

Microdeletions on the long arm of the Y chromosome and their association with male-factor infertility

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Significant advances in treatment have enabled previously infertile males to achieve fatherhood, when only a few years ago they would have had no chance of biological paternity. In contrast to the overall success of assisted reproduction, the aetiology of male-factor infertility is poorly understood. Recent studies have shown, however, that a significant proportion of men with severe infertility have microdeletions of the Y chromosome. Furthermore, reports have shown that male infants conceived through assisted reproductive techniques have inherited the same Y-chromosome microdeletion as their fathers. It has thus become important to screen men who are at risk of Y-chromosome microdeletions, as this will determine if counselling is needed prior to starting infertility treatment. This review examines the significance and limitations of the current understanding of Y-chromosome microdeletions in male infertility.

HKMJ 2000;6:184-9

Key words: Infertility, male/genetics; Oligospermia/genetics; Sequence deletion; Sequence tagged sites; Y chromosome

Introduction

The treatment of male infertility has been revolutionised by the development of intracytoplasmic sperm injection (ICSI). In this procedure, only oocyte quality and individual sperm viability have been shown to affect fertilisation rates.¹ In conventional in vitro fertilisation (IVF), approximately 50000 morphologically normal, motile sperm are used for each oocyte to achieve acceptable fertilisation rates. In contrast, ICSI requires just one viable sperm per oocyte to achieve acceptable fertilisation rates. Palermo et al² have demonstrated that ICSI is an effective method to overcome fertilisation failure in routine IVF. With the development of ICSI, there has been a rapid increase in the number and types of male infertility cases that are now treatable, to the point where only complete testicular failure is not.³ However, the rapid development of disorders being treated with ICSI has not been equalled by a rapid increase in our understanding of these disorders, in particular their aetiology and potential for inheritance.⁴

One in four infertile men will be given a diagnosis of idiopathic or unexplained infertility.⁵ Despite the

fact that the cause of the infertility cannot be identified, in most cases the partners of these men can be treated with ICSI. In effect, this approach treats the disorder with little knowledge of the possible consequences for the patient and/or their potential children.⁴ There is evidence that some cases of male infertility have an underlying genetic basis.⁶ Furthermore, severe male-factor infertility has been associated with a 10-fold increase in the occurrence of chromosomal anomalies, compared with the general male population.⁷ While the most common chromosomal anomalies are those of the sex chromosomes, which may only affect fertility, genetic diseases that are secondarily associated with male infertility may also be transmitted to children derived from ICSI.⁸ For example, the incidence of balanced-translocation carriers, either reciprocal or Robertsonian, is increased in infertile men.⁹ Infertile carriers of balanced translocations may produce unbalanced gametes with the potential for this unbalanced phenotype to be inherited by an ICSI-derived child.⁹ Hence, it has become important to be able to identify individuals who may carry a genetic defect that is responsible for their infertility.⁸

A genetic basis to some cases of male infertility was first demonstrated after microscopically visible deletions of the Y chromosome were observed in chromosomal spreads from infertile men in 1976.¹⁰ More recently, discrete microdeletions of the long arm of the Y chromosome (Yq) have been found in some azoospermic and oligospermic men.¹¹ A review by

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Simoni et al¹² suggested that the overall incidence of Yq microdeletions is more than 7% in infertile men. This conclusion supports the view of Pryor et al¹³ that Y-chromosome deletions constitute a major aetiological category of male infertility, second only to varicocele. This review aims to summarise the main findings from studies of Yq microdeletions that are associated with infertility, and to highlight the significance and limitations of such investigations.

Identification of Yq microdeletions

The literature contains more than 30 reports on Yq microdeletions in infertile men, and the number of infertile men screened for Yq microdeletions is greater than 3000. The reported incidence of Yq microdeletions in severe male-factor infertility ranges from 1% to 55.5%.^{8,13-34} All of these studies used a polymerase chain reaction (PCR)-based screening approach involving small sequence-tagged sites (STSs) in Yq11 as specific genomic Y-chromosome markers.³⁵ Vollrath et al³⁵ established the first STS interval map of the human Y chromosome, based solely on PCR analysis. By screening a panel of 96 individuals with cytogenetically visible Y-chromosome deletions with 110 STS loci in Yq11, Vollrath et al³⁵ subdivided Yq11 into 23 intervals, termed 5A to 5Q and 6A to 6F. Vogt et al¹⁵ established another STS deletion map of Yq11, dividing it into 25 intervals (D1-D25). Most of the studies of Yq microdeletions are based on the maps from these two groups.^{8,13-34} The difference in their methodologies relates to the number of STSs screened, which ranges from one to 131.^{16,18} If a small number of STSs are used to screen patient samples, there is a risk of missing some deletion sites. Of equal concern is that if a large number of STSs are used, they may contain polymorphic sequences, which are deleted in normal fertile men.¹⁴ The Yq region has been shown to contain a number of neutral deletion polymorphisms in normal fertile men.³⁶ Kent-First et al¹⁴ performed an extensive study of 112 Yq STSs in 920 proven fertile males who were tested for polymorphism and for reliability in the PCR assay. Sixty-nine STSs were shown to be non-polymorphic and reliable in the assay; they were then used to study samples from 514 infertile men.¹⁴ It was determined that a subset of 49 of the 69 STSs would have detected all of the non-polymorphic STS deletions.¹³ The study by Kent-First et al¹⁴ was the first attempt to determine which of the STSs and how many of them are required for an accurate screening protocol.¹⁴

Three studies have shown that when a Yq microdeletion is present in infertile men, ICSI-derived sons

will inherit the same deletion.^{8,37,38} One study looked for Yq microdeletions in ICSI-derived sons, regardless of the father's Yq status and found two cases in which the son had a microdeletion on Yq but the infertile father did not.⁸ The most probable explanation is that the infertile fathers were mosaic for Yq deletions and that the proportion of DNA that contained the deletion was not detectable using currently available methods.⁸

Using PCR, the STS products are amplified in an exponential manner. Hence, if a proportion of the DNA being screened had an intact copy of the STS, it would mask the detection of any deleted copies.⁸ This concept is also important for understanding how infertile men acquired the microdeletions. Edwards and Bishop³⁹ screened the fathers of infertile men who showed Yq microdeletions, but the majority did not show the same deletions. This result suggests that the microdeletions arose *de novo* in the infertile men during embryogenesis or from a meiotic error in the germ line of the presumably fertile father.³⁹ However, mosaicism for Yq microdeletions can occur,⁸ and it is possible that the fathers of some infertile men may in fact carry Yq microdeletions in a proportion of their cells. A number of fathers of infertile men who have Yq microdeletions have also shown the same or smaller deletions.^{13,15,17,19} In most of these cases, the fathers experienced long periods of infertility before conceiving their son.¹³

An interesting family history has been presented, which shows that a father and his four sons (confirmed by paternity tests) all had Yq microdeletions.⁴⁰ The father, now aged 63 years, had azoospermia and an elevated level of follicle-stimulating hormone, although he conceived five children—four sons and one daughter—in a period of 14 years.⁴⁰ All four sons had severe infertility. The father's 44-year-old brother also had azoospermia, with 16 years of infertility, but he did not have the Yq microdeletion. This was the first report of vertical transmission of a Yq microdeletion to multiple offspring and indicates that the presence of a Yq microdeletion is not an absolute marker for infertility.⁴⁰

Genotype-phenotype correlation of Yq microdeletions

An early study by Tiepolo and Zuffardi¹⁰ described the existence of a locus for spermatogenesis in the euchromatic part of Yq (Yq11), named azoospermic factor (*AZF*). The *AZF* region has been further divided into four non-overlapping regions *AZFa*, *AZFb*, *AZFc*, and *AZFd* (Fig).¹⁴ The regions were identified from

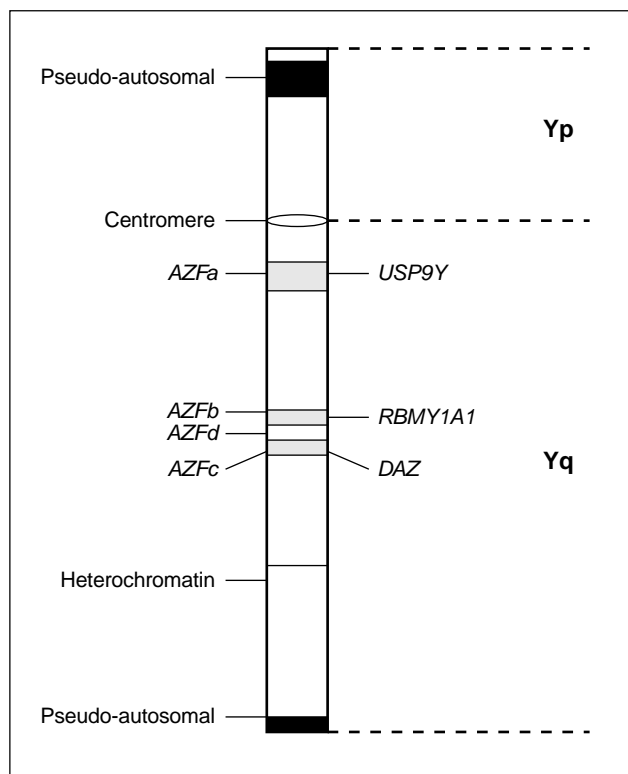


Fig. Schematic diagram of the Y chromosome, showing the AZF loci and their candidate genes

studies showing that clusters of deletions occurred within each one.^{14,15,41} Early studies attempted to assign specific infertility phenotypes to each region—for example, *AZFa* deletions would result in Sertoli cell-only syndrome, *AZFb* deletions would result in spermatogenic arrest, and deletions in *AZFc* would result in some spermatogonia being present.¹⁵ Subsequent studies, however, showed that these associations could not be made.^{13,14,16} Pryor et al¹³ showed that men with mild oligospermia and normal sperm counts but abnormal sperm morphology can have microdeletions in either *AZFa*, *AZFb*, or *AZFc* loci. Although a definitive genotype-phenotype correlation is not known for Yq microdeletions, it is generally accepted that large deletions that span multiple *AZF* regions or those restricted to *AZFa* usually result in Sertoli cell-only syndrome or severe oligospermia.¹⁴ Those microdeletions restricted to *AZFb* or *AZFc* can result in a range of phenotypes from Sertoli cell-only syndrome to moderate oligospermia,¹⁴ whereas microdeletions restricted to the *AZFd* region may present with mild oligospermia or even normal sperm counts with abnormal sperm morphology.¹⁴

The evidence provided by deletion studies indicates that at least three and possibly four distinct functional regions exist in the Yq region. But direct phenotype-genotype correlations cannot currently be made, which is not surprising when considering that the deletions

identified vary in size and thus may interrupt one or more genes present in each region. The estimated sizes of each region are: 1 Mb for *AZFa*, 1.5 Mb for *AZFb*, and 3 Mb for *AZFc*.¹⁵ Consequently, each region is large enough to contain a number of candidate genes that may be disrupted by the microdeletions observed in infertile men.

Candidate genes for spermatogenesis

A number of candidate genes for AZF have been described. However, only a few genes or gene families have been studied in detail. These include the ‘deleted in azoospermia’ (*DAZ*),¹⁶ RNA-binding motif for Y-located RNA (*RBMY1A1*)—formerly known as Y-chromosome RNA recognition motif (*YRRM*),⁴² and the Y-linked homologue of the *Drosophila* fat facets-related X gene (*USP9Y*, formerly *DFFRY*).⁴³

The *DAZ* gene family is reported to be the most frequently deleted AZF candidate gene and is located in the *AZFc* region.¹⁶ Originally thought to be a single-copy gene, *DAZ* is now known to be a multicopy gene family, which includes *DAZ2*, formerly known as spermatogenesis gene on Y (*SPGY*),¹³ and its autosomal copy on the short arm of chromosome 3 (*DAZLI*).¹³ The *DAZ* genes are expressed exclusively in testicular tissue and encode proteins that contain an RNA-recognition motif, thereby suggesting that they have a regulatory role in RNA metabolism.⁴⁴ They share a high percentage of sequence homology with the mouse gene *Dazla*⁴⁵ and the *Drosophila* gene *Boule*,⁴⁶ and it has been hypothesised that *DAZ* has been conserved throughout evolution and performs similar roles as *Dazla* and *Boule*, which seem to regulate the meiotic cell cycle.⁴⁶ This would mean that all men with *DAZ* deletions would be incapable of producing mature sperm; however, some men with oligospermia have been shown to carry *DAZ* deletions.^{8, 13,14}

Saxena et al⁴⁷ have shown that the *DAZ* gene family has evolved only recently on the Y chromosome during primate evolution and that it originates from an autosomal homologue on the short arm of chromosome 3. Immunostaining studies using antibodies to *DAZ2* have shown that *DAZ* proteins are present in the innermost layer of the male germ-cell epithelium and in the tails of mature sperm.⁴⁴ Habermann et al⁴⁴ have hypothesised that the *DAZ* protein in the germ-cell epithelium regulates the storage or transport of testis-specific messenger RNA (mRNA) in late spermatids. The originally proposed function of *DAZ* genes needs to be re-evaluated and further studies performed before their specific function can be elucidated.⁴⁴

The first *AZF* candidate gene to be isolated was *RBMY1A1*.³⁷ This gene family consists of 20 to 50 genes and pseudogenes that are distributed over both arms of the Y chromosome. The multicopy nature of *RBMY1A1* has made it difficult to assign the gene with a specific function in spermatogenesis.⁴⁸ An immunohistochemical study has shown that the RBMY1A1 protein is localised in the nucleus of human male germ cells, specifically to the *AZFb* region of the Y chromosome.⁴⁹ The *RBMY1A1* gene cluster in the *AZFb* region may thus contain the only functional copies of the *RBMY1A1* gene, and their presence in male germ cells indicates their testis-specific expression.⁴⁹ The *RBMY1A1* genes are members of the heterogeneous nuclear ribonucleoprotein G (hnRNP G) family of proteins, which are associated with nuclear polyadenylated RNA and believed to function in pre-RNA packaging, mRNA transport to the cytoplasm, and RNA splicing.⁵⁰ The *RBMY1A1* gene family has evolved from an hnRNP G-like ancestor and was copied to the Y chromosome before the diversification of mammals, as it is also present on the Y chromosome of marsupials.⁵¹ The evidence that *RBMY1A1* genes have been conserved on the Y chromosome throughout evolution and are expressed specifically in male germ cells indicates an important role in spermatogenesis.⁵² However, the relatively low frequency of deletions in *RBMY1A1*, compared with the frequency of deletions in the *DAZ* genes, appears to support the hypothesis of a rather minor role for *RBMY1A1* genes in controlling spermatogenesis.⁵³

The first major gene identified as an *AZF*a gene was *USP9Y*.⁴³ Considerably less is known about *USP9Y* than the *DAZ* and *RBMY1A1* gene families. Initially, only three infertile men were identified with *USP9Y* deletions; however, all had the entire *AZF*a region deleted, and it is possible that other, unidentified genes may have also been involved in their infertility.⁴³ The *USP9Y* gene shows homology to an X-linked gene *DFFRX*, which appears to function as a carboxyl-terminus ubiquitin hydrolase.⁴³ Unlike *DAZ* and *RBMY1A1*, the *USP9Y* protein is not confined to the testis, but is as ubiquitously expressed as the *DFFRX* protein.⁴³ A recent study found 12.5% of azoospermic men showed deletions of *USP9Y*, including one individual in whom the deletion was restricted to the *USP9Y* gene.⁵² Sun et al⁵³ have found a de novo mutation in the *USP9Y* gene, in which four base pairs are deleted from a splice donor site. This deletion would cause an exon to be skipped and the protein to be truncated.⁵³ While *USP9Y* is a functional candidate for the *AZF*a region, the exact function that *USP9Y* may play in spermatogenesis remains to be demonstrated.

Another two X-Y homologous genes have been mapped to the *AZF*a region: *DBY* (dead box on Y) and *UTY* (ubiquitous transcribed teratopeptide repeat gene on the Y chromosome), both of which are expressed as ubiquitously as *USP9Y*.⁵⁴ The centromere to telomere order of the genes—*USP9Y*, *DBY*, and *UTY*—is conserved between the mouse and human, thus suggesting an ancient organisation of these genes on the Y chromosome that predates the divergence of the human and mouse lineages.⁵⁵ Sargent et al⁵⁵ have isolated another potential spermatogenesis gene in the *AZF*a region—namely, *AZF*aT1. The expression of *AZF*aT1, *USP9Y*, *UTY*, and *DBY* have been examined in four patients with *AZF*a microdeletions.⁵⁵ In three of the patients *AZF*aT1, *USP9Y*, and *DBY* were deleted, but *UTY* was intact; all four patients had the Sertoli cell-only syndrome. The fourth patient, however, had only *AZF*aT1 and *USP9Y* deleted but retained the *DBY* and *UTY* genes; this patient had a milder oligospermic phenotype.⁵⁵ These results suggest that the deletion of *AZF*aT1 and/or *USP9Y* results in a less severe phenotype and the additional deletion of *DBY* is required for the Sertoli cell-only syndrome.⁵⁵

Other genes have been isolated from the Y chromosome and are expressed specifically in the testis, but lie outside of the *AZF* a, b, and c regions.⁵⁴ These are *BPY1*, *CDY*, and *XKRY* from the Yq arm and *PRY*, *TSPY*, *TTY1*, and *TTY2* from the Yp arm.⁵⁴ Hence, many genes are likely to play critical roles in the control of spermatogenesis.

Clinical application of Yq screening

The aetiology of many kinds of male infertility is still poorly understood. The study of Yq microdeletions will help in the development of better diagnostic methods and the expansion of the current knowledge of spermatogenesis. Many factors, including the many repetitive sequences on the Y chromosome, complicate the interpretation of the results from Yq microdeletion assays and the study of candidate genes that have critical functions in spermatogenesis.³⁶ Nevertheless, it is generally believed that men with severe male infertility should be screened for Yq microdeletions as a part of their pretreatment investigations.¹² This step is particularly important, because ICSI-derived sons are most likely to inherit the Yq microdeletion, which may result in subsequent infertility.^{8,38,39}

As more reproductive medicine laboratories start undertaking the Yq microdeletion assay, it is apparent that methods will vary greatly, particularly in the number of STSs used and the verification of deletions

seen.¹² Approximately 43 STSs may be enough to identify all the known deletion sites associated with male infertility.¹⁴ Included in this selection is the *RBMY1A1* locus, which is a member of a family of genes that is located on different sites on both arms of the Y chromosome. A deletion detected in this locus must be verified by a Southern blot assay¹⁴ In addition, all deletions of a single STS should be verified by Southern blot assay to rule out failed PCR amplification.¹² By regulating the methods used, men with infertility will receive accurate reports on which they can make informed decisions regarding their future treatment options.

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