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Transmission of male infertility to future generations: lessons from the Y chromosome*

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The introduction of ICSI and testicular sperm extraction (TESE) has allowed many infertile men to father children. The biggest concern about the wide use of these techniques is the health of the resulting offspring, in particular their fertility status. If the spermatogenic defect is genetic in origin, there is potential risk of transmitting this defect to future offspring. The most frequently documented genetic cause of male infertility is a Y chromosome deletion. The Y chromosome has acquired a large number of testis-specific genes during recent evolution, and deletions causing infertility take out a number of these genes. These deletions have been shown to be transmitted to 100% of male offspring. Also, absence of an aberration on the Y chromosome does not rule out a genetic cause of the infertility phenotype, as there are many other genes involved in spermatogenesis elsewhere in the genome, and current mapping techniques—especially on the Y chromosome—can miss many aberrations. More detailed studies of these spermatogenesis genes, which are now possible because of more precise sequence-based mapping, will lead to improved understanding of the genetic basis of male infertility and enable proper counselling of patients undergoing ICSI in the future.

Keywords: ICSI/male infertility/TESE/transmission/Y chromosome

TABLE OF CONTENTS

Introduction

Men with minute amounts of spermatozoa (oligozoospermia) or no spermatozoa at all in their ejaculate (azoospermia) are generally unable to father children through natural conception. However, with the development and popularization of ICSI and testicular sperm extraction (TESE), these men are now able to have children. Although recent reports do not show any significant difference between children conceived after ICSI and their naturally conceived peers in terms of physical health and development, the fertility status of these children is not known as the oldest children are yet to enter puberty (Sutcliffe, 2000; Aboulghar *et al*., 2001; Bonduelle *et al*., 2001; Sutcliffe *et al*., 2001). As many cases of male infertility are likely to be of genetic origin, the potential risk of transmitting infertility to future generations is of great concern.

Almost concurrently with the development of ICSI, much more is being discovered about the genetic causes of male infertility. Most research has focused on genetic aberrations of the Y chromosome. This specialized male chromosome contains many genes that are involved in spermatogenesis, and deletions involving these genes are often found in infertile males. These deletions have in fact been shown to be transmitted to male offspring via ICSI, presumably causing fertility problems in these children later in life. Future studies of genes involved in spermatogenesis, not only on the human Y chromosome but also on other chromosomes as well as in other species, will surely lead to a better understanding of the genetic causes of male infertility and enable proper counselling of couples undergoing assisted reproductive techniques (ART) such as ICSI.

The purpose of this review was to describe and explain the accumulating molecular data on Y chromosomal spermatogenesis

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genes, their role in male infertility and the transmission of deletions involving these genes to ICSI offspring. Furthermore, these data will be put in perspective of other, yet to be discovered infertility genes that are presumably widespread throughout the genome and may also be involved in transmitting infertility to future generations.

Treatment of male infertility

Until a decade ago, there were no treatment options for infertile couples when the male had severely impaired spermatogenesis. In fact, there are still no clinical therapies to correct deficient spermatogenesis (Rodriguez-Rigau *et al*., 1978; Nilsson *et al*., 1979; Baker *et al*., 1981, 1984, 1985; Schoysman and Gerris, 1983; Baker and Kovacs, 1985; Baker, 1986; Vermeulen *et al*., 1986; Silber, 1989, 2001a; Nieschlag *et al*., 1995, 1998; Devroey *et al*., 1998). Since the introduction of ICSI at the Brussels Dutch-Speaking Free University in 1992, however, there has been a revolution in our thinking about male infertility (Palermo *et al*., 1992; Van Steirteghem *et al*., 1993). Infertile couples with the most severe cases of male infertility, even with apparently 100% abnormal morphology and even just rare spermatozoa in the ejaculate, can now have pregnancy and delivery rates not apparently different from conventional IVF with normal sperm (Liu *et al*., 1994, 1995; Nagy *et al*., 1995).

In 1993, TESE and microsurgical epididymal sperm aspiration (MESA) in conjunction with ICSI were introduced for the treatment of obstructive azoospermia (Schoysman *et al*., 1993; Devroey *et al*., 1994, 1995a; Silber *et al*., 1994, 1995a; Tournaye *et al*., 1994). Later on, TESE was also found to be effective for many cases of non-obstructive azoospermia (Devroey *et al*., 1995b; Silber *et al*., 1995b, 1996, 1998). Approximately 60% of azoospermic men with presumably no sperm production at all have a minute amount of sperm production in the testis that is not quantitatively sufficient to spill over into the ejaculate, but which is adequate for ICSI (Steinberger and Tjioe, 1968; Clermont, 1972; Zukerman *et al*., 1978; Silber and Rodriguez-Rigau, 1981; Silber *et al*., 1995b, 1995c, 1996, 1997a,b, 2001a; Silber 2000). Thus, even men with spermatogenesis so deficient in quantity that no sperm at all can reach the ejaculate, could now have children with the use of TESE-ICSI (Table I) (Silber *et al*., 2001b). In fact, the pregnancy rates for these TESE cycles are more related to the age of the wife and her ovarian reserve than to either sperm count or sperm quality (Silber *et al*., 1997b; Silber 1998a).

It is with these cases of non-obstructive azoospermia and severe oligozoospermia that the greatest concern has been registered for the well-being of offspring generated by ICSI. If severe oligozoospermia or azoospermia is of genetic origin, then ICSI may create a potential problem of proliferation of male infertility in future generations (Silber, 1998b). In fact, it is estimated that if one-half of all azoospermic men were to undergo ICSI, the incidence of male infertility would double within seven generations (Faddy *et al*., 2001).

Genetic causes of male infertility: role of the Y chromosome

For several decades, it has been speculated that there is a genetic aetiology to many cases of male infertility (O'Brien *et al*., 1986, 1987; Short, 1995). The first reports with data supporting this theory involved cytogenetic studies that showed structural chromosomal abnormalities in infertile males (Jacobs and Strong, 1959; Kjessler, 1966; Olson and Magenis, 1988; Jacobs *et al*., 1992). These structural chromosomal abnormalities, such as translocations and inversions, are found in approximately 2% of infertile males (Bonduelle *et al*., 1995, 1996, 1998a,b, 1999, 2001; Van Assche *et al*., 1996; Tuerlings *et al*., 1998; Egozcue *et al*., 2000). The most common chromosomal abnormalities in azoospermic men are abnormalities involving the sex chromosomes, which are found in approximately 4% of these men. Klinefelter's syndrome, in which patients show a 47,XXY karyotype, is the most frequent form. Due to these structural chromosomal abnormalities, proper chromosome pairing during meiosis is impaired, giving rise to spermatogenic disruption and the infertility phenotype (Chandley, 1979).

The percentage of male infertility that can be explained by karyotyping alone is low, this being mainly due to the low resolution of cytogenetics studies. It was not until the development of modern molecular techniques such as polymerase chain reaction (PCR) that genetic causes of male infertility could be studied with much greater detail. Since then, many more genetic abnormalities, such as micro-deletions and point mutations, have been described in infertile males, with most research focusing on the role of genes on the human Y chromosome. As these detailed genetic studies have only been conducted for less than a decade, many cases of male infertility are still diagnosed as idiopathic, and it remains to be determined what percentage of these are, in fact, genetic in origin.

Suspicion of involvement of the Y chromosome in male infertility originally arose from cytogenetic evidence reported over 25 years ago (Tiepolo and Zuffardi, 1976). This study showed grossly obvious terminal Y chromosome deletions in a very small percentage (5 out of 1170; 0.5%) of azoospermic men who were otherwise phenotypically normal. It was then postulated that the Y chromosome contained a so-called *Azoospermia Factor* gene (*AZF*). Since then, an intense search has ensued for these *AZF* genes, i.e. genes which control spermatogenesis and which may be defective in otherwise normal but infertile males. During the mid-1990s, the long arm of the Y chromosome was shown to contain not one but at least three distinct deletion intervals which were subsequently named *AZFa*, *AZFb* and *AZFc* (Vogt *et al*., 1996). As molecular techniques used to identify these microdeletions had much greater resolution than cytogenetics, more than 0.5% of azoospermic men were now shown to have genetic defects of their Y chromosome (Figure 1)

The deletion frequency of one or more of these regions on the Y chromosome in men with azoospermia or severe oligozoospermia is approximately 5–15%, depending on the phenotypic criteria of the studied population of infertile males (Reijo *et al*., 1995, 1996; Silber *et al*., 1998; Kuroda-Kawaguchi *et al*., 2001). Many laboratories throughout the world have reported on these sub-microscopic deletions of the Y chromosome in azoospermic and severely oligozoospermic men (Ma *et al*., 1993; Bhasin *et al*., 1994; Kent-First *et al*., 1996, 1999; Morris and Gleicher, 1996; Najmabadi *et al*., 1996; Nakahori *et al*., 1996; Prosser *et al*., 1996; Qureshi *et al*., 1996; Vogt *et al*., 1996, 1997; Elliott *et al*., 1997; Foresta *et al*., 1997, 1998; Girardi *et al*., 1997; Kremer *et al*., 1997, 1998; Mulhall *et al*., 1997; Pryor *et al*., 1997; Simoni *et al*.,

1997; Van der Ven *et al*., 1997; Vereb *et al*., 1997; Chai *et al*., 1998; Grimaldi *et al*., 1998; Liow *et al*., 1998; Oliva *et al*., 1998; Stuppia *et al*., 1998; Vogt, 1998; Chang *et al*., 1999; Kim *et al*., 1999; Krausz *et al*., 1999; Seifer *et al*., 1999; Cram *et al*., 2000; Van Landuyt *et al*., 2000; Krausz and McElreavey, 2001; Van Golde *et al*., 2001). In fact, deletion screening of the Y chromosome is now considered standard practice for severely oligozoospermic and azoospermic patients undergoing assisted reproduction in most countries in the world. There is, however, still much debate on which patients to test and which specific markers, known as sequence tagged sites (STS), to use (Foresta *et al*., 2001; Liow *et al*., 2001; Simoni, 2001).

Table I. Results of ICSI in Y-deleted versus non-Y-deleted men with severe oligozoospermia or non-obstructive azoospermia. (Adapted from Silber *et al*., 2001b.)

	Y-deleted ($<2\times10^6$/ml)	Non-Y-deleted ($<2\times10^6$/ml)
Patients	23	205
Cycles	45	312
Oocytes	508	3291
Fertilization (2PN)	289 (57)	1849 (56)
Pregnancies	17 (38)	112 (36)
Ongoing pregnancies	13 (29)	81 (26)
Babies	18	99
Boys	10	43
Girls	8	46

Values in parentheses are percentages.

AZFa

The *AZFa* region differs from *AZFb* and *AZFc* because of its non-repetitive structure and its low deletion frequency. *AZFa* deletions are very uncommon and only a few patients have been described (Qureshi *et al*., 1996; Vogt *et al*., 1996; Pryor *et al*., 1997; Brown *et al*., 1998; Sargent *et al*., 1999; Sun *et al*., 1999). However, studies of this region are highly useful in understanding the genetic basis of male infertility. The *AZFa* region spans approximately 800 kilobases (kb) and contains two functional single-copy genes, i.e. *USP9Y* and *DBY* (Brown *et al*., 1998; Sun *et al*., 1999). Sequencing of these two genes in 576 infertile men and 96 fertile men revealed a de-novo point mutation in *USP9Y* in one case (a four base-pair deletion in a splice-donor site, causing an exon to be skipped and protein truncation). This mutation was absent in fertile relatives and represented the first and only case of a point mutation causing a single gene defect on the Y chromosome associated with spermatogenic failure (Sun *et al*., 1999). The lack of sequence repeats which plague the rest of the Y chromosome, made this particular region of the Y amenable to such a mutation search. Because almost all other spermatogenesis genes on the Y chromosome, such as those in *AZFb* and *AZFc*, are multicopy, searching for point mutations in these genes is virtually impossible. It is also questionable whether point mutations in a single gene of a gene family will give rise to a severe infertility phenotype as the remaining intact copies of the

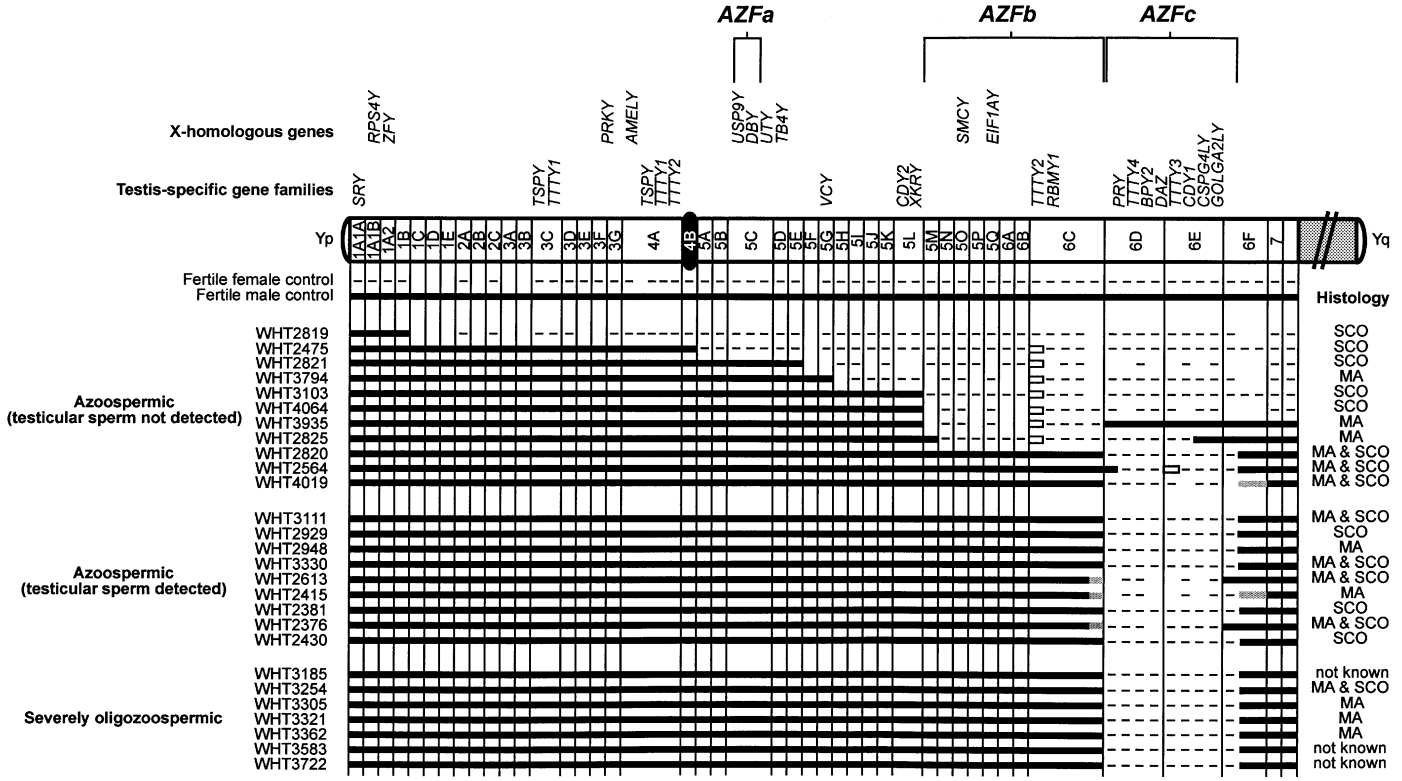

Figure 1. Deletion map of azoospermic and severely oligozoospermic men with associated phenotypes. Black bars indicate confirmed presence; minuses indicate conformed absence; grey bars indicate presumed presence but no material remaining to test; white boxes indicate disregarded positives because of similar sequences elsewhere on the Y and surrounding negative markers. Multicopy testis-specific gene families are shown as single copy for illustration purposes. Testicular histology for each patient is shown on the right. SCO = Sertoli cell-only; MA = maturation arrest. (Adapted from Silber *et al*., 2001a.)

gene could potentially compensate for the loss of function of the mutated gene.

The *AZFa* region also provides a good model for the interaction and overlapping functions of multiple genes which sheds light on the polygenic nature of the genetic control of spermatogenesis. When the entire *AZFa* region is deleted, taking out both *DBY* and *USP9Y*, the spermatogenic defect is severe and the patient is always azoospermic. In contrast, when only one gene is affected, such as in the patient with loss of *USP9Y* function due to a specific point mutation, this results in a less severe phenotype of maturation arrest with a few pachytene spermatocytes developing into mature sperm in some seminiferous tubules. Thus, the loss of *DBY* (the only other gene in the *AZFa* region) likely exacerbates the spermatogenic consequences of the loss of *USP9Y*. This finding in the *AZFa* region runs parallel to previous observations that larger Y deletions (which take out more genes) are associated with a lesser likelihood of finding sufficient sperm for ICSI (Figure 1) (Silber *et al*., 1998).

AZFb

Deletions of the *AZFb* region are slightly more common than deletions of the *AZFa* region but are still found in only a few percent of azoospermic men (Vogt *et al*., 1996; Brandell *et al*., 1998; Kim *et al*., 1999; Martinez *et al*., 2000). Interestingly, all men with deletions of *AZFb* described to date are azoospermic and show complete absence of spermatozoa in the testis (Vogt *et al*., 1996; Brandell *et al*., 1998). Therefore, similar to *AZFa* deletions, there are no reports on transmission of an *AZFb* deletion to ICSI-offspring. One of the candidate genes for the *AZFb* region is *RBMY*, which is present in multiple copies on both the short and the long arm (Foote *et al*., 1992; Ma *et al*., 1992, 1993; Kobayashi *et al*., 1994; Elliott *et al*., 1997; Lahn and Page, 1997). Several observations suggest, however, that all functionally active *RBMY* genes are clustered within the *AZFb* region and that all other genes are in fact pseudogenes (Chai *et al*., 1997, 1998; Elliott *et al*., 1997). The *AZFb* region is located distal to *AZFa* and just proximal to *AZFc* (Figure 1) (Affara *et al*., 1996; Vogt *et al*., 1996). Because of the presence of multiple sequence repeats within this region, in contrast to *AZFa*, efforts to define the *AZFb* region precisely have been hampered. Therefore, the exact content and extent of the *AZFb* region remain to be determined.

AZFc

The most commonly deleted and best-studied region on the Y chromosome is the *AZFc* region. Deletion of the *AZFc* region is found in approximately 12% of azoospermic men and in 6% of severely oligozoospermic men (Reijo *et al*., 1995, 1996; Silber *et al*., 1998; Kuroda-Kawaguchi *et al*., 2001). Recently, the complete nucleotide sequence of the *AZFc* region was published, revealing an extraordinary structure and genetic composition (Kuroda-Kawaguchi *et al*., 2001). The region is constructed from massive areas of absolute sequence identity called amplicons which are arranged in direct repeats, inverted repeats or palindromes. The *AZFc* region spans 3.5 Mb and contains seven separate families of genes with a total of 19 transcription units that are all exclusively expressed in the testis. Interestingly, nullisomy of this large 3.5-Mb *AZFc* region of the Y chromosome seems to have no other effect except upon spermatogenesis exemplifying the specialized function of this region (Kuroda-Kawaguchi *et al*., 2001).

The *DAZ* gene family, which is one of the seven gene families located within *AZFc*, was one of the first spermatogenesis genes identified on the human Y chromosome (Reijo *et al*., 1995; Saxena *et al*., 1996). The human *DAZ* genes were shown to be transcribed specifically in spermatogonia and in early primary spermatocytes (Menke *et al*., 1997). Interestingly, homologues of *DAZ* in other species were also shown to be involved in control of spermatogenesis, supporting an essential role of this gene in male fertility in humans as well. Homologues of *DAZ* have been found in *Drosophila* (termed *Boule*), in mice (termed *Dazl*), in frogs (termed *Xdazl*) and even in worms (termed *daz-1*) (Hackstein and Hochstenbach, 1995; Cooke *et al*., 1996; Eberhart *et al*., 1996; Karashima *et al*., 1997; Ruggiu *et al*., 1997; Houston *et al*., 1998). In contrast to humans, the *DAZ* gene in these other species is single copy and located on an autosome rather than on the Y chromosome. In the human, *DAZ* is present in four near-identical copies (99.9% homology) arranged in two clusters with two genes each (Saxena *et al*., 2000; Kuroda-Kawaguchi *et al*., 2001).

The human also retains an autosomal homologue of *DAZ* called *DAZL*, which is located on chromosome 3 (Saxena *et al*., 1996). During evolution, some time after the split of Old and New World monkeys approximately 30 million years ago, the *DAZL* gene was transposed to the Y chromosome (Figure 2). Once *DAZL* was transposed to the Y, it was amplified and pruned until it became the modern-day *DAZ* gene family. It was shown recently that in fact there is yet another *DAZ* family homologue in humans, termed *BOULE*, that resembles the fly homologue *boule*, even more closely than *DAZ* or *DAZL* (Xu *et al*., 2001). The exact interaction and possible functional overlap between these three members of this interesting gene family remains to be determined (Moore *et al*., 2001).

Although all *AZFc* deletions seem to be identical on a genomic scale, men with these deletions show a high diversity of spermatogenic failure in contrast to men with deletions involving either *AZFa* or *AZFb*, who are always azoospermic (Vogt *et al*., 1996; Silber *et al*., 2001a). Some men with an *AZFc* deletion are azoospermic and have no sperm in the testis, while others are only oligozoospermic (Figure 1). The specific histological defect in the testis, whether maturation arrest or Sertoli-cell-only, is also quite variable. It is therefore likely that other stochastic factors, either genetic or environmental in origin, contribute to the specific phenotype. Now that the entire *AZFc* region has been sequenced, the precise function of the genes located in this region—as well as their interaction with other genes—can be investigated further.

Most *AZFc* deletions are *de novo*, but they may occasionally be present in the 'fertile' father of the infertile male, as illustrated by two reports showing natural transmission of *AZFc* deletions from apparently fertile men (Chang *et al*., 1999; Saut *et al*., 2000). However, in these cases, the father may very well have been oligozoospermic, as 5% of even severely oligozoospermic men can father children without any infertility treatment (Silber, 2001b).

Deletions of the entire *AZFc* region result in loss of all four *DAZ* copies. Recent reports however, indicate that deletions involving only some of the *DAZ* genes are also found in infertile

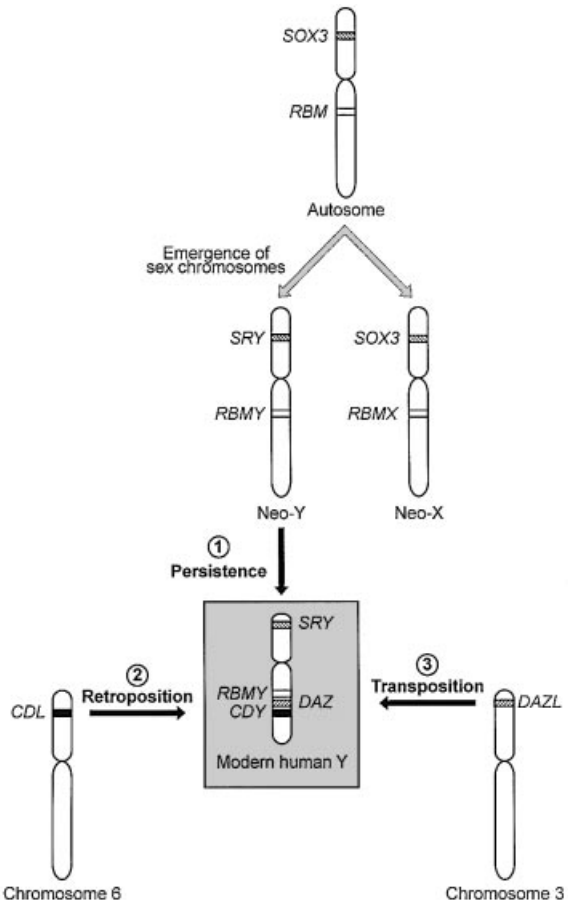

Figure 2. Evolution of the Y chromosome and acquisition of spermatogenesis genes. The Y developed from a pair of autosomes after the emergence of the sex-determining gene *SRY*. Subsequently, male-specific genes were acquired by three mechanisms: 1 persistence of genes from the original autosome (*RBMY*); 3 transposition of a block of autosomal DNA (*DAZL*); and 2 retroposition of autosomal RNA (*CDY*). (Adapted from Lahn and Page, 1999b.) With permission *Nature Genet*., **21**, 429–443.

males (Moro *et al*., 2000; Bienvenu *et al*., 2001; de Vries *et al*., 2002). In fact, some of these smaller deletions are found in men with only mild oligozoospermia, indicating a possible gene dosage effect, i.e. men with a deletion of only two *DAZ* genes are less affected than men with a deletion of all four copies (de Vries *et al*., 2002). These data illustrate that infertility is a complex multigenic disorder and that disruption of different genes or disruption of some genes of a gene family can result in different degrees of spermatogenic failure.

Although most deletions on the Y chromosome that are causative in male infertility fall into the categories of *AZFa*, *AZFb* or *AZFc*, there are also studies describing other deletions. However, because of the highly polymorphic nature of the Non-Recombining region of the Y (NRY), there are many 'Y deletions' that are in fact polymorphisms and therefore of no functional consequence. Only if these deletions are present in infertile males and not in normal controls, can they be implicated as a cause of infertility. Moreover, if the consequence of a deletion is not necessarily azoospermia but a less severe phenotype such as oligozoospermia—as seems to be the case in patients with a deletion of only two *DAZ* copies—the deletion can even be present in the 'fertile' father of the patient. As fertility is a measure of a couple and not of male gametogenesis only, a semen analysis of the 'fertile' father should be performed to see if he truly is 'unaffected', and a control population should always consist of males with normal spermatogenesis.

Mechanism of de-novo Y chromosome deletions

Research into deletions involving *AZFa* and *AZFc* has revealed interesting data on the mechanism underlying the occurrence of these deletions. Deletions of both *AZFa* or *AZFc* seem to be caused by illegitimate homologous recombination between highly similar or identical sequences which are found on the Y in great abundance. The *AZFa* region is bounded on each side by two sequence stretches of approximately 10 kb which are 94% identical to each other (Blanco *et al*., 2000; Kamp *et al*., 2000; Sun *et al*., 2000). Homologous recombination between these two sequence stretches results in dropout of the intervening *AZFa* region. Interestingly, these two sequence stretches belong to the HERV15 class of endogenous retroviruses, suggesting that viral sequences, which are ubiquitous in the human genome, can form potential targets for deletions. Although all *AZFa* deletions fall within this 10 kb region, the precise breakpoints can differ slightly between patients.

The sequence of *AZFc* reveals the same mechanism as for *AZFa*, but on a grander scale. In this case the substrates for homologous recombination are two repeats that are over 99.9% identical and are 229 kb in length (Kuroda-Kawaguchi *et al*., 2001). Precise breakpoint determination for *AZFc* deletions is virtually impossible because of the extremely high similarity between the two flanking repeats. Interestingly, the frequency with which these deletions occur seems to correspond to the length of the stretch of homology. Deletions of *AZFc*, caused by homologous recombination between 229 kb repeats, are far more common than deletions of *AZFa* which are caused by repeats of only 10 kb in length. The very repetitive nature of the Y chromosome, that made sequencing and finding small deletions or point mutations so difficult, also seems to be the cause of its instability over an evolutionary time frame, as well as in our current infertile patient population.

Evolution and genetic constitution of the human Y chromosome

What makes the Y chromosome, despite its confounding repeats, polymorphisms, and degenerating regions, such an interesting study object for male infertility? The answer lies in the evolutionary history of the X and Y chromosomes. Over the course of the past 240–320 million years of mammalian evolution, the X and Y chromosomes have evolved from what was originally a pair of ordinary autosomes (Figure 2) (Rice, 1992, 1994, 1996; Graves 1995a,b, 2000; Lahn and Page, 1997, 1999a; Graves *et al*., 1998; Jegalian and Page, 1998; Jegalian and Lahn, 2001). During that evolution, just as most of the ancestral X genes were decaying on the Y because of the lack of meiotic recombination, genes which control spermatogenesis arrived on the Y from autosomes (Figure 2). Once on the Y, these formerly autosomal genes amplified into multiple copies, and achieved greater prominence (Saxena *et al*., 1996; Lahn and Page, 1999a).

Spermatogenesis genes that arrived on the Y, but came originally from autosomes, include the *DAZ* and *CDY* genes that are among the seven gene families located in *AZFc* (Figure 2) (Saxena *et al*., 1996, 2000; Lahn and Page, 1999b). Other spermatogenesis genes on the Y, such as *RBMY*, have persisted in their original position as on the X (Delbridge *et al*., 1997, 1999; Delbridge and Graves, 1999; Mazeyrat *et al*., 1999; Vogel *et al*., 1999). The ancestral gene that remained on the X chromosome (*RBMX*) retained its widespread function, whereas *RBMY*, which persisted on the receding Y chromosome, evolved a male-specific function in spermatogenesis (Graves, 1997; Delbridge *et al*., 1999; Mazeyrat *et al*., 1999; Pask and Graves, 1999). Male benefit genes have thus arrived and accumulated on the evolving Y chromosome over many millions of years via three mechanisms: (i) persistence of genes on the ancestral X that evolved a male-specific function (RBM to RBMY); (ii) retroposition from an autosome via reverse transcription (CDL to CDY); and (iii) transposition from an autosome via translocation (DAZL to DAZ) (Figure 2).

This evolution of the modern X and Y chromosomes was initiated by the emergence of a male sex-determining gene (now known as *SRY*) on what was originally an ordinary pair of autosomes (Figure 2) (Graves 1995a,b, 1997; Lahn and Page, 1999b; Vidal *et al*., 2001). Genes associated with the non-recombining *SRY* region that were specifically beneficial for male function or antagonistic to female function, flourished on the evolving Y chromosome because it was a safe harbour, without the detrimental effect of meiotic recombination which would have otherwise allowed male-specific genes to be expressed in females (Winge, 1927; Fisher, 1931; Charlesworth and Charlesworth, 1980; Lahn and Page, 1997, 1999a,b; Silber, 1999; Brooks, 2000). Originally it was thought by many biologists that, besides this sex-determining gene, the Y chromosome contained only repetitive junk DNA. However, since the emergence of data supporting an important role of the Y in spermatogenesis, this functional wasteland model does not hold a lot of support. The modern-day Y chromosome contains many genes with cellular housekeeping functions (such as *RPS4Y* and *EIF1AY*), genes involved in spermatogenesis (such as *DAZ*, *RBMY* and *CDY*) and genes associated with other diseases such as gonadoblastoma and Turner's syndrome (such as *TSPY* and *UTY*).

The discovery of these genes was prompted by the construction of comprehensive Y chromosomal maps using yeast artificial chromosomes (YACs) and STS (Foote *et al*., 1992; Vollrath *et al*., 1992). Since then, molecular techniques such as cDNA subtraction have allowed the identification of many more genes (Lahn and Page, 1997). Although much has been done to examine the genetic constitution of the Y chromosome, these efforts were hampered by the relative crudity of STS mapping techniques and the presence of so many areas of repetitive, near-identical sequences. Once a comprehensive map of the entire human Y chromosome is available, such as is now available for the *AZFc* region, known deletions on the Y chromosome can be studied with greater detail (Tilford *et al*., 2001). This sequence- based map will also allow more precise deletion mapping than has been possible until now, potentially revealing as-yet undiscovered deletions associated with male infertility. Furthermore, more robust STS assays can be designed that enable proper deletion screening without the pitfalls of polymorphic markers.

Other infertility genes

Although most research into the genetics of male infertility has focused on the Y chromosome, many other spermatogenesis genes are present throughout the human genome. In fact there are over 4000 expressed sequence tags (EST) that are found specifically in the human testis (http://www.tigr.org/tdb/hgi/index.html). All these genes are potential candidates for male infertility, and further research is needed to elucidate their precise function and possible contribution to the infertility phenotype in our male ICSI population.

Recent studies and previous speculations indicate that the Y chromosome is not the only chromosome that accumulates genes which benefit spermatogenesis over an evolutionary time span (Fisher, 1931; Rice, 1984, 1992; Brooks, 2000; Wang *et al*., 2001). Its counterpart, the X chromosome, also seems to be an ideal locus for spermatogenesis genes. Just as the Y chromosome, the X chromosome is present as a single copy in the heterogametic XY male. Therefore, any genetic alteration on the X will have an immediate impact on the male as there is no other X chromosome to compensate or counteract the effect. Furthermore, if the gene involved is recessive and detrimental to the homogametic sex, it would be disadvantageous for XX females (sexual antagonistic gene theory). In this way, genes that are beneficial to the male (such as genes involved in spermatogenesis) would preferentially be allocated to the X chromosome. For example, a rare recessive evolutionary mutation on the X that favours spermatogenesis would be preferentially passed on to male offspring who, by virtue of a higher sperm count, would then continue to pass down this favourable X mutation to their offspring.

In fact, RT-PCR subtraction studies of spermatogonia in mice have demonstrated that a large fraction of genes which are expressed exclusively in pre-meiotic male germ cells, are indeed X chromosomal in origin (Wang *et al*., 2001). Eleven of the 36 genes that were expressed specifically in mouse spermatogonia were found exclusively on the X chromosome. Since the X chromosome is well conserved in all mammals, it seems very likely that evolution has also conferred on the human X chromosome a large portion of the burden for spermatogenesis. Thus, future research into genes on the human X chromosome and their role in male infertility is mandatory and will possibly provide new insights into the genetic basis of the infertility phenotype.

Transmission of Y deletions to ICSI offspring

Once it had been shown that many cases of male infertility were caused by deletions on the Y chromosome, concern was registered immediately about the possibility of transmitting these deletions (or other genetic causes of male infertility) to offspring via what was the relatively new technique of ICSI. Although the first boys born from ICSI procedures are yet to enter puberty, it seems likely that if they carry the same genetic defect as their father on their Y chromosome, they will be infertile just as their fathers were.

Microdeletions on the long arm of the Y chromosome do not appear adversely to affect the fertilization or pregnancy results in either severely oligozoospermic or azoospermic men, from whom sperm was successfully retrieved by TESE (Silber *et al*., 1998).

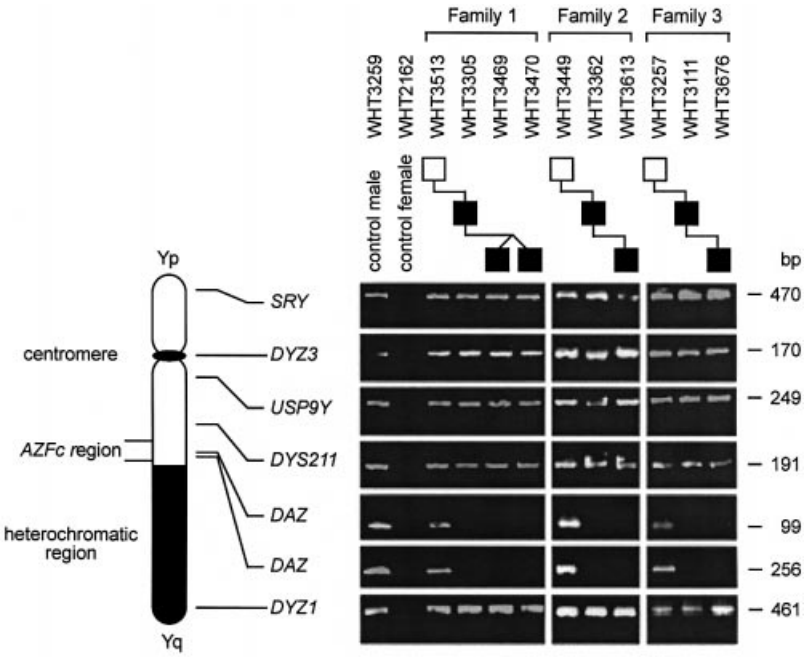

Figure 3. Transmission of *AZFc* deletions. In all three families the *AZFc* deletion in the azoospermic or severely oligozoospermic patients is not present in their fertile fathers, but is transmitted to all sons without change via ICSI. (Adapted from Page *et al*., 1999, with permission.)

Concern has been registered that ICSI results might be poorer in Y-deleted men, although in larger series that has not been the case (Table I) (Silber, 2001b; Van Golde *et al*., 2001). Thus, men with Y deletions have the same chance of obtaining offspring via ICSI as other non-Y-deleted males undergoing ICSI. Most genes involved in these Y deletions are expressed specifically in the testis during spermatogenesis and do not seem to be essential for fertilization or embryogenesis. The only problem with regard to obtaining a pregnancy is the low number of sperm available in these patients, but this is circumvented by the ICSI procedure. Of course, some deletions, such as *AZFb* deletions, give rise to complete absence of germ cells or total maturation arrest with no haploid spermatid development at all, and are, therefore, never transmitted to offspring (Brandell *et al*., 1998).

There has been some concern expressed about a possible widening of the *AZFc* deletion when it is transmitted to the next generation, although this has been reported only once (Stuppia *et al*., 1996). In our centres, all of the male offspring from Y-deleted men have had the same Y deletion as their infertile father without any expansion (Page *et al*., 1999). Fathers, brothers and paternal uncles of the infertile men were also examined for Y deletions and fertility. In all of the infertile Y-deleted men, the deletions were shown to be *de novo*, i.e. the fertile fathers of the infertile Y-deleted patients had no Y deletion, the deletion first appearing in the infertile sons. However, all male offspring of these infertile Y-deleted men derived from ICSI procedures had the Y deletion transmitted to them without change (Figure 3).

The idea that a Y deletion would always be transmitted to the ICSI-derived son is not as obvious as it might at first seem (Edwards and Bishop, 1997). If a few foci of spermatogenesis in the testis of a severely oligozoospermic or azoospermic Y-deleted man were present because of testicular mosaicism, it would seem very possible that the few areas of normal spermatogenesis within such a deficient testis of a Y-deleted man might actually have escaped from the deletion and contain a normal Y chromosome. In that event, one could have expected the sons of these patients undergoing ICSI not to be Y-deleted. However, by using fluorescence in-situ hybridization (FISH) techniques, it has been shown that all spermatozoa from *AZFc*-deleted men carry the same deletion that was originally detected in their somatic cells (de Vries *et al*., 2001). Thus, it seems that if a patient carries a deletion on the Y chromosome as determined by analysis of his blood, all of his spermatozoa will have the same deletion, as will all of his sons when these spermatozoa are used for ICSI.

As no patients are described that are mosaic for cells with and cells without a Y chromosome deletion, it can be assumed that most Y chromosome deletions arise in the testis of the fertile father, and not during embryogenesis. The deletions which arise in the testis of the fertile father are caused by an accidental homologous recombination between large sites of sequence identity causing loss of the intervening sequence. The exact frequency of the occurrence of these deletions in the testis is unknown, although it is estimated that approximately 1 in 4000 newborn boys will be Y-deleted (Kuroda-Kawaguchi *et al*., 2001). Whether these deletions might occur more frequently in the deficient testis of a patient with impaired spermatogenesis is subject to further investigation (Le Bourhis *et al*., 2000). It also remains to be determined whether non-Y-deleted men may have mosaic deletions in their testis. If so, then de-novo Y deletions would also be found more frequently in the brothers of Y-deleted

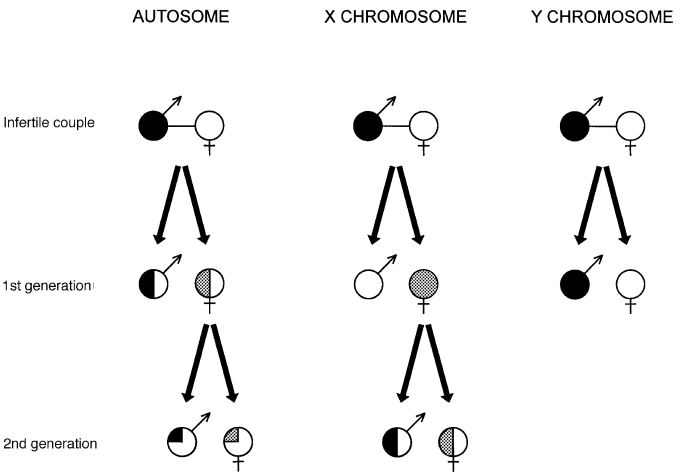

Figure 4. Transmission of male infertility through different modes of inheritance. Black illustrates an affected male, while grey indicates a female carrier. The degree of shading in the circles indicates the percentage of affected males or carrier females (100%, 50% or 25%). Assuming an autosomal origin of the infertility, a dominant mode of inheritance and heterozygosity of the index patient, 50% of boys will be affected and 50% of girls will be carriers in the 1st generation. These females can subsequently give rise to infertility in 25% of males in the 2nd generation and cause 25% of females to be carriers (50% × 50% = 25%). For an X-linked mode of inheritance, no males will be affected but all females will be carriers. These female carriers can again give rise to infertility in the 2nd generation (50% chance). Finally, when the origin of the infertility is on the Y, no females will be carriers but all male offspring will be affected.

patients, or in ICSI offspring of infertile men who have no Y deletion. There is again one study demonstrating this (Kent-First *et al*., 1996), but further research is still necessary.

The result of deletions on the Y chromosome is impaired spermatogenesis and not necessarily a failure to father offspring naturally. The degree of impairment, i.e. mild oligozoospermia, severe oligozoospermia or azoospermia, differs between different deletions and between different patients with the same deletion. Whether these males are able to induce pregnancies naturally depends on the fertility of their sexual partners. The fact that even men with *AZFc* deletions can, in some instances, father children without the use of ART, illustrates the ability of some wives of men with minute amounts of spermatozoa to conceive naturally (Sokol and Sparkes, 1987; Chang *et al*., 1999; Saut *et al*., 2000; Silber, 2001b). As some deletions, such as partial deletions of the *DAZ* clusters, potentially have a less dramatic impact on spermatogenesis, these could very well be transmitted naturally more often. The use of ICSI could contribute considerably to the spreading of these deletions and, as a consequence, reduced overall fertility within the human population (Faddy *et al*., 2001).

If a genetic aberration that leads to male infertility is located on an autosome or on the X chromosome, the possible transmission of this aberration to offspring via ICSI is different from transmission of aberrations on the Y chromosome (Figure 4). In contrast to aberrations on the Y chromosome, aberrations on the X chromosome are never transmitted to male offspring as all boys inherit an X chromosome from their mother only. All girls born from these men with X chromosome aberrations however, will be carriers. As these women are not affected by the aberration (since it only affects testis-specific genes) and are therefore fertile, they can naturally transmit the aberration to their offspring giving rise to infertility in the second generation in 50% of boys and causing 50% of girls to be carriers. If the aberration is located on an autosome, 50% of male offspring will be infertile and 50% of female offspring will again be carriers, assuming a dominant mode of inheritance and heterozygosity of the index patient. The female offspring can again transmit infertility to the second generation affecting 25% of males and cause 25% of females to be carriers (50% chance of being carrier × 50% chance of transmitting the affected chromosome = 25%). The penetrance of infertility in offspring for autosomal recessive genes will depend on the prevalence of the mutation in the general population.

Conclusions

The use of ICSI has increased tremendously over the past decade and currently even allows men with only minute amounts of spermatozoa in their testis to father children. In the majority of cases, there is no obvious explanation for the deficient sperm production in the male. If the cause is genetic in origin, the chances are that male ICSI-offspring will inherit the aberration and thus also be infertile. Studies on the role of Y deletions in male infertility have provided new insights into this transmission of male infertility via ICSI. Through evolution, the Y chromosome acquired a specialized role in spermatogenesis, and this has made it highly useful in studying the genetic causes of male infertility. Aberrations on the Y chromosome are currently found in approximately 10% of infertile males. Most, though not all, of these males still possess some degree of spermatogenesis that results in sufficient spermatozoa to perform ICSI. The presence of Y deletions does not decrease the fertilization or pregnancy rate, thereby enabling these men to father children with the same efficiency as non-Y-deleted men undergoing ICSI. However, the Y deletion is transmitted to all male offspring as all Y-bearing spermatozoa also carry the deletion.

Although standard Y deletion screening can detect deletions of *AZFa*, *AZFb* and/or *AZFc*, there might be other deletions on the Y chromosome that are not detected. The availability of the complete Y sequence will allow more precise investigations into the genomic integrity of the Y chromosome and male infertility. Furthermore, research into the many other testis-specific genes that are not located on the Y chromosome will be necessary to help elucidate the complex process of spermatogenesis and enable screening for aberrations in these genes in our male ICSI population. Of special interest are genes located on the X chromosome, as this seems also to have acquired several spermatogenesis genes during the course of evolution. However, as infertility affects the ability to transmit genes to the next generation, the mode of inheritance of genetic aberrations causing infertility is difficult to investigate. The only straightforward method of investigating the genetic basis of infertility therefore seems to be large-scale association studies comparing infertile men with idiopathic spermatogenic failure to normospermic controls. In fact, a recent study on the role of *POLG* mutations in male infertility demonstrated the usefulness of such an association study (Rovio *et al*., 2001).

In our view, genetic counselling should be provided to all infertile males, whether or not an abnormality is detected and whether or not Y deletion assays have even been performed. The

frequencies with which genetic aberrations are found in infertile males by karyotyping and standard Y deletion screening are below 15%. However, standard screening for deletions of *AZFa*, *AZFb* and/or *AZFc* is still crude and some deletions might go unrecognized. Also, some 'deletions' may in fact only be polymorphisms, and of no clinical significance. Finally, if no cytogenetic or Y-chromosomal abnormality is found in an infertile male patient, this does not at all obviate the likelihood of there being a genetic cause for his azoospermia or severe oligozoospermia.

It is apparent that there is likely to be frequent transmission of male infertility from the ICSI father to his male offspring, regardless of current testing. Every couple must decide for themselves whether they wish to consider this risk, and in our experience most such couples, even when well informed, choose to have ICSI despite this risk (Giltay *et al*., 1999). Thus, continued long-term clinical and molecular studies of ICSI offspring, together with further research into the genetic origin of male infertility, is mandatory.

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