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Y Chromosome and Male Infertility

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ABSTRACT The Y chromosome though representing only 2-3% of the haploid genome harbours about 107 genes and pseudogenes. Many of these are responsible for spermatogenesis and other male-related functions and deletion of any of these can result in infertility. The association of azoospermia with deletions involving long arm of the Y-chromosome, led to the proposition of an azoospermic factor (AZF). Further mapping of the Y-chromosome resulted in the identification of three regions viz., AZFa, AZFb, and AZFc associated with spermatogenic failure. The microdeletions involving the AZF have been extensively reported to cause male infertility. The genes of the "non-recombing region of Y-chromosome" (NRY) or the "male specific region of Y chromosome" (MSY) play an important role in male fertility. Some important genes identified in this region and associated with male infertility are: SRY, TSPY, USP9Y, DBY, UTY, E1F1AY, RBMY, DAZ, CDY1, BPY2, PRY, and TTY2. Their relative contributions to male infertility are discussed.

Y CHROMOSOME

Y chromosome is the smallest chromosome of the human genome. It has only about 60 million DNA base sequences and has the least number of genes as compared to any other human chromosome (Harris et al. 1986). About 107 genes and pseudogenes have been mapped to the Y-chromosome (http://gdbwww.gdb.com) and it has a high proportion of repetitive elements (Ali and Hasnain 2002). There are two pseduoautosomal regions, PAR1 and PAR2, on the short (Yp) and long (Yq) arms of Y chromosome that recombine, respectively, with their homologues on the X chromosome. The rest of the Y chromosome (~95 %) is know as 'non-recombining region' (NRY) or as 'male specific region' (MSY) (Fig. 1). The NRY contains intra-chromosomal repetitive elements that may be homologous to regions on X chromosome or Y chromosome (Tilford et al. 2001). The NRY region can be divided into euchromatic, centromeric and heterochromatic regions (Foote et al. 1992). The euchromatic region, cytogenetically known as Yq11 (subdivided into Yq11.21, Yq11.22, Yq11.23), is about 24 Mb. The heterochromatic Yq12 region contains 30 Mb sequences (Vollrath et al., 1992) and it has two major repeating domains viz., DYZ1 and DYZ2 (Cooke 1976). However, Ali and Hasnain (2002, 2003) divide the Y chromosome in five broad sections e.g., i) Pseudoautosomal boundary regions (PABY), ii) a Pericentric region on the short arm harbouring the sex determining (SRY) gene, iii) an Euchromatic region (DYS1) on the proximal long arm, iv) a Heterochromatic region (DYZ1) on the distal long arm, and (v) an important DYZ3 region that is critical for the survival and propagation of the Y chromosome.

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Relationship of Y Chromosome with Infertility

The Y chromosome represents only 2-3% of the human haploid genome. It along with X chromosome, originated from a pair of autosomes around 300 million years ago among reptiles, much before mammals arose (Foster and Graves 1994). These chromosomes, in mammals, most probably arose due to differentiation of SRY gene from SOX3, which has a structural homologue on the X chromosome (Foster and Graves 1994; Stevanovic et al. 1993). The lack of recombination between X and Y chromosome is thought to be responsible for the decay of Y chromosome linked genes (Lahn et al. 2001) and this hypothesis seems to explain the small size of Y chromosome as compared to X chromosome (Graves 1995; Lahn and Page 1999). However, according to Ali and Hasnain (2003), the identification of different

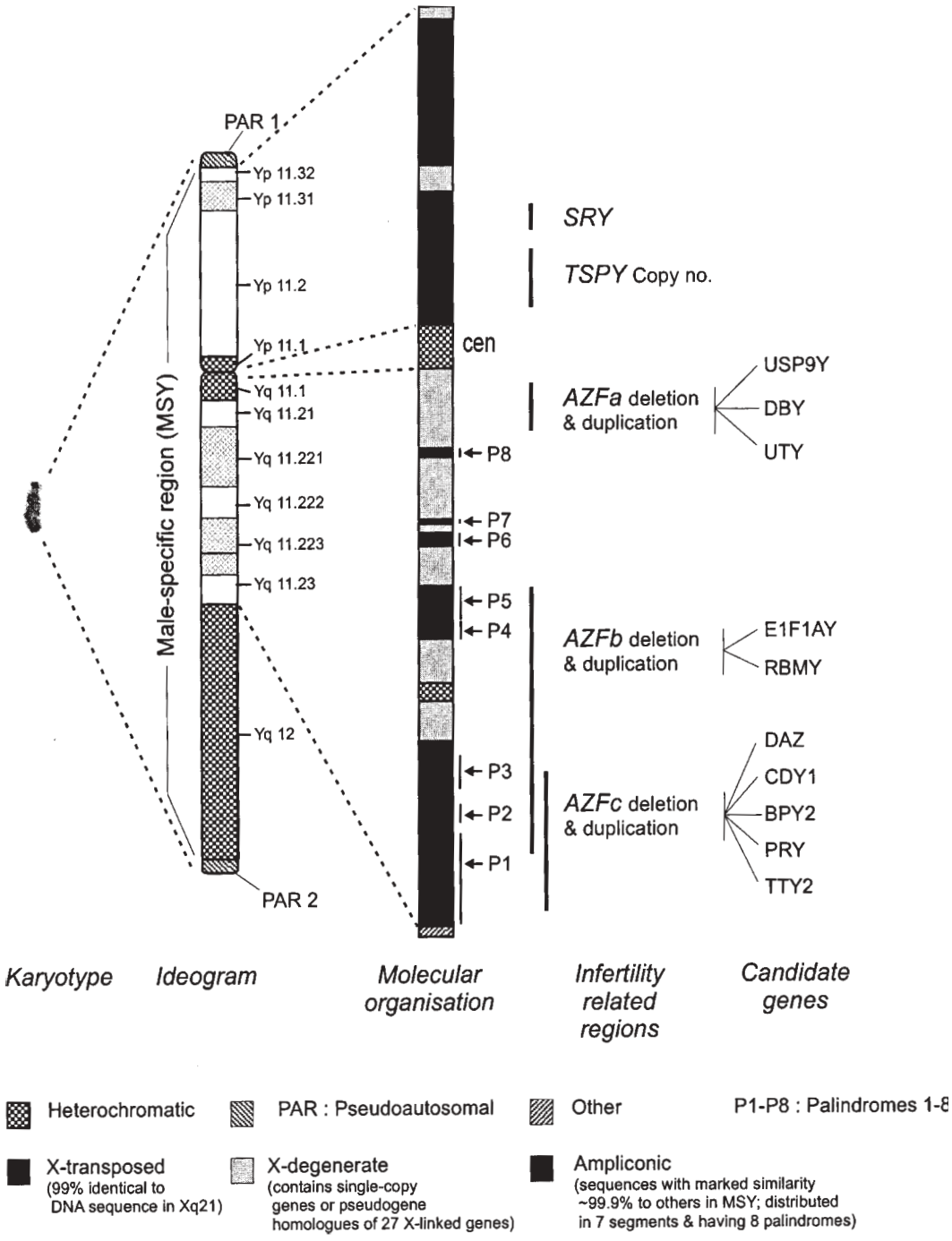

Fig. 1. The Y chromosome organization

palindromes having several different gene families (Kuroda-Kawaguchi et al. 2001; Skaletsky et al. 2003) and frequent gene conversions (Rozen et al. 2003) raises the doubt about progressive decay of Y chromosome over period of time. Y chromosome now harbours the genes that are essential for male sex determination and spermatogenesis (McElreavey et al. 2000). It was during the course of evolution that this Y chromosome acquired a large number of testes specific genes responsible for spermatogenesis and other male-reproduction-related functions (Marshall 2000; Saxena et al. 1996). The deletion of any of these genes can result in infertility (Silber and Repping 2002; Skaletsky et al. 2003). Some workers have also implicated a longer Y chromosome (Yq+) with increased risk of abortions (Genest and Genest 1985; Patil and Lubs 1977).

Mutations in Y Chromosome

In 1970's the observation of Y chromosome anomalies, particularly the deletions involving long arm of the Y chromosome, in azoospermic men lead to the proposition of an azoospermic factor (AZF) being encoded by one or more male-specific Y chromosome genes (Tiepolo and Zuffardi 1976). Later on, the Y chromosome mapping resulted in the identification of three regions viz., AZFa, AZFb and AZFc to be associated with spermatogenic failure (Vogt et al. 1996).

The MSY has at least 156 transcription units and 78 of these are protein coding genes, which collectively code for twenty seven distinct proteins or protein families. Twelve of these MSY genes are expressed ubiquitously and reside in the X-degenerate regions and 11 MSY genes are expressed predominantly in the testes (Skaletsky et al. 2003). An important observation made by Skaletsky and co-workers (2003) was that most of MSY-specific protein coding gene families, present on a single Y chromosome, are present in multiple copies ranging from a single copy to almost 35 copies, e.g., one copy (*TGIF2LY*) to two (*VCK*, *XKRY*, *HSFY*, *PRY*) to three (*BPY2*) to four (*CDY*, *DAZ*) to six (*RBMY*) to approximately 35 copies (*TSPY*) and that their numbers may vary in different populations. Earlier, Kedes (1979) had observed that the genes that are required to produce large amounts of products are characterized by high copy numbers and that these genes usually have a high number of tandem repeated sequences. He proposed that this multiplicity helps in accomplishing the organism's need for large amount of product e.g., ribosomal genes, transfer RNA and histone genes. These genes are present in the proximal and distal palindromic complexes which also have AZF factors and the recombination between palindromes P5 and P1 on the Y chromosome can cause massive deletion of these, to cause spermatogenic failure (Repping et al. 2002). Having high number of copies on the Y chromosome also increases the risk of mutations and this might be responsible for various degrees of sperm counts that are observed in different individuals (Huynh et al. 2002).

The Southern hybridization of DNA from somatic cell hybrids containing only the human Y chromosome with *Alu* specific probes has shown that the *Alu* sequences on the human Y chromosome are different form the ones present in rest of the genome (Deininger et al. 1981; Smith et al. 1987). Further, Lander and coworkers (2001) reported that *Alu* sequences are under represented on the Y chromosome as compared to the other young interspersed repeats and this might be due to the low density of somatically active genes present on the Y chromosome. The unique *Alu* sequences present on the human Y chromosome play important role(s) during spermatogenesis and are believed to be responsible for the tandem duplication and subsequent inversions of 3 Mb P1 palindromic sequences on the long arm of Y chromosome (Kuroda-Kawaguchi et al. 2001). Ali and Hasnain (2003) have opined that the SNPs found in the NRY region of Y chromosome may have some association with infertility and these in future may become a powerful diagnostic tool.

Micro-deletions in Y Chromosome

The identification of microscopically detectable deletions in the distal part of Yq in six azoospermic men, out of 1170 cases, by Tiepolo and Zuffardi (1976) provided the first evidence of a genetic cause for spematogenic failure. Vogt and co workers (1992) investigated 19 infertile men having azoospermia or severe oligospermia, and reported two men with non-overlapping micro-deletions in the distal Yq. Later in 1993, Ma and co-workers reported similar non-overlapping micro-deletions in azoospermic

males. These observations suggested the existence of multitude of loci located in this domain that might be associated with infertility. Vogt and co-workers (1996) screened 370 men with idiopathic oligospermia or azoospermia for deletions, and their findings suggested that three separate non-overlapping regions known as, AZFa, AZFb and AZFc, were required for spermatogenesis. Later on, Kent-First et al. (1999) reported the possibility of the presence of a fourth region, namely AZFd, present in-between AZFb and AZFc.

The genes of the NRY region play an important role in male fertility, but their effects are not limited only to causing azoospermia (Reijo et al. 1996). It has been reported that the deletion of a particular AZF locus results in a characteristic phenotype, and that the genes at each locus act at a particular stage of germ cell differentiation (Dada et al. 2004; Vogt et al. 1996).

AZF deletions have been extensively reported to cause male infertility while no other effects of these have yet been documented. Various authors have reported widely different frequencies, ranging from 1% to 55%, about the infertile men, who carry the AZF deletions (Foresta et al. 1998; Kent-First et al. 1999; Pryor et al. 1997; Reijo et al. 1995; Seifer et al. 1999; Stuppia et al. 1996, 1997, 1998; Vogt et al. 1996). This large inter-laboratory variation in the frequency of AZF deletions is probably due to the type of patients selected for Y chromosome analysis (Kent-First et al. 1999; McElreavey and Krausz 1999).

Candidate Genes in AZFa Region

AZFa region lies in that region of the Y chromosome which contains single copy genes with known homology to the X chromosome and very few deletions have been described in this region (Brown et al. 1998; Pryor et al. 1997; Qureshi et al. 1996; Vogt et al. 1996; Wimmer et al. 2002).

The first gene to be identified in AZFa region which was lateron found to be absent in infertile men, was DFFRY (Drosophila fat facets related Y). It was so named due to its homology to the Drosophila developmental gene fat facets (faf) (Foresta et al. 2001). DFFRY has now been renamed as USP9Y (ubiquitin-specific protease 9, Y chromosome) (Huynh et al. 2002). It is a single copy gene and seems to function as C-terminal ubiquitin hydrolase. This gene is expressed widely in different tissues and its expression is not confined only in the testes (Foresta et al. 2001). USP9Y in humans has been mapped to Yq11.2 and Xp11.4 (Brown et al. 1998; Jones et al. 1996) and its X homologue avoids X-inactivation. USP9Y makes up less than half of AZFa; but majority of the infertile males, who exhibit deletions in this section, miss whole of the interval. On the basis of such findings, it has been proposed that other gene(s) in this region, either alone or in combination with USP9Y, are responsible for spermatogenic failure (Huynh et al. 2002). Evidence for the relation of USP9Y with spermatogenesis was also provided by Sun and co-workers (1999) who showed a truncated protein in an azoospermic male having 4 bp deletion in the USP9Y gene; while this deletion was not present in his fertile brother.

Comparative mapping studies have shown the presence of two more XY homologous genes that are located in the AZFa region, thus having a possible role in spermiogenesis. These are DBY (Dead box on Y) (Lahn and Page 1997) and UTY (ubiquitous TPR motif on Y) (Mazeyrat et al. 1998). Sargent et al. (1999) localized another novel sequence AZFaT1 in this region and they proposed that deficiency of USP9Y and AZFaT1, together or individually, resulted in male infertility and the absence of DBY further manifested this condition. Foresta et al. (2000) have reported that DBY is a major spermatogenesis gene in the AZFa region. It is more frequently deleted than USP9Y and its expression is also specific for testes. Moreover, DBY is quite homologous to mouse protein PL10 which is testis specific and is expressed only in the germ cells. However, the exact function of these genes still remains unknown (Huynh et al. 2002).

Candidate Genes in AZFb Region

The deletions in AZFb region are more common than the AZFa region but these still represent only small percentage of azoospermic men (Brandell et al. 1998; Kim et al. 1999; Martinez et al. 2000; Vogt et al. 1996).

Two genes, EIF1AY and RBMY, have been mapped in the AZFb region, which corresponds to deletion interval 5O-6B (Huynh et al. 2002). The EIF1AY (translation initiation factor 1A, Y isoform) gene encodes for a Y isoform of elf-1A, a ubiquitously expressed translation initiation

Tabe 1: Candidate genes in the AZF regions of the human Y chromosome

AZF region	*Candidate genes*	*Cytogenetic location*	*Gene/ Gene family*	*Exons*	*Expression*	*Gene size (No. of bases)*	*Protein name*	*Amino acids in protein*
AZFa	USP9Y	Yq11.21-Yq11.21	Single gene	47 Exons	Ubiquitous	158,930	Probable ubiquitin carboxyl-terminal hydrolase FAF-Y (2 isoforms due to alternate splicing)	2555
	DBY	Yq11.21-Yq11.21	Single gene	17 Exons	Testis specific and ubiquitous	16,371	DEAD-box protein 3, Y chromosomal	660
AZFb	EIF1AY	Ypter-Yqter Yq11.22-Yq11.22			Testis specific and ubiquitous	17,413	Eukaryotic translation initiation factor 1A, Y chromosomal	143
	RBMY (RBMYA1)	Yq11.23-Yq11.23	Gene family	12 Exons Srgy boxes (111 bp each)	Testis specific	37,961	RNA binding motif protein, Y chromosome, family 1 member A1	496
AZFc	DAZ	Yq11-Yq11, Yq12-	Gene family	16 Exons (including multiple copies of exon 7)	Testis specific	71,817	Deleted in azoospermia protein 1 Deleted in azoospermia protein 2 (three isoforms due to alternate splicing)	Three proteins: 458; 501; 366
	CDY1	Yq11.223-Yq11.223			Testis specific	2,785	Testis-specific chromodomain protein Y 1 (2 isoforms exist due to alternate splicing)	540
	BPY2	Yq11-Yq11	Gene family	7 exons	Testis specific	5,924	Testis-specific basic protein Y 2	106
	PRY	Yq-Yq	Gene family	5 exons	Testis specific	24,240	PTPN13-like protein, Y-linked (2 isoforms exist due to alternate splicing)	147
	TTY2	Yq11.2	Gene family	7 exons		17,891 plus strand	Testis-specific Testis Transcript Y 2 [Fragment] Non coding RNA fragments or pseudogenes only	

Source: http://www.gdb.org; http://genecards.bcgsc.bc.ca; http://www.expasy.uniport.org/database/knowledge.shtml

factor, and it has an X homologue (Lahn and Page 1997). The role of EIF1AY in spermatogenesis is still not clear, as no report showing deletion of only this gene, has been observed and some feel that EIF1AY may not be the AZFb-candidate gene (Huynh et al. 2002).

The other gene, RBMY (RNA-binding motif on Y), is a multicopy gene family having 30-40 members and some pseudogenes (Ma et al. 1993; Prosser et al. 1996). RBMY, earlier known as YRRM (Y-specific RNA recognition motif), has been detected at many places across both arms of the Y chromosome. However, only the copies present in the AZFb region produce detectable protein levels (Huynh et al. 2002). RBMY genes and pseudogenes are further divided into six subfamilies, viz., RBMY1 to RBMY6. The most dominant subfamily is RBMY1 that has seven different genes, which vary from each other by 1-7 bases and are present widely in AZFb, proximal deletion interval 6 (Chai et al. 1997, 1998; Prosser et al. 1996).

Delbridge and co-workers (1999) reported the presence of a homologue of RBMY on human Xp26 and named it RBMX. RBMX appears to have retained widespread activity while RBMY became male specific (Delbridge et al. 1999). RBMY1 has been observed to co-localize with other known splicing factors at certain stages of spermatogenesis. The loss of RBMY genes in AZFb region appears to be associated with the arrest of spermatogenesis at meiosis (Huynh et al. 2002) because deletion of some members of RBMY family has been noted in infertile men having azoospermia or oligospermia. Mahadevaiah et al. (1998) observed that deletion of RBMY gene in mice produces high level of abnormal sperm development, indicating the need of further comparative studies to understand fully the role of RBMY gene family.

Delbridge and co-workers (1997) observed that in marsupials only one copy of RBMY is essential for normal spermatogenesis. However, it is still not clear whether only one copy of RBMY is sufficient or multiple copies of it are required for spermatogenesis in man (Huynh et al. 2002).

Candidate Genes in AZFc region

Five genes have been mapped in AZFc region of Yq. These are; DAZ (deleted in azoospermia), CDY1 (chromodomain Y1), BPY2 (basic protein Y2), PRY (PTA-BL related Y) and TTY2 (testis transcript Y2) (Lahn and Page 1997; Vogt et al. 1996; Yen et al. 1997; Table 1).

DAZ (Deleted in Azoospermia) Gene: The DAZ (Deleted in Azoospermia) is the candidate gene for the human Y-chromosomal Azoospermia Factor c (AZFc). Sequence analysis of Y-chromosomal DAZ by Saxena et al. (1996) suggested that it arose in primates during evolution by transposing an autosomal gene to the Y which was amplified and then its exons were pruned within the transposed gene. Originally it was thought that DAZ was a single copy gene but high resolution FISH and other molecular investigations showed multiple copies of DAZ (> 99% identical in DNA sequence) to be clustered in the AZFc region. The DAZ gene shows a high degree of polymorphism (Yen et al. 1997) and belongs to the gene family that consists of three members; BOULE, DAZ-Like (DAZL), and DAZ. This family is expressed only in germ cells, and their protein products contain a highly conserved RNA-binding motif and a unique DAZ repeat. It also has a functional DAZ homologue, DAZL1 (DAZ like-autosomal 1), on human chromosome 3 (3p24). The entire DAZ gene family appears to be expressed in the germ cells (Saxena et al. 1996). DAZ encodes for a testes specific protein containing a single RNA-binding motif with 8 to 24 copies of 24 amino acid sequence, known as 'DAZ repeat' (Reijo et al. 1995; Yen et al. 1997). At least three copies of DAZ gene viz., DAZ1, DAZ2 and DAZ3 have been reported to be functional (Saxena et al. 2000).

Ruggiu et al. (1997) reported that loss of DAZL1 gene in mouse reduced the number of germ cells and also caused complete absence of gamete production. Yen (2004) observed that both, DAZ and DAZL, are expressed throughout the life of most germ cells and are thus required for the development of primordial germ cells as well as the differentiation and maturation of the germ cells.

According to Huynh et al. (2002) though the DAZ copy number and DAZ repeats vary from person to person, it is not certain as to what is the influence of the number of copies on spermatogenesis. However, de Vries and co-workers (2002) suggested that as small deletions are present in men with mild oligospermia, there may be gene dosage effect e.g., men with 2 deleted DAZ genes be less affected than those with loss of all DAZ genes. Habermann et al.

(1998) observed that the deletion of DAZ genes did not to interfere with human sperm maturation but resulted in a gradual reduction of the mature spermatozoa.

Ferlin et al. (2005) reported that only partial AZFc deletions removing DAZ1/DAZ2 seemed to be associated with spermatogenic impairment, whereas those removing DAZ3/DAZ4 had no or little effect on fertility. Their data showed that, beside complete AZFc deletions, specific partial deletions represent a risk factor for male infertility, even though the effects on spermatogenesis would be different.

Yen (2004) reported that DAZ, most probably, has a role in the post-transcriptional regulation of mRNA expression; whereas BOULE is expressed around the time of meiosis and may have a more limited function.

CDY1, BPY2, PRY, and TTY2 Genes: CDY1, BPY2, PRY, and TTY2 genes also have multiple copies on the Y chromosome, and are expressed only in testes (Lahn and Page 1997). PRY and TTY2 have been localized in the proximal part of AZFc by restriction mapping (Yen et al. 1997). Two CDY1 genes map in the AFZc region; one within DAZ cluster and the other at the distal end. Huynh et al. (2002) observed that as most of the deletions of AZFc region involve deletion of at least one CDY1 gene, thus CDY1 is a possible AZFc candidate gene. However, the exact function of all these genes (CDY1, BPY2, PRY, and TTY2) is still not well established.

Other Important Infertility Related Genes on Y Chromosome

There are two important genes, which are out side the AZF region, but play crucial role in causing male infertility. These are discussed below:

SRY (Sex Determining Region of Y Chromosome) Gene: SRY gene (Sex determining Region on Y chromosome), also known as TDF (Testis Determining Factor), is a very important gene on the Y chromosome and it has a major role in sex determination (Goodfellow and Lovell-Badge 1993). This gene encodes for a transcription factor of about 204 amino acids from a single exon which is about 669 bp (Behlke et al. 1993). SRY is a member of mobility group DNA binding protein with highly conserved 'HMG-box' domain. Mutations in the SRY gene result in XY females with gonadal dysgenesis (one type of Swyer syndrome); and translocation of a part of the Y chromosome, containing this gene, to X chromosome results in phenotypic males with XX chromosomes. However, mutations responsible for producing Swyer syndrome may either occur in the HMG-box or in the non-HMG regions (Schaffler et al. 2000; Scherer et al. 1998).

The complete mode of action of SRY gene is still not clear but it is known that SRY-mediated regulation involves complex protein/protein interaction, depending upon cellular and promoter context, and switches on the expression of male specific genes. SRY gene also works in association with other protein partner genes like WT1, SF-1, DAX-1 and SOX9 in gonadogenesis (McElreavey and Fellous 1999). Ali and Hasnain (2003) believe that though the SRY gene is critical, it is not exclusively responsible for gonado-genesis.

TSPY (Testes Specific Protein, Y-encoded) Gene: TSPY gene is a multicopy gene and most of its copies are located in the NRY region of the Y chromosome. TSPY gene family has 20-40 gene copies that vary from individual to individual and occur in at least six locations on the human Y chromosome; with each cluster containing a unique combination of variants (Dechend et al. 2000). TSPY gene is 2.8 kb long and it has six exons and five introns. Its prototypic coding sequence is 924 bp and a sequence divergence of 10% has been reported in human TSPY sequences (Arnemann et al. 1991; Manz et al. 1993).

The first TSPY gene was localized to deletion interval 3 on the short arm of Y chromosome (Conrad et al. 1996; Vogel et al. 1998; Zhang et al. 1992). The second TSPY gene was located in the deletion interval 6E in the proximal part of long arm of Y chromosome (Ratti et al. 2000). Dechend et al. (2000) reported that majority of TSPY members map to deletion interval 3C on Yp11.2, in which each copy is embedded in a single unit of 20 kb as a constitutive part of the DYZ5 tandem repeat array. It was believed that TSPY gene does not have a homologue on X chromosome. However recently, Delbridge et al. (2004) showed that a TSPY homologue with similar gene structure lies in conserved positions, close to SMCX, on the X chromosome in humans (TSPX) and also in mouse (Tspx). TSPX is widely expressed and is subject to X inactivation. Therefore, it appears that TSPX and TSPY might have evolved from an identical gene pair on the

original mammalian sex chromosomes. The expression of TSPY sequences, by RNA analyses, has been reported exclusively in the testes, both during the prenatal and the adult stages (Arnemann et al. 1991; Jakubiczka et al. 1993; Manz et al. 1993; Zhang et al. 1992). A human TSPYL gene was earlier localized on chromosome 6 and its expression was observed in all the tissues and also during early course of development (Vogel et al. 1998).

Schnieders et al. (1996) and Vogel et al. (1998) reported that the protein coded by TSPY gene contained 308 amino acids, while Krick and co-workers (2003) put this figure as 294 amino acids. The transcript heterogeneity could, in principle, be caused either by the transcription of structurally different genes within the cluster, or by one or several structurally identical copies permitting alternative splice patterns.

The function of TSPY gene, which is only expressed in testes, is to integrate TSPY protein into spermatogenesis (Arnemann et al. 1991; Schnieders et al. 1996). The immunohistochemical data of TSPY expression suggest a function of TSPY that is specific for the proliferation of germ cells. This was reported by Schnieders et al. (1996) who observed marked variation in the immunostaining of spermatogonia, with pairs of spermatogonia showing equivalent staining indicating that TSPY was involved in spermatogonial proliferation. Furthermore, the topology of testicular expression in the adult testis and the homology of TSPY with members of the TTSN-family that are involved in cell cycle control, also suggest the role of TSPY in spermatogonial proliferation (Chai et al. 2001; Ozbun et al. 2001; Vogel et al. 1998). However, Lau (1999) opined that the expression of TSPY depends upon the spermatogenic activity and on proper hormonal environment.

On the basis of distribution of RBM and TSPY signals on the Y chromosome, Conrad et al. (1996) proposed that these two genes might have originated form a common organization on an ancient Y chromosome. As RBM is an AZF candidate gene, the possible role of TSPY in spermatogenesis is further strengthened.

Consequences of Mutations/Deletions in Y-Chromosome

Y chromosome is a likely target for increased mutational damage due to rapid division of germ cells, both during fetal and adult life (Joffe 2003). It is also not protected from mutagenic environment like the other chromosomes, by long inert periods in the ovum (Hargreave 2000). The mutations in single genes on Y chromosome are more likely to produce effects as all its genes are "haploid". According to Davis et al. (1998) the selective effects on the Y chromosome carrying spermatozoa, leading to the selective loss of male embryos; or mutations in the genes on the Y chromosome can cause a deficit in male births. AZF deletion mosaicism has also been identified as a risk factor for testicular tumor development (Bianchi et al. 2002).

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