Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility

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Among the 10% or so of men who are diagnosed as oligo- or azoospermic in the absence of any physical obstruction, research is now showing that between 8 and 15% carry a microdeletion in the long arm of the Y chromosome which, by loss of specific DNA segments, leads to loss of vital genes for sperm production. Chromosomal anomalies account for ~2% of all men who attend infertility clinics, rising to 15% among those with azoospermia. There are serious implications for couples seeking help by intra-cytoplasmic sperm injection (ICSI), since chromosomal or gene defects which might normally be lost or eliminated by natural means could be transmitted in offspring. The need for genetic testing of ICSI donors and their offspring is raised, and a requirement for counselling is recommended.

Key words: azoospermia factor/infertility/spermatogenesis/Y chromosome

Introduction

Three decades of research have provided clear evidence of the importance of genetic factors in male infertility and subfertility. Amongst all the aetiological factors involved, cytogenetic screening of patients, and the more recent DNA analysis of their Y chromosomes by polymerase chain reaction (PCR) assay, highlight an area of immense importance. A category of men with oligo- or azoospermia, previously classified as being of ‘unknown aetiology’, can now be accurately diagnosed as carriers of a chromosome anomaly or Y chromosome mutation (microdeletion), which is probably directly connected to their gametogenic failure.

Cytogenetic screening of men attending infertility clinics

The first cytogenetic screenings among men attending infertility clinics were undertaken in the early 1960s, soon after karyotyping by blood lymphocyte analysis became an established technique (McIlree et al., 1966; Kjessler, 1996). Several much larger series were subsequently published, including our own study of 2372 unselected men attending clinics in Edinburgh, 2.2% of whom were found to have a constitutional chromosome abnormality (Chandley, 1979, for review). Among the azoospermic group alone, the frequency rose to 15.4%, attributable mainly to the inclusion of the commonest category of azoospermia, men with Klinefelter’s syndrome (47,XXY). A variety of other numerical and structural anomalies, such as reciprocal and Robertsonian translocations, inversions, ring chromosomes, aneuploidies and extra (supernumerary) chromosomes, were, however, recorded (see Chandley, 1996, for review). Although little, if anything, is understood about how and why these various chromosomes anomalies could disturb germ cell development, several hypotheses have been proposed. Amongst these, two views have emerged. Burgoyne et al. (1992) suggest that pairing failures are a principle cause of germ cell breakdown, while Mittwoch and Mahadevaiah (1992) viewed these as a consequence, i.e. a symptom of developmental delay in spermatocyte progression. In the Burgoyne model, pairing is seen as a process which is strictly monitored by a ‘quality control’ mechanism at meiosis, and any defect which arises will be recognized, leading to cell atresia. In the Mittwoch and Mahadevaiah model, quality control is a pro-
cess which starts much earlier than the pairing stage itself, and indeed might operate from the onset of spermatogonial proliferation in the adult testis. Regardless of the cause of spermatogenic impairment in men carrying a chromosome anomaly, they could be at risk for producing spermatids and/or spermatozoa with an unbalanced chromosome complement. The consequence, if such germ cells were used for intracytoplasmic sperm injection (ICSI), might well be the conception of a chromosomally unbalanced offspring. This point is dealt with in more detail below (see the section ‘Genetic anomaly and ICSI’). Overall, to summarize the extensive work that has been carried out in men and other species, including the mouse, the maintenance of normal spermatogenesis appears to require an intact genome (Searle et al., 1978).

Y-linked genes and spermatogenesis

It is now realized that the fertility of oligozoospermic and azoospermic men who appear chromosomally normal by cytological examination can be disturbed by the loss, through microdeletion, of Y-chromosome genes which are vital in spermatogenic control (see Chandley and Cooke, 1994, for review). Deletions of DNA in specific parts of the Y chromosome long arm (Ma et al., 1992, 1993; Kobayashi et al., 1994; Reijo et al., 1995) appear to characterize 10–15% of all severely oligo- or azoospermic 46,XY individuals. The vital importance of the Y chromosome in the control of spermatogenesis was realized more than 20 years ago, when Tiepolo and Zuffardi (1976) described six azoospermic individuals, each of whom showed a cytologically deleted Y chromosome in an otherwise normal karyotype. Histological studies of the testes in each patient revealed a marked reduction or absence of germinal elements, and the authors proposed that a locus (the ‘azoospermia factor’, AZF) might lie at the interface between euchromatin and heterochromatin on distal Yq. The AZF locus was subsequently mapped, using collections of deleted Y chromosomes, to interval 6 on the Y chromosome map of Vergnaud et al. (1986), by Andersson et al. (1988). It lies within cytological band Yq11.23.

In 1989, a search for the AZF genes commenced in our laboratory. An initial analysis included a series of men selected from the oligo- and azoospermic categories, many having reduced testis volumes and raised serum follicle-stimulating hormone (FSH), each of which is indicative of a spermatogenic impairment. From the outset it was thought likely that, even amongst chromosomally normal individuals, a severe reduction or absence of germ cells in the testis might result from loss by microdeletion, or impairment in function of the AZF genes. Because about half of the patients who underwent screening had also undergone a testicular biopsy, it was anticipated that meaningful genotype–phenotype correlations could be made in those cases where a gene defect was found. Confirmation came in 1992 when, amongst the first 19 infertile 46,XY individuals screened, using a series of Y-specific DNA probes, two men with microdeletions were detected (Ma et al., 1992). One azoospermic man, ‘Jolar’, showed deletion of two probes in proximal subinterval I of interval 6; the second, ‘Klard’, showed deletion of 11 probes mapping across distal microdeletions XII–XIV. These proximal and distal microdeletions did not overlap, indicating either that the AZF locus is very large, or that more than one gene, or different members of one gene family, might be involved in the process of spermatogenesis. Since that time, further screening in our own laboratory (Ma et al., 1993; Qureshi et al., 1996), and by groups worldwide (Kobayashi et al., 1994; Reijo et al., 1995; Vogt et al., 1996), has confirmed the common occurrence of microdeletions in Yq amongst men whose infertility was previously unexplained.

Our initial interest in isolating genes from the Y chromosome which are important in spermatogenesis lay in the belief that, as well as opening up an area of exciting biological interest, there might also be the possibility for greatly improved diagnosis in men with oligo- and azoospermia, if the underlying cause were genetic. A major breakthrough was made in 1993, when a gene family, RBM (RNA-binding motif; previously known as YRMR), was isolated from the distal Klard deletion interval (Ma et al., 1993). The RBM family contains up to 40 members distributed across the entire length of the Y chromosome, with a major clustering in interval 6 (Schempp et al., 1995). Due to their Y-specific location, testis-
specific expression, cross-species conservation, and partial deletion in some oligo- and azoospermic individuals, they were proposed as candidates for AZF (Ma et al., 1993; Chandley and Cooke, 1994). More recently, a second Y chromosome gene, DAZ (deleted in azoospermia), which is also expressed specifically in the testis and bears an RNA recognition motif, has been isolated and proposed as another candidate for AZF (Reijo et al., 1995). So far, however, neither RBM or DAZ has yielded information concerning specific point mutations in non-deleted oligo- or azoospermic men, and the identity of AZF remains unknown.

Our most recent patient screening (Qureshi et al., 1996) was carried out by PCR analysis on 100 chromosomally normal men, all of whom were severely oligozoospermic or azoospermic. Eighty fertile control subjects were also tested, 12 of whom were fathers or brothers of the patients. Human genomic DNAs were prepared from blood or lymphoblastoid cell lines using standard techniques. A series of Y chromosome-specific sequence tagged sites (STS), which had been characterized previously and whose primer sequences had been described (Foote et al., 1992), were used. A total of 23 loci, spanning the Jolar and Klard deletion intervals, were examined, the STS having been selected to cover the intervals previously found by Southern analysis to be deleted (Ma et al., 1992).

The STS screening identified eight patients with Yq microdeletions, including the original patients Jolar and Klard. The extent of the deletions is shown in Figure 1. In the Jolar interval, three patients, Jolar, Amgal and Eltor were deleted for three consecutive STS, sY84, sY85 and sY86. A fourth patient, Sayer, had a longer deletion, which included an adjacent STS, sY87. The size of this deletion interval was estimated to be ~500 kb. In the Klard interval, the largest deletion was found in patient Kupau, who had lost nine consecutive STS; the smallest was in Hamil, who was deleted.

Figure 1. Diagram of the Y chromosome microdeletions. (a) Y chromosome, (b) Y chromosome subintervals, (c) sequence-tagged sites (STS), (d) deletion patterns of patients. In (d), a black box indicates the presence of an STS; the blank spaces indicate absence of an STS. From Qureshi et al. (1996), with permission.
for only one STS, sY152. No microdeletions were detected amongst the fathers, brothers, or other fertile control samples.

Our results show that as many as 8% of cases of unexplained infertility may have an underlying genetic aetiology. Moreover, we and others (e.g. Reijo et al., 1996; Vogt et al., 1996; Pryor et al., 1997) have demonstrated that a simple PCR-based assay can be used to determine the presence or absence of key intervals along the Y chromosome of men attending infertility clinics in whom spermatogenic impairment has been diagnosed. Attempts to correlate genotypes and phenotypes have been largely inconclusive. Of our patients with deletions in the Jolar region, Jolar, Amgal and Eltor all had ‘Sertoli cell-only’ syndrome, while Sayer, with a larger deletion, had some, albeit reduced, spermatogenesis and a mean sperm count of $4.3 \times 10^6$ per ml. Of the patients with deletions in the Klard region, Nikei and Hamil each had severely diminished spermatogenesis, only a few spermatozoa present in the ejaculate. Klard and Kupau were not biopsied. Since two cases of deletions in the Klard interval, each with Sertoli cell-only syndrome, were reported by Reijo et al. (1995), it remains impossible, given our present state of knowledge, to draw meaningful conclusions about the position or extent of a microdeletion, and its effect on fertility. On this subject, we differ from Vogt et al. (1996), who have suggested that three spermatogenesis loci (AZFa, AZFb and AZFc) might exist in $Yq$, each locus being active during a different and successive phase of male germ cell development. In our opinion, more investigation is required before the distribution and activity of the Y-linked genes which control spermatogenesis in men can be understood. Neither Jolar, Amgal, Eltor, Sayer or Hamil was shown to be deleted for $DAZ$ (STS sY254 and sY255) and, since $RBM$ genes are located at several positions along $Yq$, it was not clear whether any or all of our cases had lost critical $RBM$ genes. Based on the findings by PCR analysis, it was impossible to determine the role of $DAZ$ or $RRM$ in spermatogenesis.

Expression of $RBM$ in germ cells is dependent on a critical region of the Y chromosome long arm

In order to pinpoint regions of the Y chromosome which might be required for $RBM$ gene expression, we recently used an anti-RBM antiserum to probe sections of testis from patients carrying cytologically deleted Y chromosomes and other cases carrying a microdeletion in the Jolar or Klard regions (Elliot et al., 1997). The aim was to see whether nuclear staining could be detected in germ cells and whether, by removing copies of the $RBM$ genes from $Yq$ by deletion at various sites, expression might also be lost. For normal adult and pre-pubertal (including fetal) testes, strong staining was seen over all germ cells with the exception of adult differentiating spermatids. No expression was detectable over Sertoli cells. Two cytologically deleted patients, ‘Bitra’ (Chandley et al., 1986) and ‘Horis’, and a microdeletion patient, ‘Glips’, all failed to show $RBM$ expression in their limited number of germ cells, allowing us to define a ‘critical region’ within proximal interval 6 that appears essential for quantitative $RBM$ expression. We believe that the most active $RBM$ copy (or copies) must lie within this region and, consistent with this idea, $RBM$ genes have been mapped within the region (Reijo et al., 1995). Hence, we now believe that a comparatively small region of the Y chromosome is essential for $RBM$ gene expression and this is deleted in our three patients with severely depleted spermatogenesis. However, since they are also deleted for $DAZ$, a lack of $DAZ$ gene expression may also contribute to their spermatogenic impairment. The expression of $RBM$ in the majority of germ cells in the normal adult testis, as well as in the fetal and pre-pubertal testis, is consistent with a role for $RBM$ throughout germ cell development. However, more studies are required to clarify the exact role of the $RBM$ genes in spermatogenesis.

**Genetic anomaly and ICSI**

The ICSI technique appears to have rapidly become an accepted procedure to assist reproduction in couples where the male partner is diagnosed with very poor semen characteristics. However, relatively little is known about the ‘quality’, in genetic terms, of germ cells produced by men with spermatogenic impairment (Chandley and Hargreave, 1996). As discussed earlier, for men whose spermatogenic impairment relates to the presence in the genome of a constitutional chromosome anomaly,
there is always an attendant risk for any spermatozoon (or spermatid) to be chromosomally unbalanced. Use of such a germ cell in ICSI could inevitably lead to the production of a conceptus which might abort or be born with multiple congenital abnormalities on account of the genomic imbalance. Moreover, recent studies suggest that even oligozoospermic men who appear chromosomally normal by somatic karyotyping have a raised frequency of aneuploid (dissomic) complements present in their spermatozoa (Moosani et al., 1995). These, if used in ICSI, could lead to trisomy in the conceptus, and the recently reported increases in sex chromosome aneuploidy among ICSI pregnancies (In’t Veld et al., 1995) testify to these potential risks. Another outcome of spermatogenic impairment in a male can be the production of diploid spermatids or spermatozoa (Levy and Burgoyne, 1986; In’t Veld et al., 1997) that, at fertilization, are likely to produce a triploid conceptus. Such polyploidy amongst spermatozoa might be detectable prior to ICSI, since a larger-than-normal head size and/or multi-tailed appearance are usually characteristic features.

Finally, there remains the worrying prospect that some ICSI donors might carry a deletion of DNA sequences in those specific parts of the Y chromosome long arm containing the active genes that control spermatogenesis. There is then an obvious risk for any male child produced by ICSI to inherit the defective Y chromosome from his father, along with the phenotype of infertility. Only by careful PCR diagnostic trials amongst ICSI fathers and sons will the frequency of such occurrences be accurately assessed for the future, and these trials have already begun in some centres, including our own. It is our opinion that, should any chromosome anomaly or Y chromosome DNA defect be found in a potential ICSI donor, genetic counselling of the couple as to the possible or probable pregnancy outcome should be provided by the clinics. ICSI is a most significant therapeutic advance in male infertility treatment, and, provided such safeguards are put in place, there seems no reason why many more couples should not benefit from this new technology.

**Conclusion**

Considering that not so many years ago the causes of so many cases of oligo- and azoospermia were completely unknown, considerable advances have been made in ascribing at least a proportion to chromosomal and genetic disorders. Around 15% of all azoospermic men are known to be chromosomally abnormal, while current estimates of the proportion of men showing microdeletions on the Y chromosome long arm, which are thought to remove vital spermatogenesis genes, range from 8 to 15%. As shown by an ever-increasing number of laboratories, PCR analysis using Y chromosome STS fragments can now provide a means for rapid diagnosis amongst men with non-obstructive oligo- or azoospermia. Further refinement should lead to the eventual isolation of all the spermatogenesis genes on the Y chromosome, and a full understanding of their function will undoubtedly be revealed by assessment of phenotype–genotype correlation. There is every indication that an accurate diagnosis of many more cases of hitherto ‘unexplained infertility’ will be possible in the future.

**References**


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