INVITED REVIEW

The role of Y chromosome deletions in male infertility

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Abstract

Male infertility affects approximately 2–7% of couples around the world. Over one in ten men who seek help at infertility clinics are diagnosed as severely oligospermic or azoospermic. Recent extensive molecular studies have revealed that deletions in the azoospermia factor region of the long arm of the Y chromosome are associated with severe spermatogenic impairment (absent or severely reduced germ cell development). Genetic research into male infertility, in the last 7 years, has resulted in the isolation of a great number of genes or gene families on the Y chromosome, some of which are believed to influence spermatogenesis.

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Introduction

Defective spermatogenesis is the result of a multitude of causes, such as diseases, malnutrition, endocrinological disorders, genetic defects or environmental hazards (1). Genetic defects, such as mutations and chromosomal abnormalities, have been estimated to account for at least 30% of male infertility (2). Research in the last 20 years has indicated that the Y chromosome is necessary for sexual development and spermatogenesis. Recent genetic studies of male infertility have demonstrated that the long arm of the Y chromosome (Yq) harbours at least 15 gene families (3), of which some have been shown to be necessary for spermatogenesis. These findings have attracted great interest, not only from geneticists in their attempt to understand the mechanism of genetic control of spermatogenesis, but also from clinicians in their demand for molecular tools in the diagnosis of male infertility. As the advent of molecular-based analytical techniques are now available to investigate the Y chromosome, a variety of Y-deletions of different locations are being identified in infertile patients with different phenotypes. These findings have provided important information for our understanding of the role of the Y chromosome in spermatogenesis and may eventually allow establishing correlation of specific Y-deletions with correspondent spermatogenic phenotypes.

Male infertility

Human male infertility can result from a variety of factors. In 1987, the World Health Organization established a Task Force on the Diagnosis and Treatment

of Infertility, with the objective of creating a standard protocol for the investigation of infertile couples. Normal semen was classified as containing a sperm concentration of at least 20×10^6 /ml, of which more than 40% are progressively motile, more than 60% are alive, and over 50% show normal morphology. In addition, the semen should contain no more than 1×10^6 /ml of white blood cells (4).

A survey based on statistical studies of fecundity in human populations around the world indicated that approximately 2–7% of couples are infertile (5). Studies of 3956 infertile couples carried out in seven laboratories between 1962 and 1983 showed that male infertility was responsible for ~40% of cases (5). The report covered investigations carried out in 33 centres in 25 countries, and found that of the 6682 individuals, 717 (10.7%) were azoospermic or oligospermic ($< 5 \times 10^6$ sperm/ml) (4) (Table 1). The problem in these men appeared to be a failure of spermatogenesis, the cause of which is unknown. It is likely that the failures of spermatogenesis in some individuals are due to genetic defects.

Spermatogenesis is a long and complex process, involving a series of continuous cellular changes which are conventionally divided into the three major stages: mitotic proliferation of spermatogonia, meiosis and spermiogenesis (6).

Although the developmental process of spermatogenesis has been intensively studied and clearly described, our knowledge of the genetic control of spermatogenesis remains very limited. From a genetic point of view, it is reasonable to assume that the process of spermatogenesis is regulated by the accurately co-ordinated expression of many genes. Disruption of this process can be

Table 1 Distribution of diagnoses of male infertility (4).

Diagnosis	No. of cases	% of cases	Mean age (years)
No demonstrable abnormality	3127	46.8	31.0
Non-obstructive azoo-/oligospermia	717	10.7	31.1
Obstructive azoospermia	58	0.9	31.7
Other abnormalities*	2780	41.6	31.5
Total	6682		31.2

*Including: no demonstrable abnormality, varicocele, accessory gland infection, idiopathic teratozoospermia, idiopathic asthenozoospermia, isolated seminal plasma abnormalities, suspected immunological factor, congenital abnormalities, sexual inadequacy, idiopathic necrozoospermia, ejaculatory inadequacy, hyperprolactinaemia, iatrogenic causes, etc.

brought about by mutations of many genes. In *Drosophila*, for example, spermatogenesis is extremely sensitive to many metabolic stresses, which lead to sterility by pleiotropic effects (7). Similarly, there are also a number of mutations known to affect male fertility in mice, of which many are autosomally recessive, pleiotropic with phenotypic effects (Table 2).

Alternatively, chromosomal abnormalities, both structural (translocations mainly between autosomes and sex chromosomes) (Table 3) and numerical (such as the Klinefelter syndrome 47, XYY), constitute an important factor which can cause spermatogenic breakdown at various points, consequently resulting in 'chromosomally derived' sterility (38). In *Drosophila*, about 80% of all translocations between the X chromosome and autosomes are male-sterile (39). Reciprocal sex chromosome-autosome translocations have been

shown to cause spermatogenic arrest at the pachytene/metaphase I stage of the primary spermatocyte in mice and humans (40–42). Recent studies have demonstrated that the Y chromosome defects could also impair spermatogenesis. This review will be focused on the recent studies of the impact of the Y chromosome deletions in spermatogenesis.

Y chromosome abnormalities and their effects on spermatogenesis

In *Drosophila*

The Y chromosome has long been considered to be important in spermatogenesis in *Drosophila* since lack of the Y chromosome would result in XO male sterility. The spermatogenesis in XO males of *D. melanogaster* is

Table 2 Mutations affecting male fertility in mice.

Gene symbol	Gene name	Chromosome	Effects and references
<i>A-myb</i>	A-Myb	?	Spermatogenesis arrest at pachytene (8)
<i>azh</i>	Abnormal spermatozoon headshape	4	Abnormal sperm head formation (9)
<i>Bclw</i>	Bclw	?	Spermatogenic arrest at spermiogenesis stage (10)
<i>Bmp8b</i>	Bone morphogenetic protein 8B	?	Germ-cell deficiency, apoptosis of spermatocytes (11)
<i>bs</i>	Blind-sterile	2	Abnormal spermiogenesis (12,13)
<i>C^{3H}/C^{6H}</i>	Albino-deletion heterozygotes	?	Abnormal spermiogenesis (14)
<i>ebo</i>	Ebouriffe	?	Severely malformed spermatozoa (15)
<i>gcd</i>	Germ-cell deficient	?	Germ cell depletion (16)
<i>hop</i>	Hop-sterile	?	Polydactyly. Sperm tails absent or aberrant (17)
<i>hpy</i>	Hydrocephalic polydactyl	6	Polydactyly. Sperm abnormal and immotile (18)
<i>jsd</i>	Juvenile spermatogonial depletion	?	Azoospermia, reduced testes (19)
<i>Lvs</i>	Lacking vigorous sperm	?	Abnormal spermatogenesis at late stages (20)
<i>morc</i>	Microrchidia	?	Spermatogenic arrest in prophase I of meiosis (21)
<i>MSH5</i>	MutS homologue 5	?	Disruption of chromosome pairing (22)
<i>olt</i>	Oligotriche	?	Azoospermia (23)
<i>P^{6H}, P^{25H}, P⁵</i>	Pink-eyed, sterile	7	Abnormal spermiogenesis (24)
<i>pbs</i>	p-Black-eyed sterile	7	Coat colour diluted. Sperm abnormality (25)
<i>qk</i>	Quaking	17	Defects of spermiogenesis (26)
<i>sys</i>	Symplastic spermatids	?	Multinucleated syncytia, spermatids fail to mature (27)
<i>Tcte2</i>	t complex testes expressed 2	17	Defects in the first meiosis (28)
<i>Tctex-1</i>	t-complex testis-expressed 1	17	t complex sterility (29)
<i>t^x/t^x</i>	t-haplotypes	17	Abnormal spermatids, few spermatozoa (30)
<i>t^x/t^v</i>	t-haplotypes	17	Spermiogenic defects, failure of sperm function (31)
<i>wr</i>	Wobbler	?	Abnormal spermiogenesis (32)
<i>wv</i>	Weaver	?	Severely diminished spermatogenesis, azoospermia, severely degenerated seminiferous epithelium (33)

Table 3 Chromosome abnormalities in infertile males found in four surveys.

Survey references	Number	Sex chromosome (%)	Autosome (%)	Total (%)
Zuffardi & Tiepolo (1982) (34)	2542	175 (6.9)	40 (1.6)	215 (8.6)
Kjessler (1974) (35)	1363	70 (5.1)	20 (1.5)	90 (6.6)
Koulischer (1975) (36)	1000	27 (2.7)	6 (0.6)	33 (3.3)
Chandley (1979) (37)	2372	33 (1.4)	18 (0.76)	51 (2.1)
Total	7277	305 (4.2)	84 (1.1)	389 (5.3)

characterized by meiotic arrest at or before metaphase I (43, 44). In 1960, Brousseau (45) published a genetic map of seven fertility genes on the Y chromosome of *D. melanogaster*, five (kl-1 to kl-5) on the long arm and two (ks-1, ks-2) on the short arm. It was later demonstrated that deletions of the regions containing kl-5, kl-3 and ks-1 would prevent the formation of the outer dynein arm of the axoneme, leading to the collapse of spermatogenesis (46), while the deletion of ks-2 would result in a complex phenotype with nuclear crystal formation and abnormal meiosis (47, 48).

A lampbrush loop-like structure formed from the Y chromosome in the primary spermatocyte was first found in *D. melanogaster* (49) and later in all of the 54 *Drosophilids* studied (50). Further studies demonstrated the existence of five pairs of lampbrush loops in *D. hydei*, known as pseudonucleolus and threads, localized at the end of the long arm, loops clubs, tubular ribbon in a proximal region of the long arm close to the kinetochore, and loop nooses in the short arm of the Y chromosome. All of these loops are required for the fertility of males (51, 52). It is now known that a lampbrush loop is a male fertility gene, and it contains only one complementation group (53–56).

In *D. hydei*, the fertility genes are mainly composed of complex, locus-specific repetitive DNA sequences. These genes are transcribed stage-specifically in the primary spermatocyte nucleus as continuous long transcription units with a length ranging from 260 up to 4000 kb (57). The fertility gene on the short arm of Y chromosome of *D. hydei*, known as locus Q, forming the lampbrush loop nooses, indicated no open reading frames (58). However, this gene family did show a high capacity to form secondary structures due to internal, direct and inverted repeats, and was suggested to be suited for protein binding (59).

In 1987, a study revealed that DNA sequences of a fertility gene (locus B) of *D. hydei* were conserved on the Y chromosome in humans (60). More interestingly, seven of these DNA sequences, known as the pY6H family, were mapped to interval 6 of the human Y chromosome (61), a region which has been proved to be crucial for spermatogenesis (62). Three members of the pY6H family, pY6HP35, pY6HP52 and pY6BS65/E were found to be deleted in severely oligospermic and azoospermic men with Y chromosome microdeletions (63).

In mammals

The direct involvement of the Y chromosome in spermatogenesis in mammals was first suggested by Evans and coworkers (64). In a study of a sterile mouse with a mosaic karyotype of 39,XO/41,XXY, they found both types of cells in the bone marrow in equal number, but only 41,XXY cells were present in spermatogonia and spermatocytes, suggesting that the Y chromosome is required for normal spermatogenesis in mammals.

The most direct evidence for the presence of the mouse spermatogenesis factor on the Y chromosome came from the study of *Sxr^a* (sex-reversed, formerly *Sxr*), a dominant mutation causing sex reversal of female mice (65). Cytogenetic and molecular studies of *XY^{Sxr^a}* mice in different laboratories suggest that the *Sxr^a* region originated when a small duplicated fragment from the short arm of the normal Y chromosome was transposed to the tip of the long arm of the Y chromosome, distal to the pseudoautosomal region (Fig. 1A). Since this fragment contained the testis-determining gene *Tdy* (66) (Fig. 1B) (67, 68), when the *XY^{Sxr^a}* male mates to a normal XX female, a single recombination between the X chromosome and the *Y^{Sxr^a}* chromosome can transfer the *Sxr^a* region to the distal end of an X chromosome in an *XY^{Sxr^a}* male, leading to the generation of XY (non-*Sxr^a* carrying) males, XX (non-*Sxr^a* carrying) females, *XX^{Sxr^a}* (*Sxr^a* carrying) males and *XY^{Sxr^a}* (*Sxr^a* carrying) males (65). Adult *XX^{Sxr^a}* males are sterile as they lack germ cells. Germ cells are present in day-16 fetal gonads but, by the time of birth, the number of cells has decreased significantly, and the spermatogonia have completely disappeared 10 days after birth (65). This may be due to the presence of two X chromosomes which is incompatible with germ cell proliferation and differentiation in the testis, as seen in the XX and XXY males (65, 69, 70). The *XO^{Sxr^a}* male adults, although sterile, apparently developed as normal phenotypic males. Their testes were small but germ cells were viable. All stages of spermatogenesis were observed, but very few sperm were produced, many being immotile and showing morphologically abnormal heads (71). This observation strongly suggested the existence of a gene (or genes) involved in spermatogenesis in the *Sxr^a* region, since the germ cells entered meiosis normally.

Figure 1 Diagrams of the *Sxr* (sex reversed) mouse formation (A), and of the detailed *Sxr* region of mouse Y chromosome short arm (B). *Sxr^b* is a deletion mutation (ΔSxr^b DNA >900 kb) of the *Sxr^a*. *Smcy* is a candidate for controlling the expression of the minor histocompatibility antigen H-Y. PAR, pseudoautosomal region. Yp, short arm of the Y chromosome. Yq, long arm of the Y chromosome.

In 1984, another mutant mouse named *Sxr^b* (formerly named *Sxr'*) (72), was discovered. XX*Sxr^b* males and XO*Sxr^b* males are H-Y antigen negative, indicating the presence of *Tdy* but not the *Hya* gene in *Sxr^b* (72, 73). A complete block to spermatogenesis was found prepubertally at the onset of meiosis in XO*Sxr^b* adult testes (74). It has been shown that *Sxr^b* was derived from *Sxr^a* by a deletion of a piece of DNA which contains some portions of zinc-finger genes on the Y chromosome *Zfy-1* and *Zfy-2* (75, 76), the H-Y antigen expression gene *Hya* (77), and the spermatogenesis gene *Spy* (74) (Fig. 1B) (78).

In humans

The genetic study of spermatogenesis is more difficult in men than in animals, since the detection of correlated genetic defects and their phenotypes depends solely upon the occurrence of natural mutations within the population rather than by the artificially induced mutations in other animals. Only small regions homologous to the X chromosome at the tips of the Y chromosome undergo homologous recombination during meiosis; therefore, linkage analysis is not a feasible approach for genes on the Y chromosome.

Although it had been observed that deletions of the long arm of the Y chromosome could result in break-

down in spermatogenesis, leading to infertility in humans (79, 80), it was not recognized until 1976 when Tiepolo & Zuffardi (62) published their findings that the long arm of the Y chromosome carried genetic information essential for spermatogenesis. In the six azoospermic men studied, they found that all of these males carried a deletion of all the distal heterochromatin of the long arm of the Y chromosome (Yq) and that azoospermia was the only symptom presented. This led them to postulate that factors controlling human spermatogenesis might be located on the distal portion of the euchromatin segment of the long arm of the Y chromosome, Yq11 (62). This spermatogenesis locus, lying in Yq11.23, as demonstrated with high-resolution banding techniques, has since come to be known as the 'azoospermia factor' or 'AZF' (81). Further molecular investigations into genotype-phenotype correlation in azoospermic men led to the localization of the AZF locus to interval 6 of the Y chromosome (82) (Fig. 2) (83, 84).

The first solid molecular evidence that failure of spermatogenesis may be caused by cytologically undetectable deletions on the Y chromosome came from the identification of two non-overlapping microdeletions mapped to the distal region of intervals 5 and 6, carried by two azoospermic otherwise normal men (63). Further studies led to the proposal of the existence of three AZF subregions termed *AZF_a* (formerly JOLAR

Figure 2 Gene map of the human Y chromosome. The Y chromosome is divided into seven intervals. The azoospermia factor (AZF) is divided into AZFa, AZFb, AZFc and AZFd regions and mapped to intervals 5 and 6. PAR, pseudoautosomal region.

region), *AZFb* and *AZFc* (formerly KLARD region) respectively (85). The authors believe that deletion of *AZFa* is associated with lack of germ cells or Sertoli cell only syndrome, and that deletion of *AZFb* is associated with spermatogenesis arrest and that *AZFc* gene products are involved in the maturation process of postmeiotic germ cells (85). Although this hypothesis remains controversial and the fact may be more complex, it is generally accepted that the completion of spermatogenesis requires multiple genes not only on the Y chromosome but elsewhere as well. Very recently, the issue was further complicated by the description of another *AZF* subregion, named *AZFd*, localized between *AZFb* and *AZFc* (86). The discovery of the two separate microdeletions on the Yq in infertile men subsequently led to the identification of four candidate gene families for *AZF*. They are RNA-binding motif (*RBM*), previously named Y-linked RNA recognition motif (*YRRM*) (87), deleted in azoospermia (*DAZ*) (88), *Drosophila* fat facets related Y (*DFFRY*) (89) and chromodomain Y (*CDY*) (90) (Fig. 2).

The *RBM* gene family as an *AZF* candidate

The *RBM* family was the first Y-linked *AZF* candidate gene isolated from interval 6 by positional cloning (87). *RBM* encodes a protein that contains a highly conserved RNA recognition motif of approximately 90 amino acids, a hallmark of a superfamily of RNA-binding proteins, the heterogeneous nuclear ribonucleoprotein (*hnRNP*) family (91). Its closest member in the *hnRNP* family is

the *hnRNP*G RNA-binding protein, a widely expressed protein in the human, which is mapped to the short arm of chromosome 6, 6p12 (87, 92). The *RBM* protein consists of four tandem repeats of a 37 residue peptide, named the 'SRGY box' because of its high content of Ser-Arg-Gly-Tyr amino acids.

RBM is a multicopy gene family with an estimated 30–40 members (some of which are pseudogenes), spread over both arms of the Y chromosome but mainly in intervals 5 and 6 (87, 93). Very recently, an *RBM* homologue on the X chromosome has been identified (94, 95). The *RBM* homologues have been found on the Y chromosomes of numerous mammals, including mice and marsupials (87, 96, 97). Interestingly, the *RBM* genes in mice and marsupials contain only one SRGY box which is also the case in the *hnRNP*G gene. This led to the proposal that the Y-linked *RBM* family may have resulted from a transposition of an *hnRNP*G-like ancestral gene to the Y chromosome, followed by a series of internal amplifications of one of the exons, then a multiplication of the entire gene (97, 98). The mouse *Rbm* gene was initially thought to be a single one (87). However, it is now known that this also has multiple copies, some of which have been mapped to the *Sxr^b* region (96, 99).

The expression of the *RBM* and the *Rbm* proteins is found exclusively in germ cells in the testis. More recent studies indicate that *RBM* is a nuclear protein which may be involved in RNA processing during spermatogenesis (100). It is possible that *RBM* plays a role in modulating the splicing of pre-mRNA molecules as it has been observed that the *RBM* protein is co-localized with a number of known splicing factors at certain stages of spermatogenesis (100). Some *RBM* members were deleted in infertile men with either severe oligospermia or azoospermia (85, 87, 101–105). The deletion of *RBM* member(s) in the *AZFb* region appear to be associated with spermatogenesis arrest at meiosis (106).

It remains an unanswered question as to whether one copy or multiple copies of *RBM* are required for the completion of normal spermatogenesis in humans. In the case of the marsupial only one copy of *RBM* is functioning and required for spermatogenesis (97). Alternatively, multiple copies of genes may be necessary for producing large amounts of products needed for the production of large numbers of sperm. There are structural, functional and evolutionary arguments that repeated genes can be advantageous either through dosage repetition (107) or variant repetition or both (108). Dosage repeated genes are characterized by high copy numbers of identical sequences in tandem repeated clusters. This repetition, it is suggested, helps to fulfil the organism's need for a large amount of product; examples of dosage-repeated genes include the ribosomal genes, transfer RNA genes and histone genes (109). Variant repeated genes, for example the globin genes, actin genes and immunoglobulin genes, and

their products, are not identical but highly related, and are probably the result of the duplication and divergence of a common ancestral gene (108). Some genes show both variant repetition and dosage repetition, such as genes for 5S RNA in *Xenopus*, which have at least two large distinct sets, each representing a different gene sequence with multiple identical copies (108). Recent analysis of yeast artificial chromosome (YAC) contigs of the Y chromosome indicates that the RBM family shows both variant repetition and dosage repetition in humans (93). It is possible that different but highly related genes may be needed for controlling normal spermatogenesis in mammals. Human and other higher primates have more copies of RBM genes than other mammals. This may possibly be due to a less efficient capacity for producing sperm when compared with other mammals such as mice and rats (110), so that more copies of the genes are needed to compensate the mechanism. Hence, relatively higher numbers of the RBM genes may be necessary for sufficient production of the sperm to maintain fertility in humans. Partial loss of certain gene copies (dosage effect) may therefore lead to oligospermia rather than azoospermia because of insufficient quantities of many transcripts rather than from the lack of any particular one (111). A consequence of a high number of genes is a corresponding high rate of mutation. This may be one of the reasons for the occurrence of variable phenotypes (variable in sperm counts) seen among oligospermic patients. The dosage effect is known in other Y chromosome genes, as recently it has been reported in another multiple-copy gene Y353/B, a candidate for spermiogenesis in mice (112). There is evidence suggesting that partial loss of some Y353/B copies leads to an increased frequency of abnormal sperm heads. The RBM family contains both functional genes and non-functional pseudogenes. A study reveals that RBM2 is not present in the Japanese population and is polymorphic in some ethnic populations (113). To understand the RBM gene family, a comparative comprehensive study of the gene organization in other species will be informative. It has been shown that the deletion of most copies of *Rbm* results in high levels of abnormal sperm development (99). Although the multiple-copy nature of the RBM family makes it difficult to prove its precise role in spermatogenesis, the above findings suggest that RBM plays an important role in spermatogenic development.

The DAZ gene family as an AZF candidate

The DAZ gene was cloned from interval 6 (i.e. the AZFc region), and initially thought to be a single-copy gene (88). However, it is now clear that DAZ is a multiple gene family with at least seven copies clustered in the distal interval 6 (114). The DAZ family shares certain characteristics with the RBM family, namely it also encodes an RNA-binding protein that is also expressed in germ cells only. Another member of this family named SPGY was identified by Vogt and colleagues,

although the complete sequence of SPGY has not been released (115). The DAZ family contains 7 to 24 tandem repeats (termed DAZ repeat) of 72 base pairs with homology to the DYS1 repeat (88), the precise number of repeats varies in different individuals (116). Y-linked DAZ only exists in old world monkeys but not new world monkeys and other mammals (97, 117–119). Autosomal DAZ homologue genes have been characterized and mapped to chromosome 17 in mice and named *Dazl1* (formerly *DAZla* or *DAZh*) (117, 118) and mapped to chromosome 3p24 in human and named *DAZL1* (formerly *DAZLA*, *DAZH*) (119, 120). Only one 'DAZ repeat' was found in *DAZL1* and *Dazl1*. It has been proposed that the human Y-linked DAZ family is derived from a *DAZL1*-like autosomal ancestral gene by transposition and amplification (114). Like the Y-linked DAZ, *DAZL1* and *Dazl1* are cytoplasmic proteins expressed exclusively in testis and ovary. It has been shown that loss of *Dazl1* in knockout mice, termed *Dazl1*⁻/*Dazl1*⁻, expresses reduced number of germ cells and complete absence of gamete production in both males and females (121). This strongly suggests that *Dazl1* is required for gametogenesis. However, the role of the Y-linked DAZ in spermatogenesis has been debatable since its isolation. The Y-linked DAZ was believed to be the best candidate for AZF by some investigators because of its deletions found in a number of azoospermic or oligospermic men (88, 114). The strongest support of this view came from the finding that the disruption of *boule*, a gene identified in *Drosophila* with homology to the DAZ family, results in meiosis arrest in males (122). However, individuals lacking DAZ show various phenotypes ranging from azoospermia, oligospermia and some are even fertile (85, 104). These observations imply that the gene is not required for the completion of normal spermatogenesis. Further studies reveal that the sequence of the *boule* gene is more like that of the autosomal *DAZL1* and *Dazl1* than that of the Y-linked DAZ. For instance, a special domain of 130 base pairs is only found in *DAZL1*, *Dazl1* and *boule* but not in the Y-linked DAZ (116, 117, 119). Most, if not all, infertile individuals who lack the Y-linked DAZ carry rather large deletions of the Y chromosome which can extend from *AZFc* to *AZFα* (85, 102, 104, 123, 124). Since no point mutations of the DAZ genes have been found in any of the infertile men studied, it remains unknown whether the phenotypes of infertility are caused by the lack of the Y-linked DAZ, or by mutations or deletions of other genes lying in the *AZFc* region. It has been shown that the human Y-linked DAZ genes are highly polymorphic in the DAZ repeat region (116). A recent evolutionary study (125) on the DAZ family demonstrates that the human Y-linked DAZ exons and introns are evolving at a high male-to-female mutation rate, which is considered to be a sign of neutral genetic drift and the absence of selective functional pressure, which led the authors to postulate that the human Y-linked DAZ plays little or a limited role in spermatogenesis. In a recent transgene study by Slee

et al. (126), a human YAC of 225 kb containing a Y-linked DAZ gene was introduced to a *Dazl1* knockout mouse (*Dazl1*^{-/-}), which is characterized by severe germ-cell depletion and meiotic failure (121). Although the transgenic mice (termed *Dazl1*^{-/-}; *TgS12*) remained infertile, a partial and variable rescue of the mutant phenotype was seen with increased numbers of germ cells surviving up to the pachytene stage of meiosis (126). Since the DAZ transgene that was introduced lacked the exon-1, the experiment did not, therefore, conclusively establish the function of the DAZ gene product. However, this observation suggests that at least a certain degree of the function of the DAZL1 has been retained by the human Y-linked DAZ.

The DFFRY gene as an AZF candidate

DFFRY and DFFRX are the recently characterized homologues of the *Drosophila* developmental gene fat facets (*faf*), which have been mapped to the proximal Yq11.2 and Xp11.4 respectively in humans (89, 127). The *faf* gene is a member of a gene family encoding deubiquitinating enzymes which remove ubiquitin from protein-ubiquitin conjugates (128). It has been shown that the *faf* gene is essential for normal oogenesis (129). The mouse *Dffry* homologue has been characterized and mapped to the *Sxr*^b region on the short arm of the Y chromosome (89). Deletion of *Sxr*^b has been shown to be associated with early spermatogenic failure with an almost total loss of germ cells in meiosis (74). The expression of mouse *Dffry* is restricted to the testis and can be first detected between 7.5 and 10.5 days after birth, when type A, type B spermatogonia, preleptotene and leptotene spermatocytes are present (89). The human DFFRY gene is expressed ubiquitously in adult and embryonic tissues, including the testis. Interestingly, the DFFRY gene is mapped to AZFa and deleted in two azoospermic men lacking germ cells and one oligospermic man (89). The above observations suggest that, although the DFFRY gene does not appear to be essential for the initiation of spermatogenesis, it may still have an important impact on spermatogenesis.

The CDY family as an AZF candidate

The human chromodomain Y (CDY) was previously believed to be a gene with multiple copies mapped to Y chromosome deletion intervals 5L and 6F (90). More recent studies reveal that CDY is a gene family with at least three members identified and named CDY1 major, CDY1 minor and CDY2. The CDY family encodes a protein containing a chromatin-binding domain and a catalytic domain. The predicted coding regions of CDY1 and CDY2 were 98% identical in amino acid sequence of the predicted proteins. Most of the putative protein encoded by the CDY1 minor transcript is identical to that encoded by the major transcript except that its carboxy terminus is divergent. The CDY1 genes are

mapped to intervals 6F and the CDY2 to 5L of the Y chromosome. However, it is not known how many copies of the CDY genes are carried by the Y chromosome (90).

It is particularly intriguing that, like the DAZ family, the human CDY has an autosomal homologue, referred to as CDY-like (CDYL), mapped to the distal short arm of chromosome 6. The Y-linked CDY genes are restricted to primates, while the autosomal CDYL homologues are widely present in other mammals, including marsupials. In mice, the CDYL homologue, known as *Cdyl*, has also been identified and mapped to chromosome 13. The predicted mouse *Cdyl* and human CDYL proteins have 93% amino acid identity overall, while the predicted human CDYL and CDY proteins have only 63% amino acid identity. It has been demonstrated that, in humans, CDY is specifically expressed in adult testis, while the expression of CDYL is ubiquitous. In contrast, the mouse *Cdyl* produces two transcripts; one transcript is similar to the human CDYL, the other is similar to the human CDY. These findings have led to a suggestion that human CDYL and mouse *Cdyl* came from a common ancestor, while CDY was derived from CDYL (90). Although the function of the CDY family remains to be determined, its location on the region important for spermatogenesis and its testis-specific expression have made it a potential candidate for AZF.

Other spermatogenesis-related genes

Several additional genes, for instance *Hya* (77), *Ube1y* (130, 131), *Zfy* (132) and *Y353/B* (133) have been suggested to be involved in spermatogenesis in the mouse.

The mouse H-Y antigen gene (*Hya*) was once proposed to be the spermatogenesis gene *SpY* (74), based on the finding that *Sxr*^b males appear to be H-Y antigen negative and show absence of spermatogenesis. This view has been recently challenged by the occurrence of a new variant (134). More recently, a mouse Y chromosome candidate gene for the *Hya* locus (termed *Smcy*) has been cloned from the region encoding *SpY* and *Hya* (135). The human homologue (SMCY) has also been mapped to the same deletion interval as human H-Y antigen locus, HYA (135).

Ube1y (formerly *Sby* or *A1s9Y*) was isolated from the *Sxr*^b region independently by two groups (130, 131), and has extensive homology to the human ubiquitin-activating enzyme E1 (136). It is expressed at significant levels only in the testes of normal mice and *Sxr*^a mice, but not *Sxr*^b mice. This observation led to a proposition that the gene was a candidate for *SpY* (130, 131). Thus far, no male-specific *Ube1y* homologous sequences have been found in human or other primate species.

Zfy in mice (*ZFY* in humans) was once considered to be the best candidate for the testis-determining gene, *Tdf* (132), but is now suggested to be involved in germ cell proliferation and development. Expression of the *Zfy-1* and *Zfy-2* genes appear to be testis-specific in the adult

mouse (76, 137, 138), and the block to spermatogenesis in *XOSxr^b* mice has been attributed to the *Sxr^b* deletion which encompasses several loci including the 3' portion of *Zfy-2*, *Spy*, *Hya*, and the 5' portion of *Zfy-1* (78).

Y353/B, originally isolated from a mouse Y chromosome-enriched DNA library and mapped to the long arm of the Y chromosome (133), has recently been proposed as a candidate multiple-copy spermiogenesis gene (112, 139). *Y353/B*-related transcription is detected specifically in round spermatids in adult mouse testes (139). It has been shown that deletions of *Y353/B* lead to increased frequency of morphologically abnormal sperm and, therefore, it is mooted as a candidate gene for spermiogenesis (112). *Y353/B* is a multiple copy gene, but as yet no *Y353/B*-related sequences have been found in humans. A recent study by Lahn & Page (3) has resulted in the isolation of twelve new gene or gene families mapped to the non-recombination region of the Y chromosome, or NRY (Fig. 2). Seven of these genes are Y chromosome specific and only expressed in the testis (Table 4). Although the function of the genes remains to be investigated, it is likely that some are involved in spermatogenesis.

Clinical aspects of Y chromosome deletions

The demonstration that sperm retrieval is possible from the testes of infertile men and that these sperm can be used to achieve fertilization through intra-cytoplasmic sperm injection (ICSI) raises the possibility of genetic transmission of infertility.

The rapid expansion of our knowledge of sequences on the Y chromosome raises the prospect of a closer examination of the chromosome for deletions linked to infertility. Ideally, the assessment of the status of the Yq region should be based on a simple, reliable, reproducible, time- and cost-effective system which allows for the detection of lesions in all suspected AZF regions. With

the advent of sequence tagged sites (STSs), investigations of the Y chromosome by genomic Southern blot analysis were abandoned in favour of PCR procedures. Quicker (both in the performance and acquirement of a result), easier, less hazardous and allowing for more detailed analyses to be conducted, PCR has now become the exclusive means by which patients are screened for deletions of the AZF region/gene(s). However, unlike other PCR-based investigations (e.g. the different α thalassaemia syndromes) (140), where genetic mutations/deletions are detected by the presence of defined amplification products, all AZF screening studies to date identify deletions solely on the absence of a product after three separate amplifications in which DNA from a fertile male yields a fragment of the expected size. Although the likelihood of a deletion being missed by this approach is low (i.e. false amplification resulting in a product of precisely the correct size), reliance on a negative outcome can be misleading, because, despite optimization of the PCR conditions and the incorporation of certain safeguards (e.g. checks of template integrity by GAPDH analysis and the inclusion of female samples to detect exogenous male DNA contamination), PCR can fail. Consequently, unsuccessful amplifications which are due to factors such as the introduction of inhibitory compounds by repeated DNA sampling, variations in the template/primer concentration ratio (i.e. too little template and the primers may not find the complementary sequence, too much may lead to mispriming) or the quality of the template can occur. It has been reported that genomic DNA, when stored for a long time, inexplicably becomes less amenable to amplification of sequences that are larger than 200 bp (141), and therefore could be misinterpreted as evidence of deletions when PCR failed. Consequently, the reporting of deletions based only on the outcome of PCR is prone to overestimation.

The importance of confirmatory steps was shown in the discrepancies found in a recent study (Mallidis C,

Table 4 Genes identified in the non-recombining region of the human Y chromosome.

Gene symbol	Gene name	Tissue expression	Copies on the Y	Homologue location
DBY	Dead box Y	Ubiquitous	Single	X
TB4Y	Thymosin β 4 Y isoform	Ubiquitous	Single	X
EIF1AY	Translation initiation factor 1A Y isoform	Ubiquitous	Single	X
UTY	Ubiquitous TPR motif Y	Ubiquitous	Single	X
DFFRY	<i>Drosophila</i> fat facets-related Y	Ubiquitous	Single	X
CDY	Chromodomain Y	Testis	Multiple	
BPY1	Basic protein Y1	Testis	Multiple	
BPY2	Basic protein Y2	Testis	Multiple	
XKRY	XK related Y	Testis	Multiple	
PRY	PTP-BL related	Testis	Multiple	
TTY1	Testis transcript Y1	Testis	Multiple	
TTY2	Testis transcript Y2	Testis	Multiple	
TSPY	Testis specific protein Y	Testis	Multiple	
RBM	RNA recognition motif	Testis	Multiple	X
DAZ	Deleted in azoospermia	Testis	Multiple	3

Loveland K, McLachlan R, Baker HWG, Bhasin S, deKretser DM, unpublished observations) which utilized PCR and genomic Southern blot analysis to screen for a single STS (that for *DAZ*). After the PCR stage, 74 of the 564 patients (13.2%) were suspected of having a deletion, but after genomic Southern blot analysis to confirm the deletions, the percentage fell to 18 (3.2%), one of the lowest rates thus far reported.

Until we understand the role of *RBM*, *DAZ*, *DDFRY*, *CDY* and the other testis-specific Y located genes, it will be impossible to consider therapeutic options. The value of the action of these genes and their periods of action will influence the nature of potential intervention for therapy. A substantial number of men with deletions in the Yq region have the ability to produce sperm, albeit in relatively small numbers. Without the availability of assisted reproduction techniques it is unlikely that these men would reproduce naturally. Consequently, it is incumbent on the clinician involved in the use of ICSI to counsel these patients about the potential transmission of Y-related infertility to their male offspring.

Although a direct causality between Yq deletions and infertility has not been assigned, nevertheless it would be prudent for patients who are considering ICSI to be investigated for Yq deletions. Screening of infertile men and the detection of an intact Y chromosome does not guarantee against a child having a Y deletion (124), nor is the significance of most deletions understood. However, the information obtained from the analysis is important as it can aid both the clinician and patient to decide on an appropriate course of treatment. This is of particular importance as the decision made may have ramifications beyond fertility, and may not become apparent for a considerable time.

Ultimately, accurate information will only be available to the clinician and patient alike when diagnostic procedures that detect specific gene deletions/mutations of known significance to spermatogenesis are developed. This is predicated on the attainment of a greater understanding of the genetic basis of male infertility. To this end, studies need to be conducted on the various loci whether on the Y chromosome (e.g. testis-specific protein Y coded (142) and the 12 recently identified gene families (3), or those found on autosomes (e.g. cAMP-response element modulator (143), cAMP-response element binding protein (144), stem cell factor (145) and heat shock-protein 70) (146), all of which in gene 'knockout' studies or naturally occurring mutations cause disruption of spermatogenesis and infertility (147–149). The results emerging from target gene disruption studies are adding to the list of genes with a potential impact on human spermatogenesis and infertility.

Conclusion

Male infertility is most likely the result of deletions and/or mutations of one or more of the myriad of genes necessary for spermatogenesis. Since the proposition,

some 24 years ago, that the long arm of the Y chromosome harbours a possible *AZF* region, a multitude of studies have been conducted in search of the elusive factor. The introduction of molecular techniques has provided great insight into the genetics of infertility. Yet, despite recent advances, such as the isolation of several candidate genes, our understanding of the genetic regulation of spermatogenesis remains limited. In particular, we are still unable to establish the precise genotype–phenotype correlation between specific Y chromosome deletions and the various testicular histology patterns seen in infertile men. Using current screening methods, such as PCR screening, Yq deletions are detected in 5–10% of infertile men. This frequency, in all likelihood, is perhaps an underestimate of the true prevalence as many mutations and/or deletions of the Y chromosome might be either too small to be detected, or some deletions affecting male infertility are perhaps located outside the regions which have been studied; therefore, they cannot be detected by the present strategies. Identification of autosomal genes affecting spermatogenesis may eventually answer these questions.

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