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Y Chromosome Deletions in Azoospermic Men in India

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ABSTRACT: Genetic factors cause about 10% of male infertility. Azoospermia factors (*AZFa, AZFb, AZFc*) are considered to be the most important for spermatogenesis. We therefore made an attempt to evaluate the genetic cause of azoospermia, Y chromosome deletion in particular, in Indian men. We have analyzed a total of 570 men, including 340 azoospermic men and 230 normal control subjects. DNA samples were initially screened with 30 sequence-tagged site (STS) markers representing *AZF* regions (*AZFa, AZFb, AZFc*). Samples, with deletion in the above regions were mapped by STS walking. Further, the deletions were confirmed by Southern hybridization using the probes from both euchromatic and heterochromatic regions. Of the total 340 azoospermic men analyzed, 29 individuals (8.5%) showed Y chromosome deletion, of which deletion in *AZFc* region was the most common (82.8%) followed by *AZFb* (55.2%) and *AZFa* (24.1%). Microdeletions were observed in *AZFa,* whereas macrodeletions were observed in *AZFb* and *AZFc* regions. Deletion of heterochromatic and azoospermic regions was detected in 20.7% of the azoospermic men. In 7 azoospermic men, deletion was found in more than 8.0 Mb spanning *AZFb* and *AZFc* regions. Sequence analysis at the break points on the Y chromosome revealed the presence of L1, ERV, and other retroviral repeat elements. We also identified a ~240-kb region consisting of 125 bp tandem repeats predominantly comprised of ERV elements in the *AZFb* region. Histological study of the testicular tissue of the azoospermic men, who showed Y chromosome deletion, revealed complete absence of germ cells and presence of only Sertoli cells.

Key words: Male infertility, STS markers, repeat sequence.

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About 15% of couples are infertile because of several reasons, of which the male factor is responsible for approximately 50% of the cases. Various causative factors have been identified for male infertility, including varicocele, obstruction of spermatic ducts, agglutination of sperms, impotency, hormonal imbalance, and genetic defects. Based on the semen profile, male infertility is classified into 4 major categories, namely oligozoospermia (low sperm count), asthenozoospermia (low motility), teratozoospermia (abnormal shape and size), and azoospermia (complete absence of sperm). Genetic factors have been found to play a role in about 10% of male infertility (Pryor et al, 1997).

The male sex determining region (*SRY*) has been mapped on the short arm, whereas genes involved in the complex process of spermatogenesis are located on the long arm of the Y chromosome (Yq11), namely RNA-binding motif 1 (*RBM1*), RNA-binding motif 2 (*RBM2*), deleted in azoospermia (*DAZ*), basic protein on Y chromosome 2 (*BPY2*), chromodomain protein on Y chromosome (*CDY*), transcribed testis-specific protein on Y (*TSPY*), drosophila fat facets related on Y (*DFFRY*), or ubiquitin specific protease 9 on the Y chromosome (*USP9Y*), etc (Ma et al, 1993; Reijo et al, 1995; Kent-First et al, 1999; Sun et al, 1999; Ratti et al, 2000). Most of these genes exist in multiple copies (Delbridge et al, 1997; Dechend et al, 2000), of which only some are functional (Prosser et al, 1996; Chai et al, 1997). Although 4 azoospermic factors (*AZFa, AZFb, AZFc,* and *AZFd*) have been mapped on the long arm of the Y chromosome, *AZFa*, *AZFb*, and *AZFc* are considered to be the most important for sperm production (Affara, 2001). Major candidate genes for *AZFa*, *AZFb*, and *AZFc* are *DFFRY*/*USP9Y* (Mazeyrat et al, 1998; Sun et al, 1999), *RBMY* (Ma et al, 1993), and *DAZ* (Reijo et al, 1995), respectively.

Large numbers of sequence-tagged sites (STS) markers have been mapped to *AZFa, AZFb,* and *AZFc* regions. Incidence of Y chromosome deletion is about 1 in 1000, and these deletions are often associated with male infertility. Deletion of the growth control gene on the Y chromosome (*GCY*) interval in the *AZFa* region was found to be associated with short stature (Kirsch et al, 2000). However, some of the Y chromosome deletions are neutral, which do not affect the fertility status and phenotype of the individual (Thangaraj et al, 2002b). Several studies

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have demonstrated that microdeletion in *AZF* regions causes male infertility. Deletion of each *AZF* region has been found to have a different phenotypic effect. Sargent et al (1999) studied the *AZFa* region of 4 infertile men and found a deletion of *AZFa*. *DFFRY* and *DBY* were associated with Sertoli cell–only syndrome type 1 (SCOS) phenotype, whereas the patient, who retained *DBY*, seemed a mild oligozoospermia. However, genotype-phenotype correlation has not been fully understood. Deletions in the *AZFb* region have been found to be associated with azoospermia, oligozoospermia, and normozoospermia. Deletion of the *AZFc* region has been found to be associated with azoospermia and severe to mild oligozoospermia (Affara, 2001). It has been found in many cases that similar deletions of *AZF* regions cause quantitative loss in spermatogenesis. Among the 3 *AZF* regions, deletion of *AZFc* has been found to be the most frequent abnormality, followed by *AZFb* (Layman, 2002).

Most of the STS-based studies on male infertility have been carried out with a few markers for each *AZF* region (Martinez et al, 2000). Hence they fail to detect the Y chromosome deletion in many cases. Therefore, there is no collective opinion about the marker to be used for Y chromosome microdeletion analysis. In this study, for the first time, we have made an attempt to evaluate the association of Y chromosome microdeletion among Indian men with azoospermia using several STS makers from each *AZF* region. This study will help generate epidemiological data, which in turn, will be useful for infertility clinics for adopting an appropriate strategy to provide genetic counseling to the affected couples.

Materials and Methods

Azoospermic and Control Men

Three hundred forty azoospermic men, selected from 870 azoospermic men, attending the infertility clinic at the Institute of Reproductive Medicine (IRM), Kolkata, India, were included in the present study. The age groups of these azoospermic men ranged from 26 to 42 years. An experienced urologist at IRM carried out a detailed case history and clinical examination of every patient. Endocrinological assays (follicle stimulating hormone [FSH], luteinizing hormone [LH], testosterone, prolactin [PRL], thyroid stimulating hormone [TSH], HIV, and venereal disease research laboratory [VDRL] test); vasogram; and karyotyping were performed for each patient by respective experts. Testicular pathology was carried out wherever it was possible. Patients who showed any abnormality such as obstruction; endocrinological defect; obvious karyotypic abnormalities (eg, Klinefelter syndrome, mosaicism, translocations, marker chromosome, etc); pelvic injuries; and major illness were excluded in the present study. Two hundred thirty random fertile Indian men were included in this study as control. Blood sample (5.0 mL) from each azoospermic and fertile control man were collected with informed written consent. DNA of the above samples was isolated using the protocol described by Thangaraj et al (2002a).

Polymerase Chain Reaction Assay

Polymerase chain reaction (PCR) based studies for microdeletion on both azoospermic and control men were carried out using STS markers on the long arm of the Y chromosome. Initially the screening for *AZF* regions was done using 30 STS markers. The *AZFa* region was analyzed with sY83, sY746, sY740, sY86, sY2320, sY741, sY84, *DFFRY*, sY742, sY615, and sY743. The *AZFb* region was analyzed with sY98, sY100, sY110, sY80, sY113, sY118, sY124, sY127, sY1211, sY134, and sY143. The *AZFc* region was analyzed with sY152, sY148, sY156, sY255, sY581, sY254, sY247, and sY158. To detect the heterochromatic region, sY160 was used in all cases. Individuals who had deletions in any of these STS markers were further analyzed by using the flanking STS markers (Ma et al, 1993; Reijo et al, 1995) in order to map the deletion breakpoints. *SRY* was analyzed in all infertile men (Thangaraj et al, 2002b).

Primers were synthesized using ABI392 Oligosynthesizer (Perkin Elmer, Foster City, Calif). Each marker was amplified separately in a 0.2 mL thin wall tube using an MJ Research Thermal Cycler (Waltham, Mass) with a female negative control sample. PCR conditions used for STS markers were as follows: initial denaturation (94°C for 5 minutes) and subsequent denaturations (94°C for 45 seconds) was the same for all the samples. Extension was 65°C for 1 minute for sY127, sY134, and sY255, whereas for the other STS markers extension was 72°C for 2 minutes. Different annealing temperatures that were used for different STS markers were as follows: 60°C for 15 seconds for sY83, sY254, and sY581; 60°C for 1 minutes for sY740, sY746, sY741, *DFFRY*, sY742, sY615, and sY743; 55°C for 30 seconds for sY86; 53°C for 30 seconds for sY84 and sY158; 57.4°C for 1 minute for sY2320, sY113, sY100, sY98, and sY121; 58°C for 30 seconds for sY127, sY134, and sY255; and 55°C for 1 minute for sY110, sY143, sY124, sY156, sY148, sY247, sY118, and sY152. PCR products were analyzed by 2% agarose gel electrophoresis.

Southern Hybridization

Southern hybridization was carried out wherever a sufficient amount of DNA of the azoospermic men, who showed deletion of *AZF* regions, was available. Two Y chromosome–specific probes, such as 50f2 and 102(d)2, were used for Southern hybridization. The probe 50f2 detects 5 different loci in the euchromatic region of the Y chromosome (Jobling, 1994), whereas 102(d)2 is a Y chromosome heterochromatin-specific (*DYZ*) probe (Singh et al, 1994). Five and 2.0 μg of DNA was digested with *Eco*RI separately to hybridize with 50f2 and 102(d)2, respectively. After restriction digestion, DNA samples were size fractionated in 1.0% agarose gel and transferred onto a Hybond N+ Nylon membrane (Amersham Pharmacia, Buckinghamshire, United Kingdom) by capillary transfer using 0.4N NaOH. Southern hybridization was carried out with α^{32}P-labeled (BARC, Jonaki, India) probe. Hybridization and posthybridization washing conditions were as given in the above references.

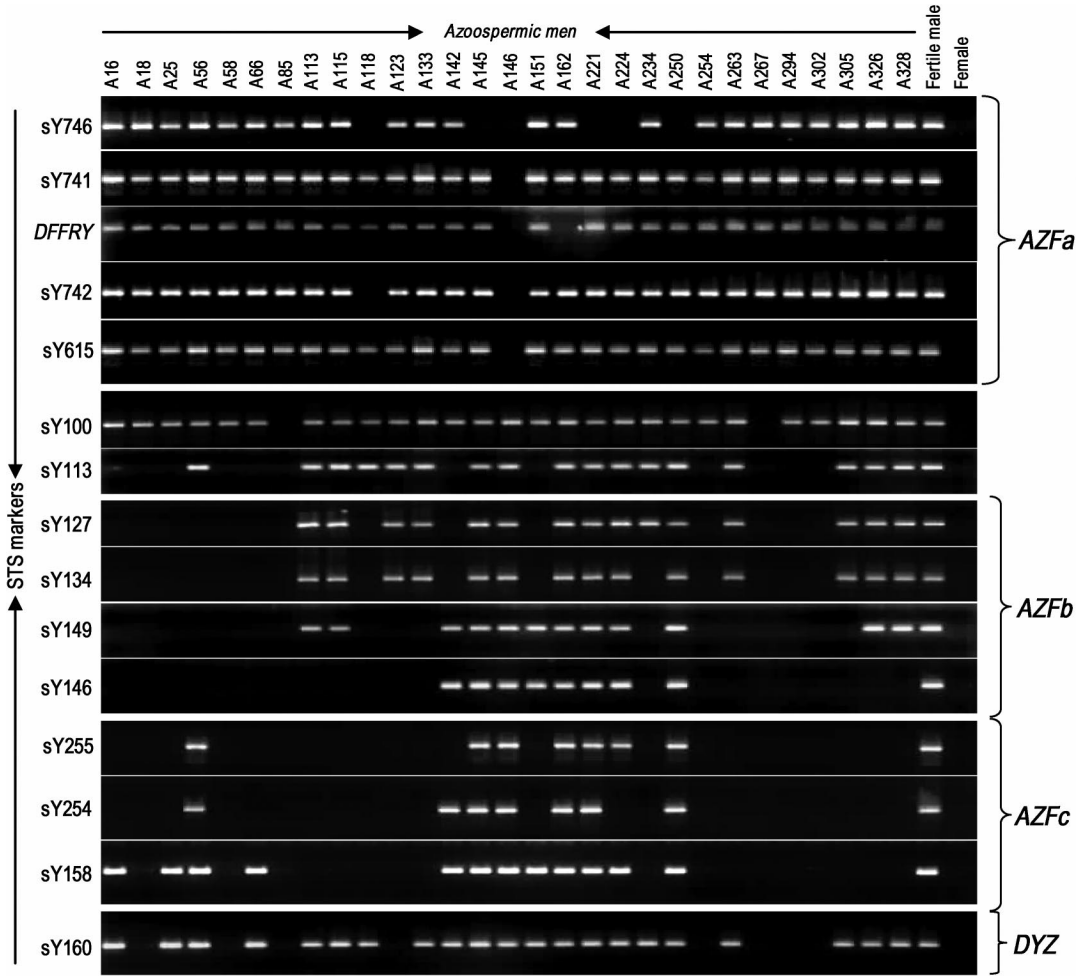

Figure 1. Gel image showing PCR products of various sequence-tagged site (STS) markers representing the *AZFa, AZFb, AZFc* and *DYZ* regions.

Sequence Analysis

To evaluate the nature of the DNA sequence present at the Y chromosome breakpoints of the azoospermic men, we have analyzed the human Y chromosome sequence, downloaded from GeneBank (available at http://ncbi.nlm.nih.gov/genbank/genomes/H_sapiens/chr_Y). The STS markers, which showed deletion in the ends of the flanking STS markers, were mapped on the Y chromosome with the help of the STS database and electronic PCR from the National Resource for Molecular Biology Information (NCBI). In the proximal region of the Yq, the deleted STS marker and its flanking STS markers (deletion interval 5C) were mapped to the contig NT_011875. Sequences that were present in *AZFb* and *AZFc* were identified on the contigs NT_011875 and NT_011903. DNA sequences of these contigs were subjected to various sequence analysis tools such as the tandem repeat finder (Benson, 1999), BLAST (Altschul et al, 1997), and alignment tools. Since the sequence information for the Yq heterochromatic region was not available in the database, we were unable to analyze the sequences from the distal heterochromatic region. The sequence and the accession numbers of the corresponding break point regions were noted, and the repeats flanking the particular break point were marked by blasting with the repeat masker program. Also, annotations that were given for each accession identification number were manually checked for the presence of repeats flanking these breakpoints. Distance between the break points was also obtained by calculating the differences between each marker position on the sequence of both the contigs and then analyzed. Approximate size of the deletion of each individual was estimated using the sequence information from the database.

Results

STS Analysis

Screening of 340 azoospermic men with the STS markers specific to *AZF* regions showed deletion in 29 individuals (Figures 1 and 2), which accounts for 8.5% of the total azoospermic men analyzed. Of these, deletion of the *AZFa* region alone was detected in 5 individuals (A145, A146, A162, A221, and A250), which accounted for 17.2% of the total deletion (Figure 3). One azoospermic man (A118) showed deletion in the *AZFa*, *AZFb*, and

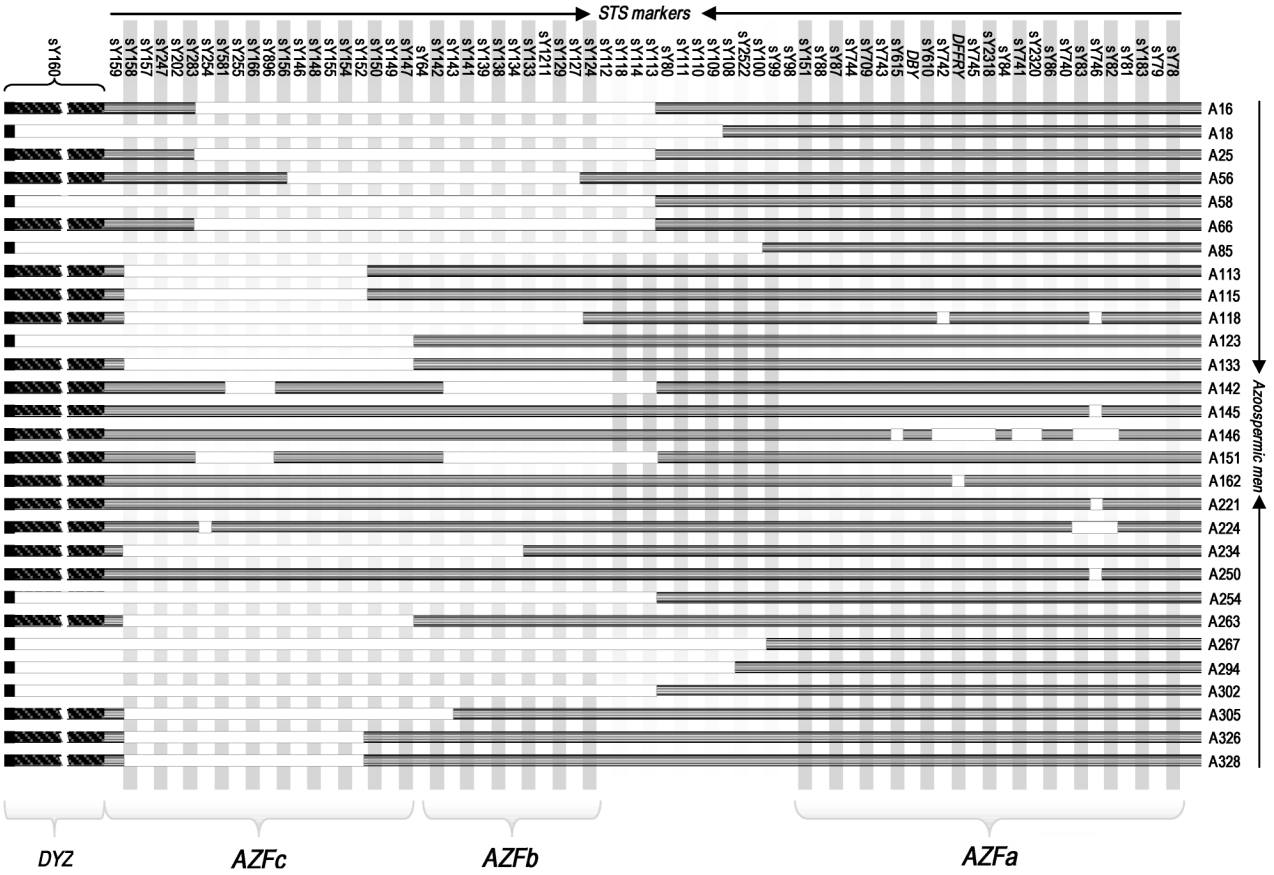

Figure 2. Y chromosome deletion map of azoospermic men generated by STS markers.

AZFc regions (3.5%). In this azoospermic man, deletion in the *AZFa* region was interstitial, whereas deletions of *AZFb* and *AZFc* were continuous (Figure 2). Another azoospermic man (A224) showed deletion in both the *AZFa* and *AZFc* regions (Figure 2), but deletion observed in this individual was microdeletion in both the regions (Figure 2). Interstitial deletion in the *AZFa* region was observed in another azoospermic man (A146); interestingly, this individual showed large deletion in the *AZFa* region (Figure 2). None of the azoospermic men showed deletion, which is unique to the *AZFb* region. Deletion of *AZFc* alone was detected in 7 individuals (A113, A115, A123, A133, A263, A326, and A328), which accounted for 24.1% of the total deletion (Figure 3). Deletion of the *AZFb* and *AZFc* region occurred for 51.7% of the total deletions observed (Figure 3). Eight individuals (A16, A25, A56, A66, A142, A151, A234, A305) showed the deletion of the *AZFb* and *AZFc* regions (Figure 2). Of these, deletions in 6 individuals were continuous for these regions. However, in 2 individuals (A142 and A151), deletions in both the *AZFb* and *AZFc* regions were independent. A total of 8 individuals showed the deletion of the heterochromatic region (*DYZ*), of which 7 azoospermic men (A18, A58, A85, A254, A267, A294, A302) showed the deletion of the *AZFb* and *AZFc* regions, and 1 individual (A123) showed the deletion of *AZFc* in addition to the heterochromatic region (Figure 2). None of the control men showed deletion for the 30 STS markers, which were used for the primary screening for the deletion of *AZFa*, *AZFb*, and *AZFc* regions.

Southern Analysis

Our STS analyses have shown that in the majority of the cases, the deletion in both the *AZFb* and *AZFc* regions were very large (macrodeletion), spanning the *AZFb*, *AZFc*, and heterochromatic regions. We therefore carried out Southern hybridization to confirm the macrodeletions. Probe 50f2 detects 3 loci (A, B, and D) on the p arm and 2 loci (C and E) on the q arm of the Y chromosome. We analyzed a total of 12 azoospermia samples representing different deletion events. Of the 12 samples, 3 (A113, A263, A326) were with the deletion of the *AZFc* region. The locus 50f2/C corresponds to the *AZFc* region, and the absence of the 50f2/C signal in all 3 individuals confirms the microdeletion in the *AZFc* region (Figure 4). Three azoospermic men (A16, A66, A118) who showed deletion of the *AZFb* and *AZFc* regions by STS analysis were also confirmed by Southern hybridization. Four azo-

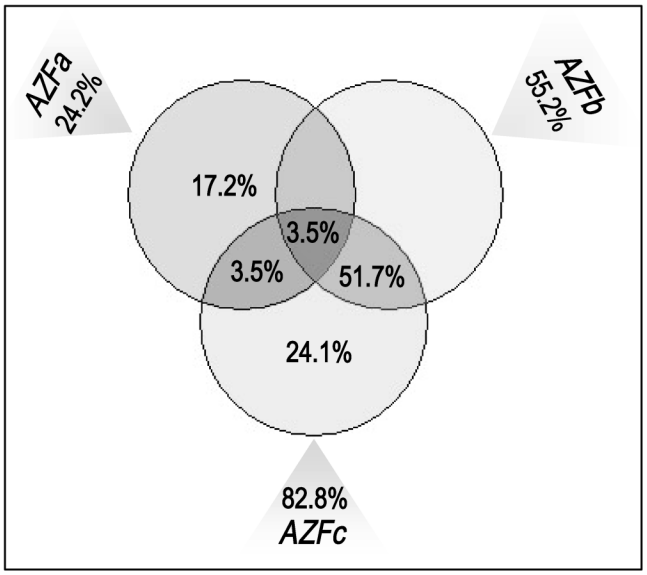

Figure 3. Vendiagram showing Y chromosome deletion in *AZF* regions. The *AZFa* region was involved in a total of 24.2%, of which *AZFa* alone was involved in 17.2%, whereas *AZFa* and *AZFc* were involved in 3.5% and *AZFa, AZFb,* and *AZFc* in 3.5%. Deletion of *AZFa* and *AZFb* was not seen in any of the azoospermic men analyzed. Deletion of *AZFb* was found in 55.2%, of which none of them showed deletion in *AZFb* alone. *AZFb* and *AZFc* were represented in 51.7% of the deletion. Deletion of *AZFc* was found in 82.8% of the deletion, of which deletion of *AZFc* (in addition to *DYZ*) was found in 17.2%.

ospermic men (A18, A85, A254, A302) who were detected as having the deletion of the *AZFb, AZFc*, and heterochromatic regions by STS analysis showed the absence of a hybridization signal from 50f2/C, 50f2/E, and 102(d)2 loci (Figure 4), confirming that these 4 azoospermic men had microdeletions involving the *AZFb, AZFc*, and heterochromatic regions. Two azoospermic men (A145, A162) who showed deletion of only the *AZFa* region were also included in the Southern analysis and revealed no deletion in the *AZFb, AZFc*, and heterochromatic regions (Figure 4). Approximate size of the deletions in the *AZF* regions, based on STS and Southern analysis, are given in Figure 5.

Sequence Analysis

All the deletion breakpoints on the human Y chromosome were mapped onto 2 contigs, namely NT_011875.7 and NT_011903.8. The accession numbers for the sequences, which showed the deletions, are given in the Table. It was interesting to note that the deletions that occurred in the *AZFa* region were mostly microdeletions compared with that of the deletions in the *AZFb* and *AFZc* regions. In the *AZFa* region, most the breakpoints were flanked by human endogenous retroviral (HERV) and L1 (LINE) type of repeat elements. Also, the breakpoints of the *AZFb* and *AZFc* regions predominantly contained L1 and retroviral sequences. It was interesting to note that there was ~240-kb region containing 125 bp tandem repeats that was identified between sY124 and sY127. These repeats spanned the sequences of GenBank accession numbers AC079157, AC079261, AC079156, AC024250, and AC009240. These tandem repeats were identified as ERVs. This region was deleted in about 50% of the azoospermic individuals (Figure 2). The markers, which failed to amplify the distal end of the Y euchromatic region, were found to be flanked by the clusters of L1-type repeat elements. However, this region did not contain 125bp tandem repeats as found above.

Comparison of the sequences of the deleted regions revealed that there were significant levels of homology, which ranged between 81% to 93% in the *AZFa* region; however, the size of these homologous sequences are about 1000 bp only. Similarly, the *AZFb* regions also showed high levels of sequence homology between the repeat sequences. Analysis of AC053490 and AC006338, which are present in the *AZFc* region, revealed that these regions had segmental duplication. A maximum of about a 40-kb sequence was duplicated with 99% homology. Approximate size of deletion in the euchromatic region of the Y chromosome of azoospermic men is shown in Figure 5. Of the 29 azoospermic men who showed deletion, 7 had a total deletion of more than 8.0 Mb, and 5 individuals had a deletion of ~4.5 Mb (Figure 5).

Testicular volumes of the infertile men who showed Y chromosome deletion ranged between 1.0 mL and 12.0 mL. However, hormone profiles of these individuals showed normal ranges, except 2 azoospermic men; one of them (A18) had high LH (27.5 mIU/mL), and the other one (A250) had low testosterone (1.5 ng/mL). Testicular biopsy of these individuals showed the presence of only Sertoli cells. There was no correlation between testicular size and quantity of sperm production, as all the infertile men analyzed were azoospermic.

Discussion

We, for the first time, made an attempt to study the genetic causes of male infertility in Indian populations. PCR-based STS analysis of 340 azoospermic men revealed micro- and macrodeletions on the Y chromosome in 29 individuals (Figures 1 and 2), accounting for 8.5% of the total azoospermic men analyzed. Earlier studies revealed that the Y chromosome microdeletions were responsible for 7% to 13% of the infertile men (Reijo et al, 1995; Pryor et al, 1997; Martinez et al, 2000), leading to azoospermia, severe oligozoospermia, and oligozoospermia. Size of the deletion on the Y chromosome, in our study, did not show any significant correlation with the amount of sperm production, as all the samples analyzed were azoospermic.

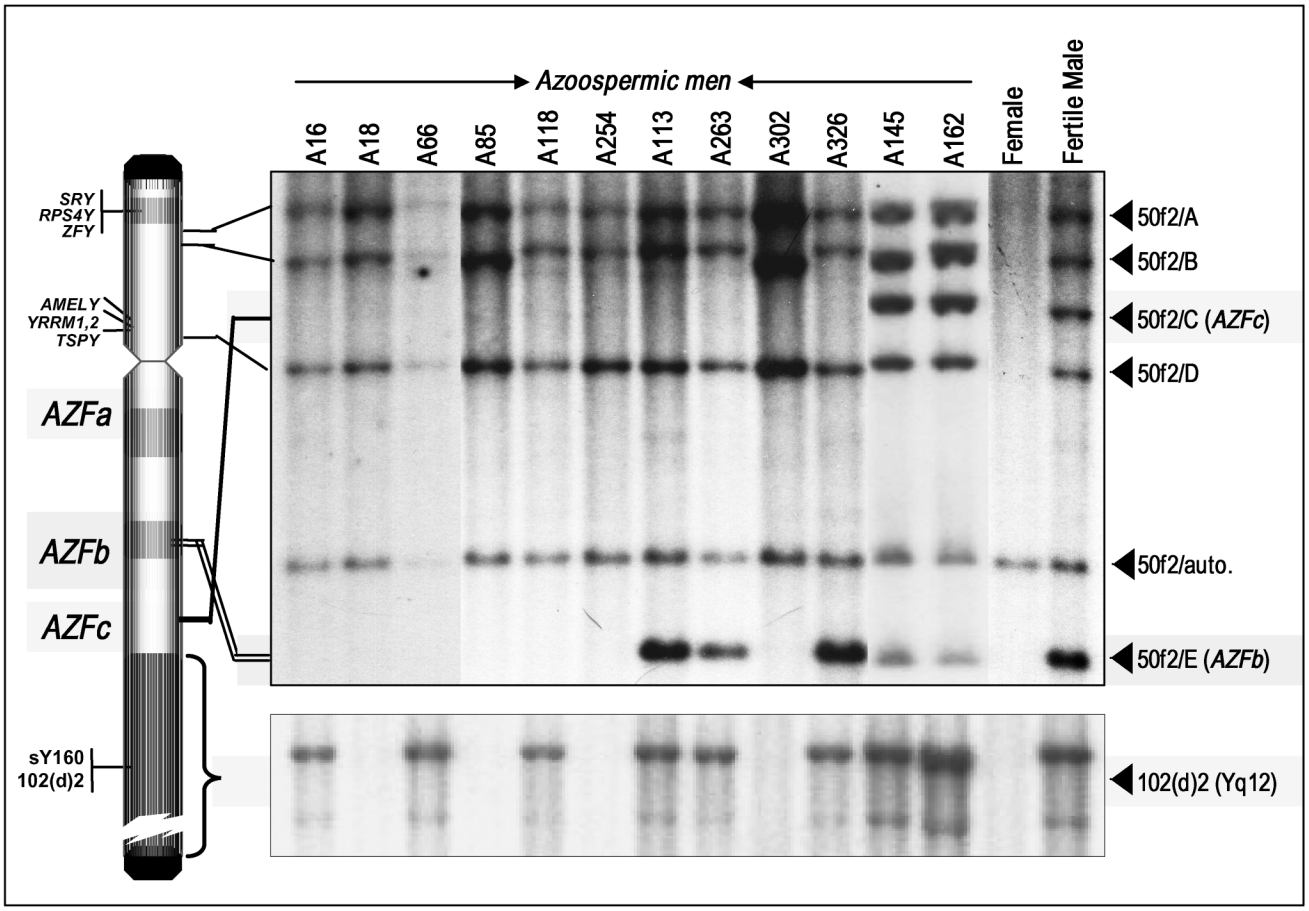

Figure 4. Southern hybridization using 50f2 showing the deletion of 50f2/C (*AZFc*) and 50f2/E (*AZFb*) loci confirming the macrodeletion on the Y chromosome (upper panel). Southern analysis using 102(d) 2 (corresponding to sY160) showing the deletion of the entire heterochromatic region (lower panel).

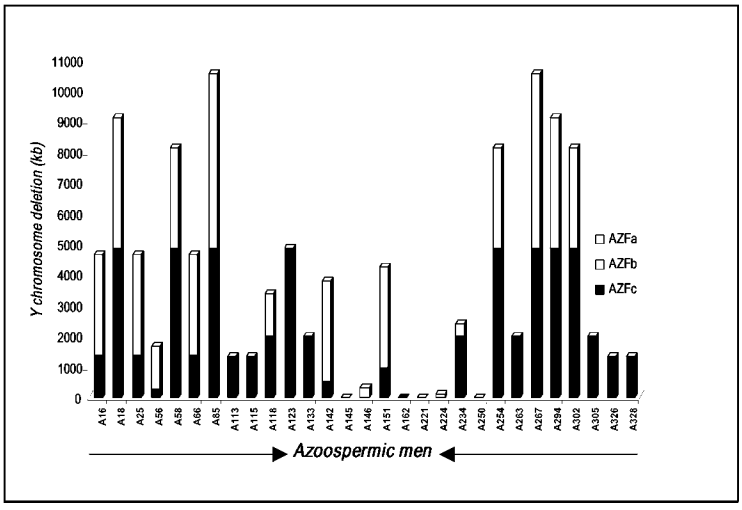

Figure 5. Approximate size of deletion in the euchromatic region of the long arm of the Y chromosome. Size of the deletion of each *AZF* region was shown in a different color.

*Type of repeat element flanking breakpoints of each STS marker and the contig and accession identification numbers**

Region	STS marker	Contig	Accession Identification Number	Repeat Type
AZFa	sY746	NT_011875	AC002992	HERV, LTR15
	sY82	NT_011875	AC013735	ERV1, L1
	sY83	NT_011875	AC002992	HERVE, HERVK, LTR15
	sY2318	NT_011875	AC004810	L1, MER
	sY745	NT_011875	AC004810	L1, MER
	DFFRY	NT_011875	AC002531	MER, LI, LTR, HERV
	sY745	NT_011875	AC002531	MER, LI, LTR, HERV
	sY615	NT_011875	AC004474	L1, MER, HERV
	sY2320	NT_011875	AC004617	LTR21B, HERV9
	sY741	NT_011875	AC004810	L1
AZFb	sY108	NT_011875	AC068541	L1, ERV
	sY100	NT_011875	AC011749	L1, Retroviral
	sY113	NT_011875	AC068541	L1, ERV
	sY124	NT_011875	AC009233	L1, Retroviral
	sY127	NT_011875	AC009240	L1, ERV
	sY129	NT_011875	AC009239	L1, Retroviral
	sY143	NT_011903	AC024236	MER, ERV
	sY147	NT_011903	AC006338	L1, ERV
AZFc	sY254	NT_011903	AC053490	L1, ERV, CR1
	sY255	NT_011903	AC006338	L1, ERV
	sY160	. . .		(GGAAT)n

* STS indicates sequence-tagged site.

In our study, approximately one fourth (24.2%) of the total Y chromosome deletion was in the *AZFa* region (Figure 3). The size of the deletion in the *AZFa* region was very narrow (microdeletion) compared with the *AZFb* and *AZFc* regions. One of the azoospermic men (A162) showed deletion of just 1 STS marker (*DFFRY*), but the majority of them (6 individuals) showed the deletion of sY746. Sun et al (1999) reported that point mutation in the genes present on the *AZFa* region can cause azoospermia. This is in agreement with the present observation that although the deletion of the *AZFa* region was very small, all the individuals with a deletion in the *AZFa* region were azoospermic. A similar type of abnormality was also detected by Kamp et al (2001). It was interesting that none of the azoospermic men studied showed the deletion of either sY84, sY86, or both. It is indeed surprising that these 2 markers were recommended for the diagnosis of the *AZFa* region (Simoni et al, 1999). Using only sY84 and sY86, we would have diagnosed as normal all 7 individuals who possess deletion in the *AZFa* region. Martinez et al (2000) have analyzed 128 infertile men with sY84, sY85, and sY86 and found none of them have shown deletion. Deletions would have been noted in those cases if more markers were used. Dohle et al (2002) also did not see any deletion in the *AZFa* region during their screening of 37 azoospermic and 113 severely oligozoospermic individuals with 2 STS markers for each *AZF* region. It may be possible that the deletion of sY84 and sY86 was predominantly seen in 1 population but not in the other populations. For instance, we found deletion of sY746 in 6 out of 7 individuals who showed deletion in the *AZFa* region, which may not be true for other populations. Considering the fact that some deletions are more predominant in certain populations (Thangaraj et al, 2002b), one has to use a large number of STS markers from each *AZF* region to detect Y chromosome deletions. In general, frequency of deletion in the *AZFa* region is less compared with the *AZFb* and *AZFc* regions (Fujisawa et al, 2001; Layman, 2002; Peterlin et al, 2002). In most of the studies, deletion of the complete *AZFa* was found to be associated with SCOS (Simoni et al, 1999; Fujisawa et al, 2001; Kamp et al, 2001; Dohle et al, 2002; Peterlin et al, 2002; Sun et al, 2000). Our study demonstrates that even deletion of a narrow region on the *AZFa* region can cause SCOS. Therefore, we suggest that sequencing of the entire *AZFa* region would help in identifying point mutations, implicating azoospermia/male infertility.

Very recently, Ferlin et al (2003) have reported the deletion of 5 single copies and 2 duplicated genes, but they found intact *RBMY1* in 4 infertile men. Our study did not show the deletion of the *AZFb* region in any of the azoospermic men analyzed. The reason that none of the infertile individuals shows deletion of only the *AZFb* region might be because of the nature of DNA sequence present in the distal end of the *AZFb* region. Altogether, deletion of the *AZFb* region was involved in 55.2% of the total

deletions, of which 51.7% were in association with *AZFc* and 3.5% with *AZFa* and *AZFc* (Figure 3).

Deletion of the *AZFc* region was detected in 24 individuals (Figure 2), accounting for 82.8% of the total deletion of azoospermic men (Figure 3). This is in agreement with the earlier studies showing that the incidence of deletion in the *AZFc* region was high compared with the *AZFa* and *AZFb* regions (Martinez et al, 2000; Peterlin et al, 2002). Reduction in the copy number of *DAZ* genes (*AZFc*) was reported to have increased risk in subfertility and infertility (de Vries et al, 2002). Of the total 29 deletions, *AZFc* alone (in addition to *DYZ*) was observed in 7 individuals (24.1%). It was interesting to note that a total of 8 Y chromosomes (27.6%) showed the deletion of the heterochromatic region (*DYZ*), which was diagnosed as having a short Y chromosome. Although the Yq heterochromatic region predominantly consists of *DYZ*1 and *DYZ*2 classes of repeat sequences (Manz et al, 1992), a few studies have shown its association with infertility and reproductive failure. Quantitative variation in heterochromatin has been found to be more in couples with fetal wastage (Buertic-Tomljanovic et al, 1997). Deletion of the entire heterochromatin and part of euchromatin was detected in an infertile man (Hartung et al, 1988). In our recent study, we observed the deletion of the entire heterochromatin, along with very little euchromatic region and duplication of the Yq euchromatic region of the Y chromosome in an individual having ambiguous genitalia and undescended testes (Thangaraj et al, 2003). We could not obtain either semen profile or assess the fertility status of the individual, as he was only 5 years old when we made the examination. But in the present study, we did not see any case showing the deletion of only the heterochromatic region of the Y chromosome.

Southern analysis with 50f2 confirmed the macrodeletion in both *AZFb* (50f2/E) and *AZFc* (50f2/C) regions (Figure 4). Deletion of only the 50f2/C locus in infertile men was earlier reported by Ma et al (1993). We are reporting for the first time the deletion of the 50f2/E region, which corresponds to the *AZFb* region and suggests that the probe 50f2 can be used to confirm the macrodeletions in both the *AZFb* and *AZFc* regions.

Sequence analysis revealed the presence of repeat elements (HERV, L1, and other retroviral sequences) at the breakpoints. Presence of a homologous repeat sequences block in this region (deletion intervals D3 and D6) suggests that the deletion in the above cases might be because of intrachromosomal recombination of homologous repetitional blocks (Sun et al, 2000; Kamp et al, 2000). As the deletion in the *AZFa* region was very small (microdeletions), it is possible that only certain repeat sequences, which are highly homologous to themselves in this region, undergo intrachromosomal recombination leading to deletion. Based on the fact that the repeat sequences are prone to deletion, the probable reason for the frequent deletion in the *AZFc* region might be due to the crossing over between the repetitive sequences in the *AZFc* region. Kuroda-Kawaguchi et al (2001) have reported that the *AZFc* region contains massive palindromes, which leads to recurrent deletion in this region. Repping et al (2002) also reported that the palindromes P5 and P1 were responsible for large deletions, up to 7.7 Mb, involving *AZFb* and *AZFc* regions of the infertile men. It can be speculated that most of the deletions involving *AZFb* and *AZFc* are mediated through intrachromosomal recombination between the ERVs and L1 repetitive elements present in both *AZFb* and distal to the *AZFc* regions. In fact, a previous study showed the presence of HERV at the heterochromatic region (Kjellman et al, 1995). Our present study does not show a single case of Y deletion involving only the *AZFb* region. Similar observation has been reported in other populations as well (Brandell et al, 1998), but Repping et al (2002) detected the deletion of only *AZFb* in 3 infertile men. If every Y chromosome consists of similar sequences, then why are the deletions occurring on Y chromosomes unique to certain individuals? This may be explained on the assumption that the retroviral or L1 repeats are involved in the deletion process, as discussed above, and their repeat blocks may be differently organized in different populations. It is possible that the 125-bp tandem repeats present in the *AZFb* region predominantly contains ERVs could contribute more to the intrachromosomal crossing over, since this region spans about 240 Kb.

Histological analysis of some of the azoospermic men with Y chromosome deletion showed SCOS. Kamp et al (2001) reported that the deletion of the *AZFa* region was associated with SCOS. Sargent et al (1999) reported that the deletion of *DFFRY* and *DBY* in the *AZFa* region was associated with SCOS. However, Fujisawa et al (2001) reported that SCOS was associated with deletion of all 3 azoospermic factors (*AZFa*, *AZFb*, and *AZFc*). Our observation of SCOS in all *AZF*-deleted individuals is in agreement with the study of Fujisawa et al (2001).

Male infertility is caused by various genetic and nongenetic factors. We have earlier studied the CAG repeat length polymorphism in the androgen receptor gene of infertile and fertile men. Although several studies found that the expanded CAG repeat was associated with male infertility in certain populations (Yong et al, 1998; Dowsing et al, 1999), we have not observed repeat length differences between infertile and fertile groups (Thangaraj et al, 2002b). The present study showed that the Y chromosome deletion was responsible for 8.5% among azoospermic men, of which deletion of the *AZFc* region was found to be of a very high frequency. We found that the deletions were up to 11.0 Mb involving the *AZFb* and *AZFc* regions. HERVs and ERVs were found to be re-

sponsible for the deletions. For the first time we have identified 125-bp tandem repeats in the *AZFb* region. However, etiologies of a large number of azoospermic men are still unknown. Analyzing the remaining azoospermic men with additional Y chromosome STS, X chromosome, and autosomal markers would help in identifying the etiology of the remaining azoospermic individuals (Brandell et al, 1998; Wang et al, 2001). In light of the above, we believe that the etiology of male infertility may differ between ethnic populations. Therefore, researchers need to keep this in mind and define the strategies for analyzing infertile samples. Our data will be useful for infertility clinics for genetic counseling by advising them to choose a female child in case of Y chromosome deletion and to adopt appropriate methods for assisted reproduction.

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