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Male infertility: analysis of the markers and genes on the human Y chromosome

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The long arm of the human Y chromosome is required for male fertility. Deletions in three different regions can cause severe spermatogenic defects ranging from non-obstructive azoospermia to oligozoospermia. Use of intracytoplasmic sperm injection (ICSI) may allow Y chromosome defects to be passed from father to son. Thus, numerous reports have stressed the need to offer genetic testing to infertile men who select ICSI and a number of reproductive clinics have begun to do so. The primary objectives of this review were: firstly, to discuss the characteristics of the published set of polymerase chain reaction markers and how these characteristics affect interpretation of Y chromosome deletion analysis and secondly, to summarize the recent literature pertaining to the genes on the Y chromosome.

Key words: azoospermia/deletion/intracytoplasmic sperm injection/oligozoospermia/Y chromosome

Introduction

Current estimates indicate that ~15% of couples are infertile; a male factor has been identified in ~50% of infertility cases (Bhasin *et al*., 1994). Although male infertility is common, little is known about genetic aetiologies that cause spermatogenic defects, except in cases linked to deletions of the Y chromosome.

In 1976, Tiepolo and Zuffardi provided the first evidence that the long arm of the Y chromosome might be required for fertility in men when they karyotyped 1170 men and found that six azoospermic men were missing most of the long arm of the Y chromosome. In two cases, they demonstrated that the fathers of the infertile men had the whole Y chromosome, an expected finding if the Y chromosome deletions were the cause of azoospermia. This indicated that the long arm deletions were *de novo* and the authors proposed the existence of a gene or gene cluster on the long arm that is required for fertility (Tiepolo and Zuffardi, 1976). Subsequently, the fertility gene or gene cluster postulated to exist on the Y chromosome became known as the azoospermia factor or *AZF*. Since this early work, many papers have reported long arm deletions in azoospermic men using both karyotypic analysis and Southern blotting to demonstrate the loss of large regions of the Y chromosome, as well as smaller interstitial deletions that might be implicated in azoospermia (Fitch *et al*., 1985; Andersson *et al*., 1988; Hartung *et al*., 1988; Johnson *et al*., 1989; Skare *et al*., 1990; Ma *et al*., 1992, 1993; Vogt *et al*., 1992; Kobayashi *et al*., 1994). These studies formed the foundation for analysis of Y chromosome deletions in infertile men. However, karyotyping and Southern blotting are laborious and so, until recently, only a few laboratories were investigating the role of the Y chromosome in human fertility.

Once the human Y chromosome was cloned and a map of polymerase chain reaction (PCR) markers which covers the entire chromosome was assembled (Foote *et al*., 1992; Vollrath *et al*., 1992), the first searches for interstitial deletions of the Y chromosome using these PCR markers on blood DNA from infertile men were published (Kobayashi *et al*., 1994; Reijo *et al*., 1995). Since that time, deletion analysis using these marker sets on DNA from several thousand infertile and fertile men have been published and three regions that are required for normal fertility have been identified (Kent-First *et al*., 1996a,b; Reijo *et al*., 1996a; Stuppia *et al*., 1996; Vogt *et al*., 1996; Foresta *et al*., 1997; Girardi *et al*., 1997; Kremer *et al*., 1997; Pryor *et al*., 1997; Simoni *et al*., 1997; Vereb *et al*., 1997; Yoshida *et al*., 1997) (Figure 1). Interest in Y chromosome deletion analysis using these markers on DNA from infertile men has stemmed largely from: (i) the likelihood that Y chromosome deletions will be inherited via intracytoplasmic sperm injection (ICSI) (Kent-First *et al*., 1996a; Reijo *et al*., 1996a); (ii) the hope that knowledge of fertility genes will lead to more rational treatments for some types of male infertility; and (iii) the ease with which these markers can be used. The objective of this review is to discuss potential problems associated with the use of these PCR markers in determining whether a particular Y chromosome deletion is the cause of defective spermatogenesis.

Deletion analysis and PCR markers

As noted above, phenotypes such as ‘maleness’, short stature, and infertility were first assigned to the Y chromosome by karyotypic analysis that revealed structural abnormalities of the Y chromosome in affected individuals (Buhler, 1980; Davis, 1981). With the use of Y chromosome-specific DNA probes on Southern blots that contained immobilized DNA from men with naturally occurring terminal deletions, a more refined map of the Y chromosome was constructed (e.g. Vergnaud *et al*., 1986). Assuming that each deleted Y chromosome has a single break, the order of DNA probes was

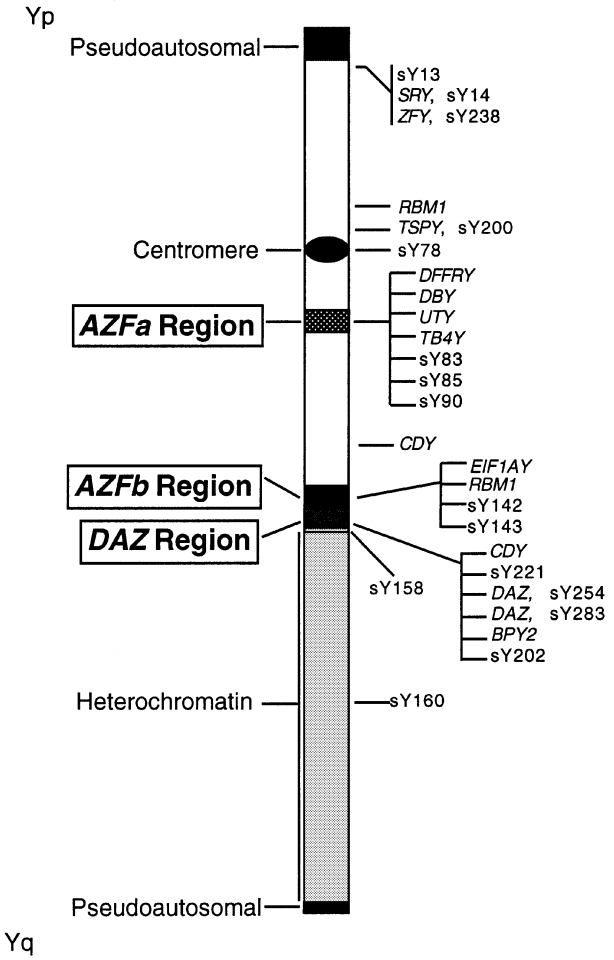

Figure 1. Three regions of the Y chromosome are required for male fertility. Four genes map to the most proximal region, the *AZFa* region. All of these genes have homologues on the X chromosome and are expressed ubiquitously (Lahn and Page, 1997). The *AZFb* region contains four gene families, as shown. The *RBM*, *PRY*, and *TTY* genes are testis-specific. These genes also have gene copies on the short arm of the chromosome (Ma *et al*., 1993; Lahn and Page, 1997). The *AZFa* and *AZFb* regions were defined by Vogt *et al*. (1996). We have termed the most distal region required for fertility, the *DAZ* region. This region is defined in Reijo *et al*. (1995). Our definition differs from that of Vogt *et al*. (1996), in that it encompasses the entire *DAZ* gene cluster and includes the sequence-tagged sites (STS) described in the text. This region contains four genes, *DAZ*, *CDY*, *BPY2*, and *PRY*.

determined by scoring the presence or absence of a marker on a large number of deleted chromosomes harbouring different breakpoints.

The first PCR-based deletion map was constructed in a manner similar to that used to construct deletion maps with Southern probes. Using a collection of DNA from men with different short and long arm deletions, markers were grouped and organized into discreet deletion intervals (Vollrath *et al*., 1992). Analysis of DNA subclones was then used to order markers over small regions that span 1 or 2 Mb (Foote *et al*., 1992). In conducting PCR-based deletion analysis of the Y chromosome, several points must be considered: (i) the most useful PCR markers are those that are single-copy or confined to a small region of the Y chromosome; (ii) multiple, discontinuous deletions along a chromosome are rare and should be verified by Southern blotting; (iii) some markers will be 'naturally' missing or polymorphic in both fertile and infertile men; and (iv) proper controls and good DNA quality are required when attempting to ascertain the significance of any suspected deletion.

Useful markers are single-copy or confined to a small region of the Y chromosome

The most useful markers for PCR-based deletion analysis are those that are single copy or confined to a small region on the Y chromosome. In other words, repetitive markers or genes that span large regions of the long and short arms of the Y chromosome will rarely be informative. When repetitive markers are used to test for deletions, negative results will occur only when a large region of the Y chromosome is missing and positive results will not indicate that all copies of that marker or gene sequence are present.

Several markers shown in Table I are single-copy, some are confined to small regions of the Y chromosome, and others contain family members that are widely dispersed along one or both chromosome arms. The copy number of a marker can be determined by PCR on yeast artificial chromosome (YAC) subclones that contain 1–2 Mb of DNA each (Foote *et al*., 1992), by PCR or hybridization of probes to cosmid, P1 or bacterial artificial chromosome (BAC) subclones that contain 40–200 kb DNA (Reijo *et al*., 1995), or by mapping of markers on the DNA of some men that have short arm deletions of the Y chromosome and on DNA from some men that have long arm deletions of the Y chromosome (e.g. Vollrath *et al*., 1992). Based on these mapping methods, markers listed in Table I that are single-copy include: sY13, sY14 (the *SRY* gene), sY238 (the *ZFY* gene), the XY homologous genes *DFFRY*, *DBY*, *UTY*, *TB4Y* and *E1F1AY*, and the markers sY83, sY85, sY90, sY142, sY143, sY202, and sY158. The absence of these markers indicates the loss of the sole member of the gene or marker family. Likewise, the presence of these markers indicates the presence of the sole member of these gene or marker families.

In contrast to single-copy markers, other markers are multi-copy and may be clustered or dispersed along the Y chromosome. Clustered markers include the *BPY2* gene family, the *DAZ* gene family, the marker sY221, and the centromeric marker sY78 (Foote *et al*., 1992; Vollrath *et al*., 1992; Reijo *et al*., 1995; Saxena *et al*., 1996; Yen *et al*., 1996, 1997; Lahn and Page, 1997). A negative PCR result when testing for the presence or absence of these markers indicates the loss of all members of the gene or marker family. The caveat in interpreting results with these markers is that the presence of a PCR band does not indicate that all members of these clustered gene families are present on the Y chromosome. In fact, it remains possible that a critical copy of these gene or marker families could be missing even though a band is amplified. Thus, until specific markers that indicate the presence or absence of a single copy of these gene and marker families are available, one must either use Southern blotting to detect the individual members of these families or interpret the results cautiously. For example, the presence of a band corresponding

Table I. Sequence-tagged sites (STS) and gene sequences for deletion analysis. Taken from Foote *et al*. (1992), Vollrath *et al*. (1992), Reijo *et al*. (1995), Vogt *et al*. (1996) and Lahn and Page (1997)

sY number (gene)	Left primer sequence	Right primer sequence	Size (bp)
sY13	GTGACACACAGACTATGCTTC	TCAAGGTTGTTGTTTAAGCT	187
sY14 (*SRY*)	GAATATTCCCGCTCTCCGGA	GCTGGTGCTCCATTCTTGAG	472
sY238 (*ZFY*)	AACAAGTGAGTTCCACAGGG	GCAAAGCAGCATTCAAAACA	350
sY200 (*TSPY*)	CGGGGAAGTGTAAGTGACCGATGGG	CTGCTCTTCAAAAAGATGCCCCAAA	189
sY78	TCCTTTTCCACAATAGACGTCA	GGAAGTATCTTCCCTTAAAAGCTATG	180
DFFRY	GAGCCCATCTTTGTCAGTTTAC	CTGCCAATTTTCCACATCAACC	111
DBY	CATTCGGTTTTACCAGCCAG	CAGTGACTCGAGGTTCAATG	83
UTY	GCATCATAATATGGATCTAGTAGG	GGAGATACTGAATAGCATAGC	65
TB4Y	CAAAGACCTGCTGACAATGG	CTCCGCTAAGTCTTTCACC	102
sY83	CTTGAATCAAAGAAGGCCCT	CAATTTGGTTTGGCTGACAT	275
sY85	TGGCAATTTGCCTATGAAGT	ACAGGCTATTTGACTGGCAG	369
sY90	CAGTGCCCCATAACACTTTC	ATGGTAATACAGCAGCTCGC	176
CDY	CCTCAAAATCCACTGACG	CAAGCGATATCTCACCACC	72
E1F1AY	CTCTGTAGCCAGCCTCTTC	GACTCCTTTCTGGCGGTTAC	84
RBM1	CTTTGAAAACAATTCCTTTTCC	TGCACTTCAGAGATACGG	800
sY142	AGCTTCTATTCGAGGGCTTC	CTCTCTGCAATCCCTGACAT	196
sY143	GCAGGATGAGAAGCAGGTAG	CCGTGTGCTGGAGACTAATC	311
sY254 (*DAZ*)	GGGTGTTACCAGAAGGCAAA	GAACCGTATCTACCAAAGCAGC	350
sY283 (*DAZ*)	CAGTGATACACTCGGACTTGTGTA	GTTATTTGAAAAGCTACACGGG	375
BPY2	CCCAGATTTTCACAGGTGCT	CTCATTTGTATGCTGGGCCT	209
sY221	GTAAGCCCCAGATACCCTCC	AAATTGTTTGGAAAAGGACACC	113
sY202	ACAGTTTGAAATGAAATTTTAAATGTGTT	TGACAAAGTGAGACCCTACTACTA	121
sY158	CTCAGAAGTCCTCCTAATAGTTCC	ACAGTGGTTTGTAGCGGGTA	231
sY160	TACGGGTCTCGAATGGAATA	TCATTGCATTCCTTTCCATT	236

to the *DAZ* gene does not indicate that all members of this gene family are present on a particular Y chromosome. It merely indicates the presence of at least one member of the *DAZ* family containing the marker sequence.

Finally, some markers are multicopy and dispersed across large regions of the Y chromosome. Markers in this category include sY200 (the *TSPY* gene family), CDY, RBM1 and RBM2, and the genes *PRY, TTY1, TTY2*, and *XKRY.* The presence of a PCR product corresponding to these dispersed marker families does not indicate that all members of these families are present on a particular Y chromosome. In addition, the absence of these markers would indicate that very large deletions of the Y chromosome have occurred, perhaps even encompassing the centromere. Thus, the complete absence of these markers should be suspected of being a spurious result in many instances and should be verified by additional PCR and by Southern blotting, e.g. Vogt *et al*. (1997), for methods of analysing the presence and absence of the *RBM1* genes.

Multiple, discontinuous deletions along a chromosome are rare and should be verified by Southern blotting

Multiple, discontinuous deletions along a particular Y chromosome are rare. When such events commonly occur, then the order of the markers or the reliability of the PCR reactions are highly suspect. For example, some Y chromosomes have been reported to contain both deletions removing the most-centromeric region implicated in infertility in men, the *AZFa* region, and deletions removing the most distal region, the *DAZ* region, but not removing the intervening *AZFb* region (Foresta *et al*., 1997). These types of discontinuous Y chromosome deletions should be extremely rare. In other reports, a majority of patients with deletions have multiple, discontinuous deletions of one or a few markers (Najmabadi *et al*., 1996). This could indicate polymorphism of the markers tested, a need to reorder the markers on the deletion map, or spurious negative results associated with particularly poor markers. In any case, the presence of multiple, discontinuous deletions in a substantial percentage of a clinical population prevents interpretation of the significance of any deletions in that population.

Some markers will be 'naturally' missing from both fertile and infertile men

Markers missing in both fertile and infertile men are termed 'polymorphic' and are unlikely to represent significant deletions since a correlation between marker or gene loss and fertility cannot be established. Since genetic variation is inevitable in human populations of different ethnic backgrounds or geographical histories, polymorphisms are expected. They can be distinguished from functional mutations by examining the distributions of the markers in both affected and unaffected individuals. Recent examples of polymorphic markers include sY207 and sY272, markers that can be absent with no consequence to fertile men since they appear to be missing from fathers and infertile men (Pryor *et al*., 1997).

Proper controls and good DNA quality are required when attempting to ascertain the significance of any suspected deletion

Nearly all reports of deletion analysis indicate that a suspected deletion of a marker is tested at least two or three times before

the deletion is recorded. In addition, most reports indicate that normal male and normal female DNA samples are included as controls for marker and PCR performance (Reijo *et al*., 1995, 1996a; Kent-First *et al*., 1996b; Stuppia *et al*., 1996; Vogt *et al*., 1996; Foresta *et al*., 1997; Girardi *et al*., 1997; Kremer *et al*., 1997; Pryor *et al*., 1997; Simoni *et al*., 1997; Vereb *et al*., 1997; Yoshida *et al*., 1997). Methods of preparing the DNA samples from all patients and fertile individuals vary considerably. Common methods of preparing DNA include extraction with the Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA), the QIAamp Blood Kit (Qiagen Inc, Santa Clarita, CA, USA), salting-out procedure (Miller *et al*., 1988), or cell-lysis and dialysis (Reijo *et al*., 1995; Najmabadi *et al*., 1996; Pryor *et al*., 1997; van der Ven *et al*., 1997). Undoubtedly, the purity of the DNA affects the rate of PCR failure; the more pure the DNA, the less often markers must be retested and the fewer false negatives. Laboratories setting up DNA testing of Y chromosomes should investigate which DNA preparations work well through marker testing on fertile men.

Genes on the Y chromosome

Ultimately, the best PCR markers and assays will be derived from DNA sequences from *bona fide* fertility genes – genes in which an internal deletion or mutation has been shown to cause infertility. Although no internal deletions or mutations have been identified in any of the genes currently implicated in Y-linked infertility including the *DAZ, RBM1, RBM2, CDY, BPY1, BPY2,* and *PRY* genes (Ma *et al*., 1993; Reijo *et al*., 1995; Lahn and Page, 1997), it is likely that several of these genes are required for fertility. No significant open reading frames have been identified in two other Y chromosome genes, *TTY1* and *TTY2*, making them less likely to be implicated in infertility. Finally, genes such as *DFFRY, DBY, UTY, TB4Y* and *EIF1AY* are not specifically expressed in the testis (Lahn and Page, 1997). These groups of genes may affect fertility but are also likely to be required for more ubiquitous functions.

In the last several years, data have been accumulating that suggest that members of both the *RBM* and *DAZ* gene families are required for fertility. In addition, several members of newly-identified gene families map to intervals implicated in infertility including the *CDY, BPY2,* and *PRY* genes. These families are discussed in more detail below.

RBM *genes*

The *RBM* genes, *RBM1* and *RBM2*, were identified in 1993 (Ma *et al*., 1993). Approximately 15–30 copies of these genes are dispersed along the short and long arms of the Y chromosome (Delbridge *et al*., 1997). It is likely that many copies are functional (Chai *et al*., 1997) and that many are non-functional (Prosser *et al*., 1996). Some authors report that the *RBM2* gene is polymorphic, in that both fertile and infertile men of Japanese ancestry frequently have no *RBM2* genes (Nakahori *et al*., 1994), whereas others report that Japanese men are not polymorphic for *RBM2* genes (Chai *et al*., 1997).

Deletions of the *AZFb* region of the Y chromosome encompass at least one functional copy of the *RBM1* gene. This *RBM* gene is translated and produces a protein that localizes to the nucleus of all spermatogenic cell types (Elliot *et al*., 1996, 1997). It is probable that the *RBM1* genes are required for normal fertility in men. However, whether the loss of the *RBM1* genes in men with *AZFb* deletions causes infertility is not clear. Deletions of the gene(s) have been reported using Southern blotting and PCR (Ma *et al*., 1993; Kobayashi *et al*., 1994; Nakahori *et al*., 1996; Foresta *et al*., 1997; Pryor *et al*., 1997). However, since *RBM1* and *RBM2* genes are dispersed across the short and long arms of the Y chromosome, it is unclear how a negative PCR result should be interpreted; how does the loss of a subset of the *RBM* genes lead to a negative PCR result when other family members, presumably with the same or similar sequence, remain on the Y chromosome? To overcome this ambiguity, Vogt *et al*. (1996) used both PCR and Southern blotting with the *RBM1* cDNA to ascertain *RBM1* deletions. Eventually, this will not be necessary when markers that are specific to each *RBM* gene become available.

DAZ *gene cluster*

Deletions encompassing the *DAZ* region of the Y chromosome, the most distal region implicated in infertility, are the most common molecularly-defined cause of infertility in humans. They occur in ~13% of azoospermic men and 6% of severely oligozoospermic men (Najmabadi *et al*., 1996; Reijo *et al*., 1995, 1996a,b; Stuppia *et al*., 1996; Foresta *et al*., 1997; Mulhall *et al*., 1997; Simoni *et al*., 1997; van der Ven *et al*., 1997).

The identity of the critical fertility gene(s) within the *DAZ* region has been the subject of considerable debate. Several putative genes map within or near the *DAZ* region (Ma *et al*., 1993; Reijo *et al*., 1995; Lahn and Page, 1997). No point mutations have yet been identified in any of these genes including *DAZ* although it is difficult to identify point mutations in multigene families especially when the populations are small (Vereb *et al*., 1997). Thus the evidence that indicates that infertility is caused by the loss of one or more of the *DAZ* genes is indirect. Firstly, in contrast to several gene families that are dispersed along large regions of the Y chromosome (examples include the *RBM*, *CDY,* and *PRY* gene families), the *DAZ* genes map completely within the commonly-deleted *DAZ* region. Deletions of the *DAZ* region remove all of the Y chromosome *DAZ* genes (Reijo *et al*., 1995, 1996a; Saxena *et al*., 1996). This is an important observation since, if multiple functional copies of a gene are present and dispersed across the Y chromosome, then perhaps deletion of a single gene or subset of genes will be phenotypically silent or lead to only mild defects in spermatogenesis. Secondly, no deletions of the *DAZ* genes have been observed in men with normal sperm counts, whereas 6–13% of men with few or no spermatozoa have deletions that remove all or most of the *DAZ* gene cluster (Reijo *et al*., 1995, 1996a; Pryor *et al*., 1997; Simoni *et al*., 1997). Thirdly, the *DAZ* genes have maintained their exon/intron structure in spite of the tendencies for genes to degrade on the Y chromosome (Saxena *et al*., 1996). This suggests a selective pressure to maintain functional *DAZ* genes. Other gene families may no longer contain introns and exons including the *CDY, BPY* and *PRY* genes which are not recovered from the

DAZ region by exon trapping (Reijo *et al*., 1995). Fourthly, disruption of *DAZ*-related genes in model organisms causes infertility. The *DAZ* genes were transposed to the Y chromosome during primate evolution. The ancestral *DAZ* gene, the *DAZL* (DAZ-Like) gene, maps to chromosome 3 in humans (Saxena *et al*., 1996; Shan *et al*., 1996; Yen *et al*., 1996) and has autosomal homologues in many organisms including flies, frogs, *Caenorhabditis elegans*, and all mammals (Cooke *et al*., 1996; Eberhart *et al*., 1996; Maiwald *et al*., 1996; Reijo *et al*., 1996b; Shan *et al*., 1996). Disruption of fly *DAZL* homologue, *Boule*, causes infertility via arrest at the pachytene stage of meiosis I. Disruption of mouse *DAZL* homologue, *Dazl*, results in dosage-dependent infertility during prenatal germ cell development (Ruggiu *et al*., 1997). Mice with no functional *Dazl* genes are sterile but heterozygous mice with one intact copy of the *Dazl* gene are only subfertile, exhibiting reduced germ cell numbers. This indicates that the number of copies of the *DAZ/DAZL* genes may decide germ cell number. Infertility in men with deletions of the *DAZ* gene cluster resembles that of both flies and mice that lack *DAZ* homologues. Men lacking the *DAZ* gene cluster may be sterile (lacking all germ cells), have meiotic arrest in pachytene I, or simply have fewer germ cells and be subfertile ($<5\times10^6$ spermatozoa/ml; Reijo *et al*., 1995, 1996a; Girardi *et al*., 1997).

Immunohistochemical localization of the *DAZ* family of genes is controversial. In mice, the protein encoded by the *DAZ* homologue, *Dazl*, is found most abundantly in spermatocytes and to a lesser degree in spermatogonia (Ruggiu *et al*., 1997; R.A.Reijo *et al*., unpublished data). In humans, one report suggests that the DAZ protein is confined to sperm tails (Habermann *et al*., 1998), whereas other data using multiple antibodies indicates that the localization of DAZ protein largely overlaps the localization of mouse *Dazl* in that it is found in the immature germ cell populations (R.A.Reijo, D.M.Dorfman, A.A.Renshaw, K.R.Loughlin and D.C.Page, unpublished data). The latter is also consistent with the localization of DAZ and DAZL messages in both humans and mice (Menke *et al*., 1997; Neiderberger *et al*., 1997).

Since the *DAZ* gene cluster spans 1–2 Mb of the distal Y chromosome, it is not surprising that many PCR markers map to the *DAZ* gene cluster. These include those that have been published as specific DAZ primers and also a large number of primers that have been mapped to the *DAZ* genes by PCR of cosmid subclones (R.A.Reijo; unpublished data) or sequence analysis (a genomic *DAZ* sequence is available under GenBank Accession No. AC000021). Some of the PCR markers that map within the *DAZ* genes have the following sY numbers: 146, 147, 148, 149, 152, 154, 155, 201, 203, 205, 206, 208, 231, 232, 241, 242, 245, 253, 254, 255, 257, 258, 259, 262, 277, 279, and 283. When some of these markers (e.g. sY149 or sY203) are reported as absent from patients, authors may suggest that this is proof of deletions in the *DAZ* region that do not include the *DAZ* genes (Najmabadi *et al*., 1996; Pryor *et al*., 1997). Instead, these markers are within the *DAZ* genes and encode exons and introns of the gene. Since each of the markers listed above is present in 3–5 copies of *DAZ* within the deletion interval, one would rarely expect to see small deletions that remove only a few markers in *DAZ* when PCR analysis is done with these markers.

CDY, BPY2 *and* PRY *genes*

These genes were identified by cDNA selection of cosmids from a Y chromosome-specific genomic DNA library (Lahn and Page, 1997). Since these genes were only recently discovered, little has been published about them other than their general position on the Y chromosome and that they are testis-specific in expression. None of these genes maps to the most proximal region implicated in infertility, the *AZFa* region, although several other genes that are ubiquitously expressed including the *DFFRY, DBY, UTY,* and *TB4Y* genes do map to *AZFa* (Figure 1) (Lahn and Page, 1997). PRY is a multigene family that contains members that map to both the short and long arms of the Y chromosome but at least one copy of the gene family maps to the *AZFb* region and at least one copy maps to the *DAZ* region. Finally, at least one copy of another dispersed multigene family, the *CDY* gene family, maps to the *DAZ* region next to a small multigene sequence, *BPY2* that is exclusive to the *DAZ* region (Lahn and Page, 1997). Hopefully, gene-specific or region-specific PCR assays able to detect deletions in these gene families will be reported soon and homologues will be identified in other organisms in which genetic manipulation can be used to uncover their functions.

Should Y chromosome analysis and genetic counselling be offered to infertile men?

Has basic research concerning the role of the Y chromosome in infertility progressed to a point where it should be translated to clinical settings? A recent study reported that 96% of infertile couples in which a man has a severe defect in spermatogenesis, choose Y chromosome testing when it is offered. Furthermore, 94% of men who accepted testing returned for counselling and 21% of couples deferred assisted reproduction to await further information about genetic risks. This indicates that the transmission of infertility to offspring is weighed carefully by infertile couples (Rucker *et al*., 1998) and that there is a desire for information. However, consideration should be given to which patients should be offered Y chromosome testing and what information should be conveyed.

The population of infertile men who may warrant Y chromosome testing is not yet precisely defined. However, we can assume that the following groups of men are at risk for Y chromosome deletions: (i) men with idiopathic (unexplained) oligozoospermia who have sperm concentrations of $<5\times10^6$ spermatozoa/ml, the highest sperm count associated with any reported deletion (Girardi *et al*., 1997), and who are considering the use of in-vitro fertilization (IVF) and ICSI are likely to be at risk; (ii) men with oligozoospermia ($<5\times10^6$ spermatozoa/ml) and a defined anatomical problem (e.g. varicocele) and who are considering IVF and ICSI (see Pryor *et al*., 1997 for a report of a man carrying a Y chromosome deletion who also presented with a varicocele); and (iii) men with non-obstructive azoospermia who are to undergo testicular sperm extraction (TESE) in association with IVF and ICSI (since spermatozoa

from men undergoing TESE with Y chromosome deletions can fertilize oocytes and lead to pregnancies). In addition, some men with non-obstructive azoospermia who do not elect IVF and ICSI might elect to have Y chromosome testing to determine the underlying cause of their infertility.

Once genetic testing for Y chromosome deletions is conducted, what constitutes a meaningful deletion? Numerous studies report the deletion of one or two markers that have not been proven to be linked to infertility. It is important that the relevance of such deletions be explored completely before any information is conveyed to patients. Is the deletion real or does it represent failure of a poor assay? Is the deletion *de novo* or is it carried from father to son? Is the marker frequently missing in a particular ethnic group? In most cases, when a panel of markers is used to test deletions in *AZFa*, *AZFb*, and the *DAZ* region, there will be little evidence to support reporting the apparent deletion of a single marker missing on the Y chromosome. However, the loss of a group of markers that physically map to a contiguous region of the Y chromosome that includes the *AZFa*, *AZFb*, or *DAZ* regions can safely be assumed to have consequences for the fertility of men since numerous studies have reported their loss in infertile but not fertile men (Kent-First *et al*., 1996; Reijo *et al*., 1996a; Vogt *et al*., 1996; Stuppia *et al*., 1996; Foresta *et al*., 1997; Girardi *et al*., 1997; Kremer *et al*., 1997; Pryor *et al*., 1997; Simoni *et al*., 1997; Vereb *et al*., 1997; Yoshida *et al*., 1997). There is still incomplete information to allow prediction of the degree of spermatogenic failure associated with any specific deletion. Some men with *DAZ* deletions present with no germ cells whereas others present with sperm counts of $1–5\times10^6$ spermatozoa/ml (Reijo *et al*., 1995, 1996a; Girardi *et al*., 1997; Pryor *et al*., 1997). Although it has been suggested that isolated deletions of the *AZFa* region and *AZFb* regions are likely to cause the most severe defects in spermatogenesis (Vogt *et al*., 1996), the number of deletions reported is generally fewer than those for the *DAZ* region. This suggests that the ability to predict the consequences of individual deletions is impaired by the limited number of observations currently available.

In summary, infertile men with severe spermatogenic failure are at risk for Y chromosome deletions. If Y chromosome testing is offered to these men and a deletion is observed, the most conservative prediction is that the deletion can be passed from father to son. The most likely outcome is that the son will be infertile since no deletions have been shown to occur in normally fertile men but the degree of severity of the spermatogenic defect the son will display cannot be predicted reliably at this time.

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