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Y Chromosome Microdeletions and Alterations of Spermatogenesis*

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ABSTRACT

Three different spermatogenesis loci have been mapped on the Y chromosome and named "azoospermia factors" (*AZFa*, *b*, and *c*). Deletions in these regions remove one or more of the candidate genes (*DAZ*, *RBMY*, *USP9Y*, and *DBY*) and cause severe testiculopathy leading to male infertility. We have reviewed the literature and the most recent advances in Y chromosome mapping, focusing our attention on the correlation between Y chromosome microdeletions and alterations of spermatogenesis. More than 4,800 infertile patients were screened for Y microdeletions and published. Such deletions determine azoospermia more frequently than severe oligozoospermia and involve especially the *AZFc* region including the *DAZ* gene family. Overall, the prevalence of Y chromosome microdeletions is 4% in oligozoospermic patients, 14% in idiopathic severely oligozoospermic men, 11% in azoospermic men, and 18% in idiopathic azoospermic subjects. Patient selection criteria appear to substantially influence the prevalence of microdeletions. No clear correlation exists between the size and localization of the deletions and the testicular phenotype. However, it is clear that larger deletions are associated with the most severe testicular damage. Patients with Y chromosome deletions frequently have sperm either in the ejaculate or within the testis and are therefore suitable candidates for assisted reproduction techniques. This possibility raises a number of medical and ethical concerns, since the use of spermatozoa carrying Y chromosome deletions may produce pregnancies, but in such cases the genetic anomaly will invariably be passed on to male offspring. (*Endocrine Reviews* **22:** 226–239, 2001)

I. Introduction

THE Y chromosome is not essential for life and until recently most regions of it were assumed to be functionally inert. Sex determination (controlled by the *SRY* gene) (1) has long been viewed as the sole function related to the Y chromosome (2), but this theory changed in recent years when another important function (the control of spermatogenesis) (3) was discovered and many genes were mapped to the Y chromosome.

Spermatogenesis is a long and complex process requiring about 70 days and involving an elaborate succession of distinct cell types (4, 5) generated by mitotic and meiotic divisions. In the initial stages, spermatogonia divide via mitoses, giving rise to primary spermatocytes, which in turn undergo the first meiotic division leading to secondary spermatocytes. Through the second meiosis these cells produce haploid cells (round spermatids), which elongate during the spermiogenesis process (elongated spermatids) and finally differentiate into mature spermatozoa, by condensation of the chromatin, substitution of histones with protamines, and formation of the acrosome and the other sperm components. However, our knowledge of the mechanisms regulating spermatogenesis is still poor, and only recently has research focused on the identification of genes specifically involved in its regulation.

Nevertheless, infertility is a major health problem affecting 10–15% of couples seeking to have children, and a male factor can be identified in about half of these cases (6). A significant proportion of infertile males are affected either by oligozoospermia (reduced sperm production) or azoospermia (lack of any sperm in the ejaculate). Such alterations in sperm production may be related, in turn, to different underlying testicular histological pathologies, ranging from the complete absence of germ cells (Sertoli cell-only syndrome) to hypospermatogenesis and maturation arrest. The alteration of spermatogenesis can be the consequence of many causes, such as systemic diseases, cryptorchidism, endocrinological disorders, obstruction/absence of seminal pathways, or infections. However, the cause of male infertility is unknown in up to 50% of cases, and until recently relatively little research focused on the possible genetic etiologies. The explosive growth of assisted reproduction techniques and, in particular, of intracytoplasmic sperm injection (ICSI) (7) has contributed to the development of such research. The study of Y chromosome microdeletions is particularly important because of the potential for transmission of genetic abnor-

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malities to the offspring, as these techniques bypass the physiological mechanisms related to fertilization.

II. Structure and Gene Content of the Y Chromosome

The Y chromosome is the smallest human chromosome and consists of a short (Yp) and a long (Yq) arm. The pseudoautosomal regions (PARs), which pair with the X chromosome during meiosis, are located at both ends. The region outside the PARs that does not recombine is called the nonrecombining region of the Y chromosome (NRY) (Fig. 1). This part consists of several repetitive sequences that may be homologs to regions on the X chromosome or Y-specific. The Yp and the proximal part of Yq consist of euchromatin, while the distal part of the long arm is made of heterochromatin, and this region may vary in length to constitute about one-half to two-thirds of Yq (Fig. 1). Therefore, the Y chromosome long arm may be cytogenetically divided in an euchromatic proximal region (Yq11, subdivided into Yq11.1, 11.21, 11.22, and 11.23) and a heterochromatic distal region (Yq12), whereas the euchromatic short arm is called Yp11 (Fig. 2a).

The absence of meiotic recombination within most parts of the Y chromosome has hindered the construction of a linkage map of the chromosome. Therefore, Y chromosome mapping has been based on naturally occurring deletions. The initial Vergnaud interval map (8) divided the Y chromosome into seven intervals (Fig. 2b): the short arm and the centromere contain intervals 1–4, distal to proximal; the euchromatic part of Yq is represented by intervals 5 and 6, proximal to distal; the heterochromatic region is defined as interval 7. Deletion interval 5 corresponds approximately to Yq11.21 through the middle part of Yq11.22, and deletion interval 6 corresponds to the middle part of Yq11.22–Yq11.23. On this basis, Vollrath *et al.* (9) further divided the seven-interval map in 43 subintervals, and this is the most commonly used map (Fig. 2c).

Initial attempts to draw a physical map of the Y chromosome led to the isolation of overlapping yeast artificial chromosome (YAC) contigs (10, 11). More recent efforts in sequencing came from a systematic approach within the Human Genome Project. With the major contribution of the two leading centers involved in this project (Washington University and Whitehead Institute), nearly 40% of the euchromatic region of the Y chromosome (about 13 Mb out of the estimated 35 Mb) have been sequenced, and more than 40 contigs have been isolated (data updated at the end of June 2000; official site www.ncbi.nlm.nih.gov/genome). Even if only half of them have been physically mapped to date, a finished sequence of the entire Y chromosome will be available in the near future. Together with this sequence map, more than 300 sequence-tagged sites (STSs) have been generated and mapped. STSs are known sequences of genomic DNA that can be amplified by PCR. These STSs may be specific for a gene or may overlap anonymous regions of the Y chromosome, and their use in Y deletion screening will be discussed in *Section VII*.

Many genes on the Y chromosome have been identified only recently, and perhaps novel genes will be described once sequencing has been completed; so far, more than 30 genes and gene families are known. As summarized by Yen (12), these genes can be classified into three groups on the basis of their location on the Y, their copy number, and their pattern of expression: 1) pseudoautosomal genes (such as *ASMTL*, *MIC2*, and *IL9R*): their sequences are identical on the Y and X chromosomes and, with few exceptions, they are expressed in different tissues; 2) genes located within X-Y homologous regions on the NRY (such as *USP9Y*, *DBY*, and *UTY*): these have homologs on the X chromosome encoding for proteins with very high amino acid identity. These genes are ubiquitously expressed, although some have testis-specific transcripts in addition to ubiquitous transcripts; 3) Y-specific gene families (such as *DAZ*, *CDY*, and *TSPY*); these are multicopy genes, widely distributed on the Y chromosome or clustered within a small region, and they are expressed only in the testis. One exception to this classification is *SRY*, the gene that determines testis development: it is Y-specific, but it is in single copy and has a different pattern of expression, limited to the genital ridge and in fetal and adult Sertoli cells and germ cells (13–15).

III. The Azoospermia Factors

Tiepolo and Zuffardi in 1976 (3) were the first to hypothesize a correlation between Y chromosome deletions and male infertility. These authors examined the karyotype of 1,170 men; in six sterile males with azoospermia they observed large deletions including the entire heterochromatic region (Yq12) and an undefined amount of the adjacent euchromatic part (Yq11). In two cases they demonstrated that the fathers of the patients with deletions carried a normal Y chromosome, indicating that these mutations were *de novo* events. This suggested that the deletions were the cause of the azoospermia and they postulated that a genetic factor located in Yq11 was important for male germ cell development. This gene or gene cluster was defined as "azoospermia factor" (*AZF*). However, the genetic complexity of the *AZF*

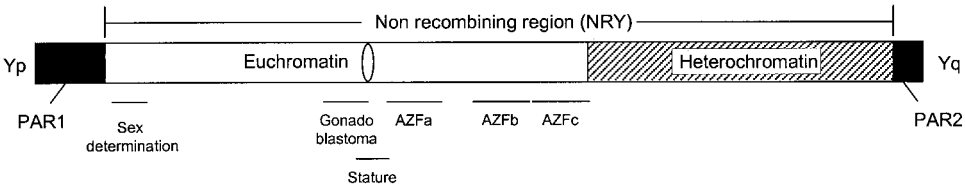

FIG. 1. Schematic representation of the Y chromosome. The PARs are represented in *black*, the euchromatic region in *white*, and the heterochromatic region is *striped*. Outside the PARs is the nonrecombining region of the Y chromosome (NRY). Below are schematically represented the phenotypes associated with alterations of the Y chromosome (sex determination, risk of gonadoblastoma, small stature, and the three spermatogenic failure loci *AZFa*, *b*, and *c*). Yp and Yq, Short and long arm, respectively.

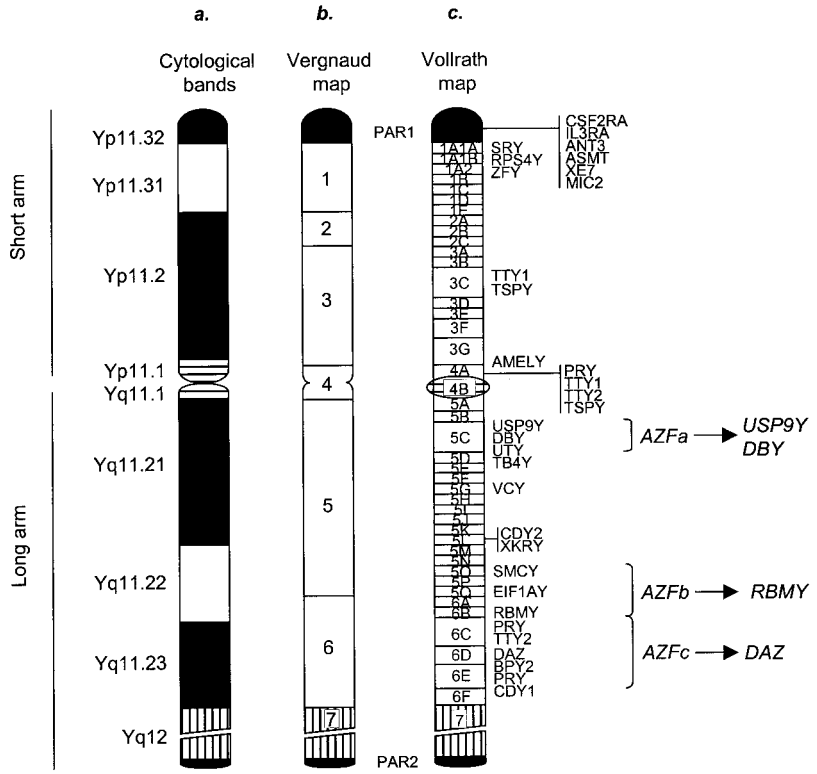

FIG. 2. a, Representation of the cytological bands of the Y chromosome. The short arm is called Yp11, and the long arm is Yq11 (euchromatic region) and Yq12 (heterochromatic region, striped). b, The seven intervals of the Vergnaud map of the Y chromosome (8), where intervals 1–4 span the short arm and the centromere, intervals 5 and 6 span the euchromatic region, and interval 7 spans the heterochromatic region. c, The 43 interval map of the Y chromosome (9). On the *right* are represented a list of genes mapped to the Y chromosome, the localization of *AZF* regions and the corresponding candidate genes.

locus could be revealed only with the development of STS- and YAC-based mapping. These analyses permitted the detection of interstitial submicroscopic deletions not visible at the cytogenetic level and detectable only by STS-PCR or Southern hybridization. Such deletions are called microdeletions. Molecular mapping analyses on patients with microdeletions have complicated the original hypothesis of a single locus for spermatogenesis on Yq, suggesting that three nonoverlapping regions in deletion intervals 5 and 6 may be deleted in infertile men. These spermatogenesis loci are termed *AZFa*, *AZFb,* and *AZFc* (16) from proximal to distal Yq. Furthermore, a fourth region (*AZFd*) has been proposed between *AZFb* and *AZFc* (17), but this finding must be confirmed. According to Vogt *et al.* (18) (Fig. 2c), *AZFa* is located at the proximal portion of deletion interval 5 (subinterval 5C), *AZFb* spans from the distal portion of deletion interval 5 to the proximal end of deletion interval 6 (subinterval 5O–6B), and *AZFc* is located at the distal part of deletion interval 6 (subintervals 6C–6E). Several genes located in *AZF* regions are expressed in the testis and could therefore be viewed as "*AZF* candidate genes." However, based on studies of infertile patients, only a few genes can actually be considered responsible for the *AZF* phenotype. The first *AZF*-candidate gene was isolated in 1993 from a region subsequently shown to correspond to *AZFb*. Two years later the second *AZF*-candidate gene was identified from the *AZFc* region. Both genes have been quite well studied both at the molecular level and in terms of deletions in infertile patients. The structure of *AZFa* and its gene content have only recently been described, and analyses of this region in infertile males suggested that two genes may be considered *AZFa*-candidate. However, it should be noted that recent findings of many genes or gene families outside the proposed *AZF* regions (19) suggest that even this classification may be an oversimplified picture.

IV. *AZFb* Region and Candidate Genes

A detailed sequence and gene map of *AZFb* is still not available, and only nonoverlapping YAC and BAC clones have been described. Even if the *AZFb* interval, as usually defined, spans the subintervals 5O–6B, the precise boundaries can differ and its exact extension is unclear. This depends on different deletion events between individuals and differences in screening procedures. Microdeletions can remove *AZFb* alone, parts of *AZFb*, or also include flanking regions (*e.g.*, *AZFc*). The extent of deletions will obviously affect which genes are removed: for example, deletions may remove the block *SMCY-XKRY-CDY2* (19), or the block *PRY-TTY2* (19) if they extend proximally or distally, respectively.

To date, two genes have been mapped to subintervals 5O–6B: *EIF1AY* (translation-initiation factor 1A, Y isoform) and *RBMY* (RNA binding motif on the Y). *EIF1AY* encodes a Y isoform of eIF-1A, an essential translation initiation factor, which has an X homolog and is ubiquitously expressed (19). Its role in spermatogenesis is completely unknown, and no deletion specifically removing this gene has been reported. Therefore, it is not considered an *AZFb*-candidate gene. However, *EIF1AY* possesses abundant testis-specific transcripts in addition to ubiquitous transcripts (19), suggesting that this gene may contribute to the *AZFb* phenotype.

RBMY was the first among the *AZF* candidate genes to be identified and was cloned using patient DNA with a deletion

in the proximal region of interval 6 (20). Initially, two similar cDNAs were isolated and named *YRRM1* and *2* (Y-specific RNA recognition motif) as they were predicted to encode a protein with an RNA-binding motif. Subsequently it was shown that, in fact, there is a family of 20–50 genes and pseudogenes spread over both arms of the Y chromosome, including a cluster within the *AZFb* region (21, 22), and *YRRM* was renamed "*RBMY* gene family" (18). Such copies belong to at least six subfamilies (23, 24), but *RBMY-I* is the only actively transcribed gene, and the most functional copies are located on interval 6B (22), thus making it a major candidate for the *AZFb* region (18). *RBMY* is present as multiple copies in all eutherian ("placental") mammals (25) and has an active X-borne homolog recently discovered both in humans and marsupials (26, 27). It has been proposed that *RBMX* retained a widespread function while *RBMY* evolved a male-specific function in spermatogenesis.

The RBMY proteins (Fig. 3) consist of a single RNA-binding domain of the RRM (RNA recognition motif) type at the N terminus and an auxiliary C-terminal domain containing four 37-amino acid (aa) repeats. This domain is known as the SRGY box, since it contains a serine-arginine-glycine-tyrosine sequence (20, 28). The gene consists of 12 exons, and the 37-residue repeats are encoded by single exons (exons 7–10) (Fig. 3), which show more than 85% homology between their nucleotide sequences (28).

Consistent with a role in spermatogenesis, the *RBMY* genes are expressed only in the germline in the testis (spermatogonia, spermatocytes, and round spermatids) (22). The actual function of RBMY in male germ cell development is not clear; it is a nuclear protein with dynamic modulations in its spatial location in the various spermatogenic cells, suggesting that it possesses different functions related to pre-mRNA splicing (29).

RBMY is considered the major *AZFb* candidate gene given its testis specificity, its absence in a fraction of infertile patients, and its homology with the mouse *Rbm*, deletion of which causes male sterility (30–32). However, the multicopy nature of this gene has complicated attempts to prove its role in human spermatogenesis, as detrimental mutations in patients have not yet been identified.

V. *AZFc* Region and Candidate Genes

In an attempt to correlate severe spermatogenic defects with frequent and consistent *de novo* microdeletions of the Y chromosome, Reijo and colleagues (33) cloned a novel gene from the distal portion of deletion interval 6 shown to be deleted in men with azoospermia. This gene was named *DAZ* (deleted in azoospermia) and was originally thought to be single copy, whereas it is now clear that it is a member of a multigene family with more than one copy on the Y chromosome, clustered in the *AZFc* region (18, 34–36). Therefore, *DAZ* was renamed "*DAZ* gene family" (18). The exact number of genes in the family is still not clear, although at least three copies have been described by Southern blotting or restriction mapping (34, 36, 37), and seven copies, representing either active genes or pseudogenes, may be seen by fiber-fluorescent *in situ* hybridization (35).

The structure of the *DAZ* gene is somewhat similar to that of *RMBY* (Fig. 4). *DAZ* encodes a protein with a single RNA-binding domain at the N terminus and a C-terminal domain containing an internally repeated sequence of 24 aa, the so-called DAZ repeats (33, 34). The *DAZ* transcription unit appears to contain at least 16 exons and to span about 42 kb. Exon 1 consists of the initiator codon, exons 2–5 encode the RNA-binding domain, and each of exons 7a–7 g encodes a single DAZ repeat (34).

The number of *DAZ* transcripts is unclear, since RT-PCR studies showed that each individual carries two or more species of *DAZ* transcripts, which differ in both the copy number and the order of the DAZ repeats (37). Such *DAZ* transcripts could have derived from the same gene through alternative splicing or from different *DAZ* genes. Like *RBMY*, *DAZ* is transcribed and translated into proteins only in male germ cells (38–41), even if a discrepancy exists between the findings of Page's group (expression mainly in spermatogonia) (38) and that of Vogt's group (detection of DAZ proteins in late spermatids and sperm tails) (39).

DAZ is found on the Y chromosome only in humans, Old World monkeys, and apes (34, 42–44). In all other mammals it is represented as an autosomally located, single copy gene (42, 45–47). *DAZ* was acquired by the Y chromosome from an autosomal homolog *DAZL1* located on chromosome 3p24 and with a single DAZ repeat (34, 48–51) (Fig. 4). A complete *DAZL1* copy was transposed to the Y chromosome, a 2.4-kb genomic sequence encompassing exons 7 and 8 was tandemly repeated, and, finally, the whole transcription unit was amplified, giving rise to a multicopy gene family (34, 37).

Although *DAZ* is not the only gene present in the distal Yq interval 6 (19, 36, 52), its high prevalence of deletions in infertile men makes it the major *AZFc* candidate. This possibility is further strengthened by the high homology of *DAZ* with a *Drosophila* male infertility gene, *boule*, mutation of which causes spermatogenic arrest (53, 54). Furthermore, more recent proof of the spermatogenic role of the *DAZ* gene product arises from the observation that a human *DAZ* transgene is capable of partially rescuing the sterile phenotype of

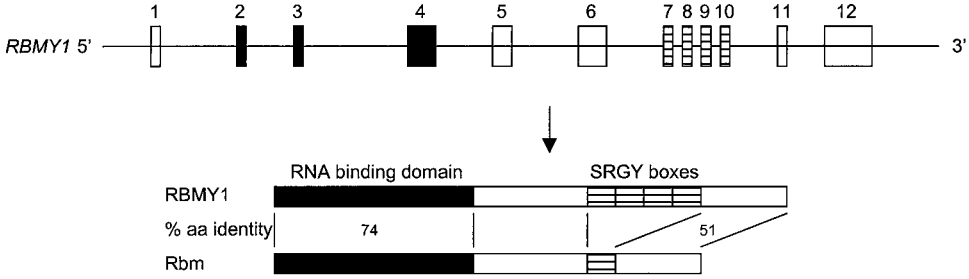

FIG. 3. Genomic structure of the *RBMY1* gene and relative protein. Exons 2–4 (*black*) encode for the RNA-binding domain of the protein, while each of exons 7–10 (111 bp) encode for a single 37-aa SRGY box (*striped*). Identity at the amino acid level between RBMY1 and mouse Rbm is shown in the *lower part* of the figure.

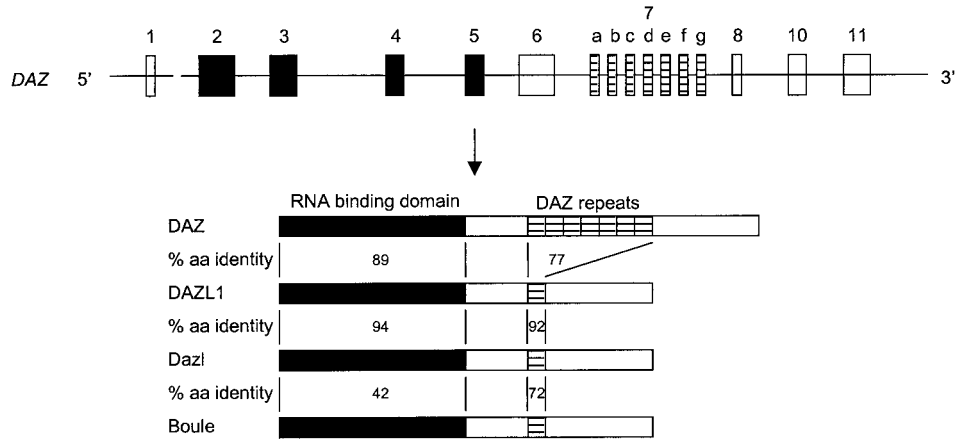

FIG. 4. Genomic structure of the *DAZ* gene and relative protein. Exons 2–5 (*black*) encode for the RNA-binding domain of the protein, while each of exons 7a–7g (72 bp) encode for a single 24-aa DAZ repeat (*striped*). Identity at the amino acid level among DAZ, autosomal human DAZL1, mouse Dazl, and *Drosophila boule* is shown in the *lower part* of the figure.

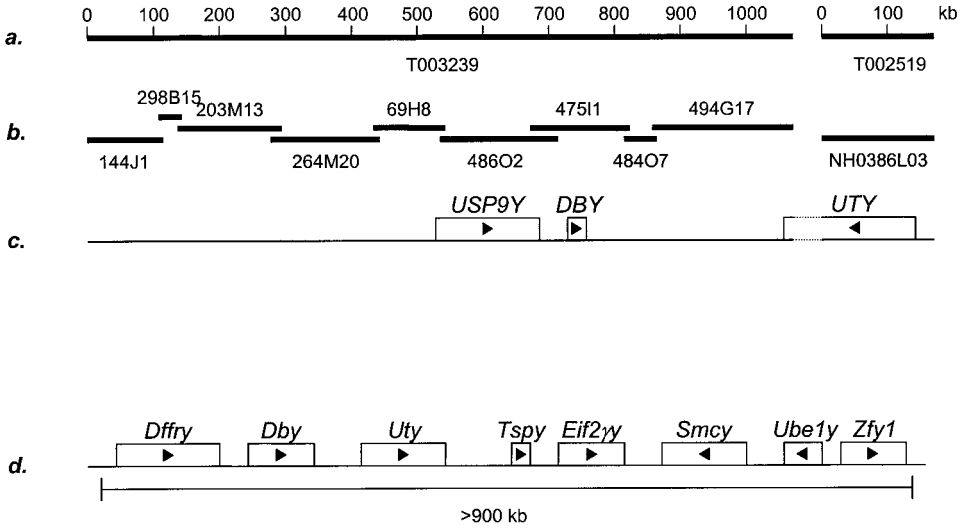

FIG. 5. The human *AZFa* region and the mouse Sxr^b interval. a, The *AZFa* region is included in two nonoverlapping contigs, spanning more than 1.2 Mb. b, Ten overlapping clones covering the *AZFa* region. c, Localization of the three genes, *USP9Y*, *DBY*, and *UTY*, with respect to the clones. The *arrows* indicate the 5′–3′ orientation of the genes. Note that *UTY* is not completely included in the clone sequences and therefore the complete genomic structure of this gene is still unknown. d, Transcription map of the mouse Sxr^b interval (spanning more than 900 kb), showing the syntenic homology with the human *AZFa* interval: the block *Dffry*, *Dby*, and *Uty* is highly conserved between human and mouse. The numbers below the clones and contigs represent the GenBank accession numbers.

a mouse knockout for the homologous gene *Dazl* (55). However, most difficulties in understanding the biological function of *DAZ* and the genotype-phenotype relation probably arise from the multicopy nature of this gene. Like *RBMY*, deletions of *DAZ* in infertile patients are generally screened by PCR on genomic DNA extracted from peripheral leukocytes. Therefore, only deletions removing the whole of the *DAZ* gene cluster can be detected, and intragenic deletions or deletions not involving all the *DAZ* copies, as well as *de novo* point mutations in affected patients, have yet to be discovered. Therefore, there is still no definitive proof for a requirement of *DAZ* in spermatogenesis.

Although a sequence map of *AZFc* is not yet available, several genes other than *DAZ* have been mapped to this region (19, 36): *CDY1* (chromodomain Y 1), *BPY2* (basic protein Y 2), *PRY* (PTA-BL related Y), and *TTY2* (testis transcript Y 2). The function of these genes is unknown, but they share similar characteristics: they are in multiple copies on the Y chromosome, they are expressed in the testis only, and they are Y specific (19). In particular, three *PRY* and *TTY2* genes have been identified in the proximal part of *AZFc* by restriction mapping (36), and therefore they are probably not involved in the spermatogenic disruption observed in patients with deletion limited to *DAZ*. Two *CDY1* genes map in the *AZFc* region, one within the *DAZ* cluster and the other one at the distal end (36). This finding is intriguing since at least one *CDY1*copy in invariably absent in patients with *DAZ* deletion. Therefore, *CDY1* can be considered an *AZFc*-candidate gene, but deletions removing this gene specifically should be identified in patients to confirm this hypothesis. No mapping and deletion data are available for *BPY2*, which, therefore, is not included at present among the *AZFc*-candidate genes.

VI. *AZFa* Region and Candidate Genes

The most recent data suggest that more than one gene may be responsible for the *AZFa* phenotype. However, the characterization of the critical interval is still under way, and the structure and gene constitution of this region have only recently been described (19, 56–59).This region contains three genes and has a syntenic homology with the mouse ΔSxr^b interval (56) (Fig. 5) located on the Y chromosome short arm, deletion of which causes a severe spermatogenic impairment (60), very similar to that observed in patients with the *AZFa* deletion.

The first gene identified in *AZFa* and subsequently shown

to be absent in infertile patients was *DFFRY* (*Drosophila* fat facets related Y) (56, 61), recently renamed *USP9Y* (ubiquitin-specific protease 9, Y chromosome). This gene differs substantially from the other *AZF* candidate genes *DAZ* and *RBMY*: it does not encode for an RNA-binding protein, but seems to function as a C-terminal ubiquitin hydrolase; it is a single copy gene, it has an X-homologous gene that escapes X-inactivation, and it is ubiquitously expressed in a wide range of tissues, rather than testis specific (19, 61) (Table 1). Furthermore, a nine-residue peptide derived from *USP9Y* has been shown to represent a new minor histocompatibility antigen (H-Y antigen) presented by HLA-A1 and involved in graft rejection (62). *USP9Y* occupies less than half of the *AZFa* interval (57), while the majority of infertile males carrying *AZFa* deletions show the absence of this entire interval (16–18, 56, 63–66). These findings suggested that other gene(s) in this region may be responsible, either singly or in combination with *USP9Y*, for the spermatogenic disruption observed in *AZFa*-deleted patients. In fact, comparative mapping studies showed that two further X-Y homologous genes are located both in the Sxr^b interval and in *AZFa*, suggesting a possible role in spermatogenesis: *DBY* (dead box on the Y) and *UTY* (ubiquitous TPR motif on the Y) (19, 56). More recently, a novel expressed sequence (AZFaT1) was mapped to this region (57). All such genes appear to be ubiquitously expressed (19, 56, 57), therefore differing from their mouse homologs, since mouse *Dby*, for example, is expressed in several tissues (56, 57) and *Dffry* is testis specific (61).

Initial studies on patients with deletions clearly limited to *AZFa* suggested that deficiency of *USP9Y* or AZFaT1 or both cause male infertility and that the additional loss of *DBY* may make the phenotype worse (57). Substantial proof for *USP9Y* as a spermatogenesis gene has been recently published (58), since a 4-bp deletion leading to a truncated protein was discovered in an azoospermic man. An extensive deletion and expression analysis of deletion intervals 5 C/D in highly selected infertile patients allowed us to assemble a refined map of *AZFa* and to demonstrate that *DBY* may represent the major spermatogenesis gene of this region (59). It is more frequently deleted than *USP9Y* , and it shows a testis-specific transcript in addition to ubiquitous transcripts. The role for this gene in human spermatogenesis is further supported by the significant homology of *DBY* with the mouse protein PL10 (59), which is testis-specific and expressed only in germ cells. *DBY* consists of 17 exons (59) and encodes for a putative ATP-dependent RNA helicase, as it belongs to the DEAD box proteins (67, 68). However, its specific function in male germ cell development is still unknown.

VII. Y Chromosome Microdeletions in Infertile Men

A. Methods of detection

Once a map of PCR markers covering the Y chromosome was assembled (9, 69), the first searches for interstitial deletions in infertile men were published (33, 70, 71). Such PCR markers are known as STSs, and to date more than 300 have been physically mapped. Each STS detects known sequences of genomic DNA, and its normal amplification by PCR indicates the presence of this DNA sequence in the Y chromosome, while its absence may indicate a deletion. An STS may be specific for a gene or gene family or may detect anonymous sequences.

Most problems with the STS-PCR technique in screening for microdeletions derive from the intrinsic nature of the Y chromosome, which largely consists of repetitive elements and gene families widely dispersed along the chromosome. As described by Kostiner *et al.* (72), STS markers fall into three categories: 1) markers that are single copy, such as those for *SRY*, *USP9Y*, *DBY,* or *UTY*; these STSs are the most informative given their specificity, and their absence indicates the loss of the specific gene sequence; 2) markers that are multicopy but clustered in a small region of the Y chromosome, such as those for the *DAZ* gene family; in such a case a negative PCR result indicates the loss of all members of the gene family. However, the presence of a PCR band is less informative, since a normal amplification merely indicates the presence of at least one gene copy containing the marker sequence; 3) markers that are multicopy and dispersed across large regions of the Y chromosome, such as those for *RBMY*, *CDY,* or *TSPY* or other multicopy gene families widely distributed on the chromosome; such repetitive markers may be informative only when their absence indicates that very large deletions of the Y chromosome have occurred, *e.g.*, encompassing the entire Yq11.

Some markers may represent normal polymorphisms, as they may be absent in both fertile and infertile patients. Polymorphic markers (such as sY207 and sY272) are not

TABLE 1. Comparison of the characteristics of the *AZF*-candidate genes *DAZ*, *RBMY*, *USP9Y*, and *DBY*

	DAZ	*RBMY*	*USP9Y*	*DBY*
Localization	Yq (*AZFc*)	Yp and Yq Functional copies in *AZFb*	Yq (*AZFa*)	Yq (*AZFa*)
Gene organization	Gene family (cluster)	Gene family (20–50 genes and pseudogenes, belonging to almost 6 subfamilies)	Single gene	Single gene
Gene structure	16 Exons (including multiple copies of exon 7) DAZ repeats (72 bp each) in variable number and order	12 Exons SRGY boxes (111 bp each)	46 Exons	17 Exons
Expression	Testis-specific (germ cells)	Testis-specific (spermatogonia, spermatocytes, round spermatids)	Ubiquitous	Ubiquitous and testis-specific transcripts
Protein	RNA binding	RNA binding	Ubiquitin hydrolase H-Y antigen	RNA helicase

useful in screening since their absence does not represent significant deletions (73).

The STS-PCR technique is performed on genomic DNA extracted from peripheral leukocytes; although rapid and simple, certain precautionary measures should be applied in Y chromosome deletion screening before assuming that a deletion exists; these measures include the use of high quality DNA and internal and external positive and negative controls (*SRY* or *ZFX/ZFY* gene, fertile male, normal woman, and blank controls). Furthermore, European guidelines for the molecular diagnosis of Y chromosome microdeletions (74) suggest that the screening should be performed by multiplex PCR amplification in which an internal control is amplified together with the selected STS(s). The number of STSs to be used for a first screening is not well established, and it varies substantially among the authors. However, as a general rule, at least two or three STSs for each *AZF* region should be used; if a deletion is found and confirmed, the number of STSs should be increased to determine the deletion breakpoints.

Given the repetitive nature of some STSs and the quite frequent finding of noncontiguous deletions, the results obtained by PCR should be sometimes confirmed by Southern blotting experiments, especially if a deletion not encompassing known spermatogenesis loci is found. Furthermore, a deletion may be assumed to have a pathogenic role only if it is demonstrated that it is of *de novo* origin and not present in other fertile family members. Therefore, when available, male relatives of patients should be analyzed.

The rapid progress in molecular biology technologies will allow us, in the near future, to improve both the quality of the diagnosis and the time needed for a Y deletion screening. For example, fluorescent PCR will probably take the place of the conventional PCR method, since it is 1,000-fold more sensitive, less time consuming, and would allow the accurate testing of a small amount of DNA (*e.g.*, for single cell analysis). The analysis of Y chromosome deletions may also shift to other more automated methods of detection, such as the microarrays technique. However, time is needed to increase our knowledge of Yq genes and mapping before this method may completely replace traditional methods.

B. Selection of patients, genotype-phenotype relation, and origin of deletions

The recent progress in molecular biology and Y chromosome mapping has rendered the analysis of Y chromosome microdeletions in infertile patients a routine diagnostic step. As a result, numerous studies on this topic have been published: more than 4,800 infertile men have been studied. Taken together, these data suggest that Y chromosome microdeletions constitute one of the most common specific causes of male infertility. In fact, based on studies from 1992, the overall prevalence in infertile men is 8.2% (401/4,868) (16, 17, 33, 66–66, 70, 73, 75–102) (Fig. 6). In contrast, only 12 of 2,663 (0.4%) fertile males were found to carry a deletion, probably reflecting polymorphisms, as discussed below. However, among the various studies, remarkable differences in the prevalence of microdeletions exist, ranging from 1% (85) to 35% (63), reflecting above all different patient selection criteria. Therefore, the actual incidence of clinically relevant microdeletions in infertile men is still unclear. In this review we have summarized the studies published up to May 2000, grouping them on the basis of the various patient classifications used. In fact, male infertility is a heterogeneous diagnostic category that may be classified only on clinical and historical data, or on seminological data (normo-, oligo-, azoospermia) and/or on testicular structure (Sertoli cell-only syndrome, hypospermatogenesis, spermatogenic arrest, obstructive forms) (103). Figure 6 shows the categories of infertile men, as described in the original studies: in some reports other or nonuniform classifications are used, and therefore only 3,640 of 4,868 patients may be clearly grouped (16, 17, 33, 62, 63, 73, 75–98). To clarify a genotype-phenotype relation, the different classification of infertile patients is even more crucial.

Most studies have focused on azoospermic and severely oligozoospermic men (sperm count $<5 \times 10^6$/ml) with a

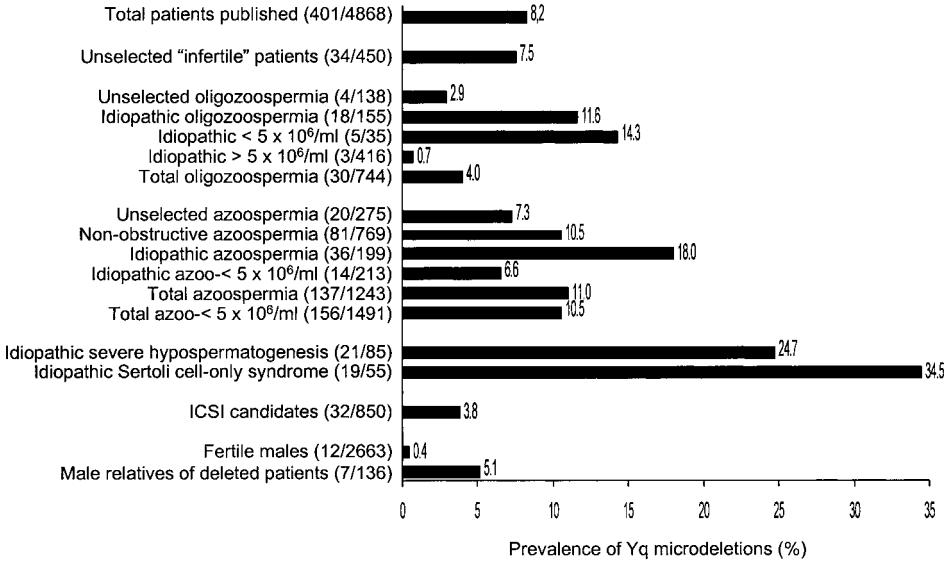

FIG. 6. Summary of the literature on Yq microdeletions in infertile males from 1992 to May 2000 (16, 17, 33, 63, 64, 66, 70, 73, 75–102). The first category at the *top* of the figure represents all patients published, while the other categories have been grouped from homogeneous studies in which a clear description of patients was reported. The prevalence of Yq microdeletions was calculated regardless of the number of STSs used or genes analyzed.

total of 1,491 patients published (deletion in 156 subjects, prevalence of 10.5%), and more recently on ICSI-candidates (32/850, 3.8%). However, also in such categories of infertile men we must distinguish between true azoospermic and severely oligozoospermic men, and among patients with idiopathic infertility, known causes of spermatogenic alteration, obstructive azoospermia, or unselected patients. Figure 6 clearly shows that the prevalence of deletions increases with more strict patient selection criteria: in unselected oligozoospermic men the prevalence is 2.9% but rises to 11.6% if idiopathic oligozoospermia is selected, and to 14.3% in idiopathic severe oligozoospermia; similarly, unselected azoospermic patients show a deletion rate of 7.3%, but if we exclude obstructive azoospermia the prevalence rises to 10.5%, and to 18% if only idiopathic forms are considered. Furthermore, if patients are selected on the basis of their testicular structure, the prevalence is 24.7% in idiopathic severe oligozoospermia with a testicular picture of severe hypospermatogenesis, and 34.5% in idiopathic azoospermia with a testicular histology of Sertoli cell-only syndrome. A further consideration is that the prevalence in patients with a sperm count greater than 5×10^6/ml is very low (0.7%), suggesting that Y deletions most frequently determine severe damage in sperm production. As shown in Table 2 and considering only homogeneous studies in which sperm count of infertile patients is reported (16, 33, 63, 64, 73, 76, 78–80, 82–94), deletions more frequently determine azoospermia (84.3%) than severe oligozoospermia (14.1%) or moderate oligozoospermia (1.6%). These data further strengthen the hypothesis that Y chromosome microdeletions produce a severe loss of spermatogenic cells up to their complete absence, and that a deletion screening should be offered mostly in azoospermic and severely oligozoospermic patients.

The relative prevalence of deletions in *AZFa*, *b*, and *c* regions in infertile men, as results from the analysis of homogeneous studies, is shown in Table 3. Deletions most frequently involve the *AZFc* region including *DAZ* (59.6%), less frequently the *AZFb* region including *RBMY* (15.8%), and in only 4.9% of cases the *AZFa* interval. Larger deletions involving two or three *AZF* regions are observed in 13.6% of patients, while in 6% of cases the deletions are located in regions not overlapping the *AZF* intervals. These data suggest a predominant role in spermatogenesis of the *DAZ* gene family with respect to the other *AZF* candidates. However, such results may also reflect our current knowledge of the *AZF* genes, and we have, for example, detected a high prevalence of deletions in the *AZFa* region in selected infertile patients affected by idiopathic severe testiculopathies (9/133, 6.8%) (59). The higher incidence of deletions in *AZFc* may be due to the fact that it is nearly always included in the screening program while *AZFa* and *AZFb* are more rarely investigated. Furthermore, the problems in analyzing multicopy genes may explain the low rate of deletion found, for example, for *RBMY*. The higher frequency of deletion in *AZFc* may also be due to the presence of long direct and inverted repeats in this region (36), rendering this interval particularly prone to deletion events.

TABLE 2. Seminal features of patients with Yq microdeletions, as determined from homogeneous studies where sperm count was reported (16, 33, 63, 64, 73, 76, 78–80, 82–94)

	n	%
Azoospermia	156/185	84.3
$<5 \times 10^6$ sperm/ml	26/185	14.1
$5–20 \times 10^6$ sperm/ml	3/185	1.6

TABLE 3. Relative prevalence of deletions in *AZFa*, *b*, and *c* regions in infertile men (16, 33, 63, 64, 73, 76–78, 80, 82–95, 100, 102)

	n	%
AZFa	13/265	4.9
AZFb	42/265	15.8
AZFc	158/265	59.6
AZFa + b	4/265	1.5
AZFb + c	22/265	8.3
AZFa + b + c	10/265	3.8
Outside *AZF*	16/265	6.0

The phenotypes associated with deletions are variable, and in general there is no clear correlation between the localization of the deletions (*AZFa*, *b,* or *c*) and the clinical phenotype. Although one group reported different phenotypes in association with each of the three deleted regions (16), such correlations have not been observed by others. The analysis of the literature (Table 4) shows that *AZFc* deletion may be associated both with azoospermia (54%) and severe oligozoospermia (46%), and the testicular histology (where available) may vary from Sertoli cell-only to spermatogenic arrest and hypospermatogenesis. Tubules with variable defects may be found in the same individual. The absence of *DAZ* appears therefore to be insufficient to determine the complete loss of the spermatogenic line, but rather seems to produce a reduction in the number of these cells or an alteration of their maturation process. It is possible that the testicular damage caused by *DAZ* deletions is progressive and that oligozoospermic patients may become azoospermic later in life. Alternatively, small differences in the extent of apparently identical *DAZ* deletions may explain this variable phenotype, but none of these hypotheses have yet been demonstrated.

Deletions in *AZFa* and in *AZFb* cause azoospermia in two-thirds of all cases, and more rarely severe oligozoospermia. Therefore, the phenotype associated with such deletions seems to be more severe than that observed in *AZFc*-deleted patients, even if in some cases an *AZFb* deletion (above all not including *RBMY*) may be associated with moderate oligozoospermia. The testicular histology of *AZFa* patients with azoospermia always shows Sertoli cell-only, while in patients with severe oligozoospermia it resembles severe hypospermatogenesis, *i.e.*, no maturation arrest is seen. Our study on the *AZFa* region (59) showed that the loss of *DBY* may be associated both with Sertoli cell-only syndrome and severe hypospermatogenesis, suggesting that this gene might regulate the first phases of the spermatogenic process or the activity of the stem cells. On the contrary, the deletion of *USP9Y* (59) as well as its mutation (58) have been found to determine only severe hypospermatogenesis. *AZFb* patients could have more variable defects, and in about half of cases a spermatogenic arrest is observed. The variable spermatogenic alterations observed in *AZFb* patients may indicate multiple functions of *RBMY* during spermatogenesis or,

TABLE 4. Seminal and testicular phenotypes associated with Yq microdeletions, as determined from homogeneous studies (16, 33, 57, 63, 73, 77, 78, 80, 81, 83–85, 87–94, 99)

	AZFa	*AZFb*	*AZFc*	*AZFa + b*	*AZFb + c*	*AZFa + b + c*
Seminal pattern						
Azoospermia	8/12 (67%)	21/31 (68%)	54/100 (54%)	2/4 (50%)	24/25 (96%)	15/15 (100%)
Severe oligospermia	4/12 (33%)	7/31 (23%)	46/100 (46%)	2/4 (50%)	1/25 (4%)	
Moderate oligospermia		3/31 (9%)				
Testicular histology						
SCOS	5/9 (56%)	2/13 (15%)	6/28 (21%)	1/1 (100%)	2/4 (50%)	10/10 (100%)
Severe hypospermatogenesis	4/9 (44%)	4/13 (31%)	16/28 (58%)		1/4 (25%)	
Spermatogenic arrest		7/13 (54%)	6/28 (21%)		1/4 (25%)	

SCOS, Sertoli cell-only syndrome. Severe oligospermia: $<5 \times 10^6$ sperm/ml, moderate oligospermia: $5–20 \times 10^6$ sperm/ml.

alternatively, that other genes located in this region may act in combination to *RBMY* and that their presence or absence modulates the phenotype.

The variable phenotype observed both in patients with *AZFb* and in patients with *AZFc* deletions may be explained by different hypotheses:

1. Different extension of the deletion. Deletions may completely remove *AZFb* or *AZFc* or they may be smaller, extending, for example, only one gene or gene cluster or few STS markers. It can be speculated that larger deletions may be associated with a more severe phenotype. However, neither a review of the literature nor our experience support this hypothesis. For example, deletions removing only *DAZ* and not extending to flanking regions may cause both azoospermia and severe oligozoospermia. Furthermore, the combined deletion of *DAZ* and *CDY1* do not seem to cause a phenotype worse than that observed in patients who retained the distal *CDY1* copy (A. Ferlin, E. Moro, A. Rossi, and C. Foresta, submitted). However, it should be kept in mind that apparently identical deletions, as assessed by STS-PCR, may be actually slightly different in size and this may, at least in part, justify a different phenotype.

2. Role of homologous genes and genetic background. Each of the *AZF* candidate gene has homologs on the X chromosome (*DBX*, *USP9X*, *RBMX*) or on autosomes (*DAZL1*), as many other genes on the Y chromosome have (*e.g.*, *EIF1AY* has a homolog on the X chromosome, and *CDY* has a homolog on chromosome 6). Even if no direct evidence of a role of these genes in spermatogenesis exists, their status may modify the phenotypic expression of Y-deleted patients. This is particularly suggestive for *DAZL1/DAZ*, since *DAZL1* is expressed exclusively in the gonads and may therefore synergistically act in combination with *DAZ* during spermatogenesis. Therefore, the genetic background may modulate the phenotypic effect of a given deletion, and the absence of an *AZF* gene may be differently compensated by other genes of the family.

3. Progression of the spermatogenic failure. It has been suggested that Yq microdeletions could result in progressive worsening of sperm production (88, 104, 116), and that oligozoospermic men may become azoospermic with time. This is an intriguing hypothesis that, if confirmed, has important prognostic consequences, since cryoconservation of spermatozoa in these cases could avoid more invasive techniques, such as ICSI, in the future.

From the analysis of the literature the only clear correlation that has been found is with very large deletions involving more than one *AZF* locus. These deletions are associated with the most severe phenotype, and this is exemplified by the invariable finding of azoospermia and Sertoli cell-only in patients with deletions of *AZFa-c*. Such large deletions probably show the additional effects of each single gene deletion. These data are also in agreement with the report that smaller deletions are associated with finding some sperm at TESE (testicular sperm extraction) during ICSI treatments, while larger deletions are not (92).

For 401 Y-deleted patients, 136 male relatives (father and/or brothers) were analyzed, and in 95% of cases (394/401) no microdeletions were found (Fig. 6). In the other seven cases a deletion was found both in the father and in his infertile son (16, 17, 70, 73, 105); in some cases the deletion involved STS markers subsequently shown to represent polymorphisms, but in other descriptions the father was found to carry a clinically relevant deletion of apparently identical size to that of the son, or even a smaller deletion (105). These findings demonstrate that such nonpolymorphic deletions can, in rare cases, be passed on from father to son, and may become larger (with possible additional negative effects). In most cases, these naturally occurring transmissions involved the *AZFc* region, and this was confirmed by a recent study that reported an identical *AZFc* deletion including *DAZ* in four infertile brothers and their father (104). The father was azoospermic at the time of analysis, but he evidently possessed some degree of fertility when he fathered, suggesting that the loss of germ cells caused by *DAZ* deletions may be progressive over the years. However, as seen above, it is possible that apparently identical deletions, as determined by STS analysis, are actually different in size, and that the father, for example, could have a smaller deletion than his infertile sons. Alternatively, a different exposure to the environment or a different genetic background may have determined the phenotypic variation.

Apart from the few inherited cases described above, the major part of Yq deletions are of *de novo* origin, which means that they are not present in the father's DNA and probably originated in the germ line of the father (106). The spermatogenic stage at which the deletion occurs is not known: even if primary spermatocytes in meiotic prophase are probably the most sensitive cells, deletions may arise during other steps of DNA replication or even during spermiogenesis (106). The mechanism by which a Y deletion arises is not clear, since unlike autosomes, only limited parts of the Y chromosome pair with the X chromosome and no recombination occurs within the *AZF* regions. Therefore, it has been

proposed that Y deletions are likely to be the consequence of the presence of many highly repeated DNA elements causing illegitimate intrachromosomal recombination. Specific genetic or environmental factors may predispose certain individuals to produce higher proportions of sperm with *de novo* deletions that may compete successfully with nondeleted spermatozoa to fertilize an egg and give rise to a Y-deleted child. This hypothesis could only be demonstrated by deletion analysis on single spermatozoa of the fathers. The other stage at which a Y deletion could originate is in the fertilized egg or during the first embryonic developmental phases of the infertile son (106). This should give rise to a mosaicism (normal Y chromosome in leukocytes, deleted Y chromosome in the germ line) and also this hypothesis should be analyzed in the future. Whatever is the case, the *de novo* origin of a Y deletion in an infertile patient is fundamental to assess its pathogenic role in determining the spermatogenic disruption.

The causative role of Y chromosome microdeletions in spermatogenic impairment is also supported by the evidence that male infertility caused by well known etiologies, such as Klinefelter's syndrome, previous chemo-radiotherapy, orchitis, or testicular trauma, are rarely associated with Y deletions (63, 107). However, a recent study (83) reported a high prevalence of deletion also in azoospermia and severe oligozoospermia associated with other apparent causes (5/72, 6.9%). These data suggest that such severe testiculopathies may actually be due to Y chromosome deletions. In particular, sporadic observations showed that patients with cryptorchidism or varicocele and azoospermia or severe oligozoospermia could carry deletions (17, 73, 80, 88, 93). We reported a high incidence of deletion in unilateral ex-cryptorchid patients affected by azoospermia or severe oligozoospermia due to severe bilateral testicular damage (11/40, 27.5%), while we found no deletions in ex-cryptorchid men with normal function of the descended testis (108). Furthermore, it is possible that the severe testiculopathy caused by Y chromosome microdeletion may have rendered the testis unresponsive to the normal stimuli that regulate testicular descent. These results suggest that the bilateral testiculopathy observed in patients with unilateral cryptorchidism may be related to the deletion of the Y chromosome and not to the abnormal location of the testis. Similarly, seven of 40 (17.5%) patients affected by left varicocele and severe oligozoospermia sustained by severe hypospermatogenesis were found to have a Y deletion, while no abnormalities were detected in patients with varicocele and mild oligozoospermia (109). Also in this group of patients, the bilateral testicular damage is due to the underlying genetic anomaly and not to varicocele itself. The finding of Y chromosome microdeletions in such highly selected patient groups strongly suggests that the phenotype associated with a Y chromosome microdeletion may be also cryptorchidism and varicocele other than idiopathic infertility. All patients affected by severe testiculopathies should be screened for microdeletions, regardless of other concomitant causes of testicular damage (110).

From the clinical point of view, hormonal values and testicular volumes in Y-deleted patients indicate a severe testiculopathy involving only the spermatogenic system. In fact, the testes are generally reduced in size, FSH concentrations are high, and LH and testosterone plasma levels are within the normal range. The major part of studies reported no significant differences in testicular volumes and FSH levels between patients affected by severe testiculopathy with and without Y deletions. However, one study reported that FSH levels in patients with deletions, although higher than controls, were lower with respect to patients with similar tubular alterations but without deletions (80), and another study showed that Y-deleted patients had normal FSH plasma concentrations (87). If a difference in hormonal concentrations between deleted and nondeleted patients really exists, further studies are necessary to understand the mechanisms responsible for such differences.

As previously noted (111), there is no correlation between the frequency of microdeletions detected and the number of STSs analyzed. For example, if we consider only homogenous studies in which idiopathic azoospermic and oligozoospermic patients are reported (63, 64, 73, 80–83, 87, 94, 95, 101), the number of STSs used varies from eight (80) to 85 (73), but the prevalence of deletions does not increase if more STSs are used ($P = 0.9$, not significant). Therefore, at the present time, the diagnostic performance is not improved by using large sets of STSs, and good results can be obtained using two to three STS markers for each *AZF* region, as recently suggested (74). What is really important for a careful diagnosis is that the panels of STS chosen amplify tracts of the Y chromosome containing genes that are known to be deleted specifically in infertile men and are not polymorphic. Furthermore, research in this field is growing and if a gene of the Y chromosome becomes a strong spermatogenesis candidate it should be included in the screening; in general, it is better to choose STS markers that amplify specific regions of a gene than those amplifying anonymous tracts of the Y chromosome.

C. The concern of assisted reproduction techniques

ICSI is the direct introduction of a spermatozoon into an oocyte to achieve fertilization and pregnancy when the number of spermatozoa in the ejaculate is very low or even absent. In the latter case, ICSI can be performed using spermatozoa obtained from the epididymis or directly extracted from testicular tissue. Furthermore, techniques of spermatid injection into oocytes may be performed and first term pregnancies have been achieved in humans (112, 113). Despite the worldwide diffusion of ICSI in recent years, the possible risks that might ensue from its indiscriminate use have been considered only recently. These concerns arose especially with the recent advances in genetically determined male infertility (114, 115). ICSI arouses more fears of the transmission of genetic abnormalities to offspring than other forms of assisted reproduction techniques because it bypasses all the physiological mechanisms related to fertilization, which need an active motile spermatozoon to undergo normal capacitation and acrosome reaction and to start all mechanisms required to penetrate the oocyte. By bypassing these steps, ICSI allows an altered spermatozoon to fertilize an oocyte, thus increasing the risk of genetic defects in the offspring. In other words, a genetic defect giving rise to abnormal spermatogenesis that can be surmounted by ICSI could be trans-

mitted to the children produced. The concerns are most remarkable for male infertility related to Y chromosome microdeletions, since Y-deleted patients are strong candidates for ICSI, as in most cases spermatozoa or spermatids suitable for the procedure can be recovered from semen or the testis, but all male offspring will invariably inherit the deleted Y chromosome from the father.

Mulhall *et al.* (90) first reported the fertilization and pregnancy achieved utilizing ICSI with testicular spermatozoa from azoospermic patients presenting deletions in the *DAZ* region, suggesting that Y-deleted spermatozoa are fully competent for fertilization. Subsequently, the same group reported the birth via ICSI of male offspring from an *AZFc*-deleted man (116), and other authors reported similar findings (117, 118). Following Mendelian expectations, all the boys inherited their fathers' deleted Y chromosome. These observations provide a concrete foundation for alerting couples to the likelihood of transmitting infertility-causing Y deletions by ICSI. Furthermore, since Y microdeletions are the most common molecularly defined causes of spermatogenic failure, one might expect that significant numbers of Y-deleted boys will be fathered through ICSI. From the analysis of the literature (Fig. 6), the prevalence of Y chromosome microdeletions in the ICSI candidate group is relatively low (3.8%), but it should be kept in mind that all severely oligozoospermic and azoospermic men with spermatozoa or spermatids in the testis represent the best candidates for this procedure, and the prevalence in these groups of patients is much higher. These findings provide a compelling rationale for the screening of all infertile men before ICSI.

Y-deleted spermatozoa may also transmit the deletion to male offspring via *in vitro* fertilization (IVF). In fact, it has been demonstrated that spermatozoa from an oligozoospermic subject carrying a Yq deletion are able to fertilize oocytes *in vitro* (119), suggesting that sperm carrying a deletion possess all the characteristics required to regulate capacitation, acrosome reaction, and the ability to penetrate and fertilize the oocyte. These findings are also supported by the evidence that Y deletion may be transmitted from father to sons also by spontaneous conception (16, 105, 106), even if very rarely (see above). In our opinion, Yq microdeletion analysis should be considered not only in patients undergoing ICSI, but also when undergoing other IVF programs.

The actual consequence of inheriting a Y deletion is still not clear, and we will have to wait until babies with the Y deletion conceived by IVF/ICSI become adult. Since descriptions of larger deletions in the sons with respect to the fathers have been reported (105), careful counseling of patients is mandatory, especially as to the risk of inheriting a deletion the clinical consequences of which are presently unknown, but, at the very least, include infertility. An identical deletion may be associated with different phenotypes, and therefore we cannot foresee the actual defects in the sons. Of greater concern is the possibility that additional unknown genetic problems may be present in infertile men whom nature has deemed unable to reproduce until now. Obviously, after extensive counseling, the couple must make the final decision about further treatment, but important ethical considerations arise since severe male infertility has now become a hereditary disorder. The clinician should be aware that the use of such techniques may lead to an increase in the number of infertile subjects in future populations. The primary rationale of medically assisted reproduction is the opportunity for parents to have a healthy baby, and a mandatory condition for achieving this goal is that the genetic characteristics of the gametes must be normal.

VIII. Conclusions

The rapid growth of molecular biology has determined that microdeletions of the Y chromosome represent an important cause of male infertility and the most frequent genetic etiology of severe testiculopathy. Such findings are fundamental both for a careful diagnosis of male infertility and for its treatment, and Y chromosome screening is now a reality in the major andrological and infertility centers. The detection of a deletion in an infertile man provides a proper diagnosis of the disease, allows the clinician to avoid empirical, unnecessary, and often expensive treatments to improve fertility (*e.g.*, hormonal treatments), and has important ethical consequences if the patient is a candidate for assisted reproduction techniques. Furthermore, it is now clear that a molecular diagnostic test of Y chromosome microdeletions should be at least performed in all men with a sperm concentration of less that 5×10^6/ml, regardless of the presence of other apparent concomitant causes of testicular damage, such as varicocele or cryptorchidism.

The identification of the actual role played by the *AZF*-candidate genes in spermatogenesis will provide significant advances to our understanding of the biology of spermatogenesis, as well as the analysis of novel Y-chromosomal genes with a potential role in male germ cell development will clarify other important features of this important chromosome.

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