gene and several other transcriptional units causing a decrease in the sperm count (de Vries et al. 2002; Fernandes et al. 2002; Repping et al. 2003). Those deletions removing *DAZ1* and *DAZ2* were found to be associated with spermatogenic impairment whereas those removing *DAZ3/DAZ4* were found to have no or little effect on fertility (de Llanos et al. 2005; Ferlin et al. 2005).

However Machev and co workers (2004) observed gr/gr deletions among 2 control individuals with sperm count more than 40 million. They concluded that gr/gr deletions have little or no effect on sperm production. Their work emphasized that sequence dependent loss of function

differences between AZFc gene copies may frequently be the basis of spermatogenic failure. Through sequence family variant (SFV) analysis, they were able to conclude that the loss of *CDY1* variants (variant a, variant b) is significantly higher in infertile men with no evidence of partial deletions, than control men. Following this several workers have reported gr/gr deletions among control subjects as well as in infertile patients (Hucklenbroich et al. 2005; Carvalho et al. 2006; Zhang et al. 2006). This leads to the conclusion that genetic redundancy might exist in the AZFc region as number of gene copies residing in the palindrome P1 were also found to be deleted among fertile males (Vogt 2005).

AZFd

Kent-First and co workers (1999) designed 9 multiplex PCR reactions scanning about 48 Y linked STS and came up with the fourth spermatogenic loci, AZFd. The loci were proposed to exist between AZFb and AZFc. Interestingly Kent-First and co workers (1999) do not rule out the possibility that the STS mapped to the AZFd region could be very well detecting undescribed copies of *DAZ* or *RBM*. The existence of the fourth AZF region received support as well as disapproval from other workers involved in analyzing Y microdeletions. Simoni and co workers (2004) in his best practice guidelines for diagnosis of Y microdeletions stated that the sequence of MSY and the mechanism of microdeletion clearly showed that there is no AZFd locus between the AZFb and AZFc regions. However Muslumanoglu and co workers (2005) reports microdeletions in the putative AZFd loci by deletion analysis through multiplex PCR reactions employing 4 STS namely sY133, sY145, sY152 and sY153 spanning the proposed AZFd region. Few other analyses also reports deletions in the putative AZFd region (Cram et al. 2000; Yao et al. 2001)

Apart from the genes governing spermatogenesis confined to the AZF locus, Lin and co workers (2006) have identified ten novel genes through microarray analysis. Six of them encode proteins with predictable functional domain and is believed to correlate with spermatogenesis and/or spermiogenesis. Four others were found to encode nonfunctional proteins while two lacked mouse orthologues.

GENOTYPE PHENOTYPE CORRELATION

Human spermatogenesis is a complex process involving several genes during each phase of division and differentiation. Histological studies were carried out among infertile patients with microdeletions mapped to any of the three AZF regions. The results enabled to correlate microdeletions occurring at a particular AZF region to the spermatogenic phase affected, extent of damage caused and resulting condition (Vogt et al. 1996).

AZFa Deletions

Testis histology of patients confirmed to have microdeletion at the proximal Yq11 region revealed Sertoli Cell Only (SCO) syndrome (Vogt et al. 1996; Foresta et al. 2000; Ferlin et al. 2007). SCO is characterized by the absence of germ cells in all the seminiferous tubules analyzed or presence of germ cells in only very few of the tubules. The former is referred to as SCO type 1 and the latter as SCO type 2 (Terada and Hatakeyama 1991). The disruption of spermatogenesis is confirmed to have occurred pre-meiotically before or during the proliferation phase of spermatogonia (Vogt et al. 1996). The results and correlation were further confirmed by series of reports by various other groups. Brown and co workers (1998), reported two patients with deletion of the *USP9Y* gene to have SCO type 1 syndrome while one person with similar deletion had hypospermatogenesis. Similar observations were also made by other workers (Sargent et al. 1999; Sun et al. 1999). These reports served to conclude the associated phenotype for AZFa deletions.

AZFb Deletions

Unlike AZFa microdeletions, the patients with AZFb microdeletions were found to have spermatogonia and primary spermatocyte normal in all the tubules observed (Vogt et al. 1996). However Vogt and co workers (1996) were unable to trace post meiotic germ cells which led to the conclusion that the disruption occurred before or during meiosis at the spermatocyte stage. The observation helped in clearly demarcating the resulting phenotype from that of AZFa deletions Complete AZFb and AZFc deletions(P5/

ProximalP1) were found to be associated with SCO syndrome and alteration in spermatocyte maturation where as partial deletion in this region is associated with milder phenotypes and frequent presence of sperms (Ferlin et al. 2007)

AZFc Deletions

Patients with AZFc microdeletions were found to be having less severity with regards to spermatogenic disruption. Observed testis histology among five patients by Vogt et al. (1996) revealed the presence of germ cells at different developmental stages in some tubules although majority of the tubules which had sertoli cells were devoid of germ cells. Four of the patients were found to produce small number of motile sperms ranging from 0.1 to 2 million/ml. Germ cells were found at different stages in different tubules resembling SCO type 2 conditions. This reveals that a post meiotic spermatid or sperm maturation defect is the primary cause of the spermatogenic failure. Partial deletion occurring in the AZFc region, involving the *DAZ* genes have been reported to cause hypospermatogenesis (Ferlin et al. 2005).

AZFd Deletions

Infertile men with deletions in the putative AZFd may have mild or even normal sperm count but associated with abnormal sperm morphology (Kent-First et al. 1999). Musluhanoglu and co workers (2005) reported 3 azoospermic (semen devoid of sperms) men with maturation-arrested testicular histology, who had only single locus (sY152) microdeletion confined to the putative AZFd region.

MECHANISM OF MICRODELETION

Homologous recombination is the molecular event leading to the Y chromosome deletions (Sun et al. 2000). In case of the AZFa deletions, the proximal and distal breakpoints were mapped to long retroviral sequence block HERV 15yq1 and HERV15yq2, located at the Yq11 intervals D3 and D6 respectively according to the deletion map of Yq11 by Vogt et al. (1996). These sequences would have originated from germ cell infection by exogenous retroviruses through reverse transcription and genomic integration (Kamp et al. 2000). In breakpoint

analysis performed on 6 infertile individuals with AZFa deletions, it was possible to perform PCR experiments bridging both retroviral sequence blocks which map a distance of 781.557 Kb in proximal Yq11 (Kamp et al. 2000). Kamp and co workers (2000) were able to locate the AZFa breakpoint fusion region on the recombined HERV15yq1/ HERV 15yq2 sequence blocks.

Further STS mapping revealed the proximal and distal breakpoints to be 94% similar. The sequences were found to be a 10Kb provirus of the HERV15 class of endogenous retroviruses. In the two men analyzed the breakpoints were very well within the HERV15 proviruses (Sun et al. 2000).

In order to elucidate the molecular mechanisms involving AZFb deletions, Repping and co workers (2002) studied three deletions of AZFb and eight deletions of AZFc. They were able to localize the breakpoints and also sequence 9 of the deletion junctions. Seven of these deletions were confirmed to have occurred as a result of homology based recombination but the remaining two deletions could not be ascertained to homologous recombination. This demonstrated that the factors other than homology based recombination underlie these deletions. Later Ferlin and co workers (2003) were able to successfully map the proximal and distal breakpoints. They have reported the complete sequence and genomic organization of the AZFb region. The proximal breakpoint was mapped to the junction A2/C1 family of repeats. The distal break point was mapped to a region of 173bp between STS sY1211 and sY1207. But unlike the sequence homology between proximal and distal breakpoint sequence, in the AZFa region, the AZFb deletion breakpoints did not show any significant homology though interspersed repetitive sequences exist near the breakpoints. But Ferlin and co workers (2003) were able to identify a 112kb segment corresponding to the proximal AZFb to have 2 identical copies in AZFc region. Recombination between these direct repeats of AZFb and AZFc regions could be one possibility for AZFb deletions.

The AZFb deletions are massive and extend from palindrome P5 to proximal arm of palindrome P1, 1.5 Mb within AZFc. The deletions encompass 6.2 Mb removing 32 genes and transcripts. The deletions involving AZFb and AZFc

extend from P5 to distal arm of P1 sparing distal AZFc. The deletions encompass 7.7Mb removing 42 genes and transcripts. These are 'one of the largest of all human interstitial deletions' for which deletion junctions as well as the sequence encompassed are available. (Repping et al. 2002).

Mechanism of microdeletions in the AZFc region is found to be similar to that of AZFa region. In one study the proximal breakpoints was found to be between the STSs sY1192 and sY1197 spanning 349kb whereas the distal boundary was mapped between STSs sY104 and sY1125 spanning 229kb (Kawaguchi et al. 2001). The breakpoints correspond to b2 and b4 members of the blue amplicon family. Both b2 and b4 show 99.9% homology strongly suggesting the role of homologous recombination involved in deletions of AZFc genes. Repping and colleagues (2004) also described b2/b3 recombination of a 1.8-Mb deletion that deletes half of the AZFc region, including 12 members of 8 testis-specific gene families. Apart from this gr/gr (green-red/green-red) recombination characterized by removal of 1.6Mb sequences that removes *DAZ1* and *DAZ2* copies has also been reported in the AZFc region (Repping et al., 2003). However recent works reports novel partial deletions in the AZFc region that were due to Non Homologous Recombination (NHR) (Noordam et al., 2011). The authors hypothesize that as palindromes are inherently unstable and as the AZFc region harbors 3 palindromes (P1,P2 and P3) it might render the region prone to DNA breaks leading to deletions via NHR. Previously Repping and coworkers, 2002, have also reported two P5/distal-P1 deletions that occurred by means of NHR.

CONCLUSION

Human spermatogenesis is a complex process involving a series of coordinated events. The process is regulated and governed by several genes arrayed in the azoospermic factor regions. The AZFa region harbors three important candidate genes namely *USP9Y*, *DBY*, and *UTY* which upon deletion are reported to cause azoospermic and oligospermic condition. *RBMY* is considered to be the candidate gene of AZFb region but recent deletion analysis shows the possible role of other genes present in this region governing normal spermatogenesis. AZFc

region is the hotspot and frequent deletions are found in this locus. It harbors the *DAZ* gene which is found to be deleted in most of the cases involving AZFc deletions. However in much of the cases other genes are also removed along with the *DAZ* rendering it difficult to consider *DAZ* as the sole candidate gene of AZFc region. Homologous recombination accounts for majority of the deletions observed. But recently deletions via non homologous recombination has also been reported which can also explain the reports indicating absence of sequence homology among the flanking regions involved in deletions among few cases.

ACKNOWLEDGEMENT

We thank Prof. S.N. Hegde, Chairman of our Department, Ms. Kavitha P, Mr. Shivaprasad, Ms. Chaitra P. T, Mr. Mohsen Najafi, Mr. Sreenivasa G and Dr. C. Sharat Kumar for their support and help during the course of the preparation of the manuscript. We thank UGC, New Delhi, for the financial support rendered.

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