REVIEW
The Y chromosome and male fertility and infertility[1]

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Summary

Since 1995, thanks to a large number of studies, Y chromosome microdeletion screening has become part of the routine diagnostic work-up of severe male factor infertility. Many initial contradictory issues such as variability in deletion frequency, markers to be tested, presence of deletions in 'fertile' men, and genotype–phenotype correlation has been resolved. Past and present unresolved issues are discussed in this review.

Keywords: Y chromosome, fertility, male infertility, microdeletions, spermatogenesis, genetics

Although it has been established in 1976 that deletions of the long arm of the Y chromosome are associated with spermatogenic failure (Tiepolo & Zuffardi, 1976), it is only in the last few years have these regions been defined at a molecular level. Vogt et al. (1996) observed that Y chromosome microdeletions follow a certain deletion pattern, with three recurrently deleted non-overlapping subregions in proximal, middle, and distal Yq11, designated 'AZFa', 'AZFb' and 'AZFc', respectively. Since 1994 several combined clinical and molecular studies have been performed in order to define the role of Y chromosome microdeletions and Y chromosome genes in male infertility. However, published data has been often confusing and contradictory concerning the deletion frequencies and genotype–phenotype correlations. The pathogenetic role of Y deletions in male infertility has been even questioned by reports describing Y microdeletions in 'proven fertile men' (Pryor et al., 1997; Kent-First et al., 1999). Although Y chromosome microdeletions represent the most frequent molecular genetic cause in infertile men, as many as 85% of azoospermic and 90% of severe oligospermic men do not have deletions. Other Y chromosome related factors such as variations in repeat sequences in multicopy gene families, mutations or

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polymorphisms in Y specific genes, rearrangements such as duplications, could contribute to the infertile phenotype.

Y chromosome and fertility

Although the concept of 'fertility is not a synonym of normozoospermia' is known to andrologists, most studies used 'proven fertile men' with unknown sperm count, as a control group rather than 'normospermic men'. In 12 men of 2295 controls, small deletions outside the DAZ region have been reported (for review see Krausz & McElreavey, 2001 and references therein). They may be rare polymorphic variants (Kostiner et al., 1998) or related to technical errors, or the fertile men may themselves have been oligozoospermic. Natural transmission of deletions removing the entire AZFc deletions has been also reported (Vogt et al., 1996; Pryor et al., 1997; Chang et al., 1999; Saut et al., 2000), but sperm analysis was available only in two cases. Two fathers (one with reduced and the other with unknown sperm count) were able to father only one child (Vogt et al., 1996; Pryor et al., 1997) whereas the father of four infertile sons was azoospermic many years later after the natural conception of his sons (Chang et al., 1999). These findings indicate that the natural transmission of this genetic defect is likely to be related to a situation where the father had oligozoospermia with progressive decrease of his sperm count over time associated with a high fertility state of the female partner. Consistent with this hypothesis, a progressive decrease of sperm number over time has also been reported in infertile men with AZFc deletions (Girardi et al.,

1997; Simoni *et al.*, 1997; Saut *et al.*, 2000). Environmental effects or different genetic backgrounds (compensation for the absence of Yq genes, by autosomal or X-linked factors) may also account for these variable phenotypes.

Recently, a total of 392 normospermic men have been tested and no Yq microdeletions have been reported for this category of men (see Krausz & McElreavey, 2001 and references therein). This data is in accordance with a specific role for Y chromosome microdeletions of the AZF regions in spermatogenic failure.

Frequency of Yq deletions

The incidence of Y microdeletions in infertile men varies between studies, from 1% (van der Ven *et al.*, 1997) to 55% (Foresta *et al.*, 1998). Factors related to (i) study design (inclusion criteria, clinical definition of patients); (ii) lack of rigorous testing of negative results (false deletions), and (iii) genuine population variances may explain this variability. We studied four different populations (three of European and one of non-European origin) with a similar set of primers and using homogeneous criteria for the definition of 'idiopathic' and 'non-idiopathic' infertility (Krausz *et al.*, 1999a, b, 2001a; C. Krausz, L. Quintana-Murci, H. Sayer & K. McElreavey, personal communication). The deletion frequency calculated for the overall study population of each study ranged from 1.5–10.8% (Fig. 1). The highest frequencies were found in two studies with the largest number of azoospermic men included. The calculation of deletion frequency for uniformly defined group of idiopathic severe oligo- and azoospermic men

showed a more homogeneous figure, of 10–18%. Our results indicate that the main factor influencing deletion frequency is the composition of the study population and ethnic or geographical differences apparently have no influence on it, although the number of populations studied is small and needs to be expanded.

In four study populations we tested a total of 318 patients (250 severe oligo and azoospermic men) and 200 normospermic controls for the absence of gene-specific markers in the three regions: DBY and USP9Y (AZFa); eIF-1AY (AZFb); BPY2 and DAZ (AZFc; Krausz *et al.*, 1999a, b, 2001b; Krausz *et al.*, submitted). No isolated gene deletions were found. Interestingly, a group (Foresta *et al.*, 2000) have reported isolated gene-specific deletions within the AZFa region. This finding has not been confirmed in more than 1300 infertile males tested for AZFa genes by different groups [Krausz *et al.*, 1999a, 2001a; Sun *et al.*, 1999 (only USP9Y tested), Peterlin *et al.*, 2002; Frydelund-Larsen *et al.* (only DBY tested)]. Considering that no specific phenotype has been reported for patients bearing 'isolated gene' deletions (Foresta *et al.*, 2000), it is unlikely that the lack of isolated gene deletions in the other studies is related to patient selection criteria.

Based on our own data and data published elsewhere (for review see Simoni *et al.*, 1998; Krausz & McElreavey, 1999; Foresta *et al.*, 2001) it can be concluded: (i) Y deletions have been found almost exclusively in patients with <1 million spermatozoa/mL; (ii) deletions are extremely rare with a sperm concentration >5 millions of spermatozoa/mL (approximately 0.7%) and in certain cases deletions removed only single STSs which without further confirmation by
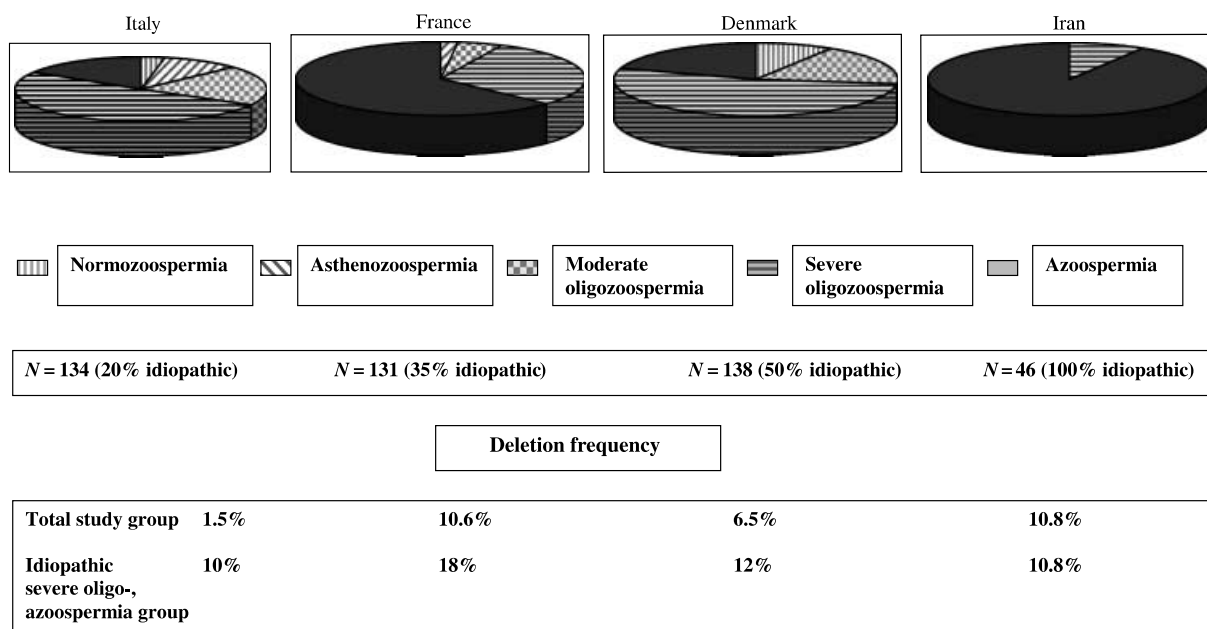
Figure 1. Schematic representation of the results of the four studies cited in the text: Italian (Krausz *et al.*, 1999a), French (Krausz *et al.*, 1999b), Danish (Krausz *et al.*, 2001) and Iranian (submitted), using a similar set of markers and clinical definition. The deletion frequency of the total study population and the deletion frequency of the subgroup comprising idiopathic azoospermia and severe oligozoospermia are indicated.

other techniques, such as Southern blotting, are of dubious significance; (iii) the most frequently deleted region is AZFc (approximately 60%), followed by deletions of the AZFb and AZFb + c or AZFa + b + c regions (35%) whereas deletions of the AZFa region are extremely rare (5%); (iv) isolated gene-specific deletions must be extremely rare and found only for AZFa genes so far, and (v) Deletions have also been found independent of the concomitance (chance associations) of abnormal andrological findings (7%) such as varicocele, cryptorchidism, hypogonadotrophic hypogonadism, obstructive azoospermia, etc.

Genotype–phenotype correlation

Deletions removing the entire AZFa or AZFb regions ('*complete*' deletions) are associated with Sertoli Cell only syndrome (SCOS) and spermatogenic arrest, respectively (Krausz *et al.*, 2000; Kamp *et al.*, 2001). *Partial* deletions of these regions or *complete* or *partial* AZFc deletions are associated with a variable phenotype ranging from hypospermatogenesis (oligozoospermia) to SCOS. A possible explanation for such a variable phenotype is a progressive regression of the germinal epithelium over time which has been reported in patients with AZFc deletions (Warchol *et al.*, 2000; Calogero *et al.*, 2001). An alternative explanation for the variable phenotype is related to influences of the genetic background and environmental factors in different individuals.

In two studies of the Danish population complete hormonal analysis was available in all patients (Krausz *et al.*, 2001a; Frydelund-Larsen *et al.*, 2002) FSH levels were above the mean value in all cases. Serum Inhibin B concentrations were uniformly below the normal range in each patient, indicating that abnormally low levels of Inhibin B should be included among the indications for a Y chromosome microdeletion screen. Testicular endocrine function, in a total of 89 oligo-azoospermic patients with and without microdeletions, have been recently evaluated by an other group (A. Tomasi, personal comunication). Inhibin B and FSH levels were undistinguishable in patients with idiopathic and microdeletion-associated oligo-azoospermia (A. Tomasi, personal comunication). These data do not support the hypothesis proposed by an other group (Foresta *et al.*, 2002), that microdeleted patients have a less severe impairment of Sertoli cell function than patients with idiopathic oligo-azoospermia. The relatively high level of Inhibin B, found in the group of men with AZF deletions presenting SCOS by Foresta *et al.* (2002) may be related to the different biopsy procedures used in the studies. Fine needle biopsy used by Foresta *et al.* may lead to overlooking of areas of spermatocytic arrest or to a false overrepresentation of SCOS.

Clinical significance of Y deletions

The identification of Y deletions has a diagnostic, prognostic and preventive value. In azoospermic men, the presence of a *complete* AZFa or AZFb deletion has a negative prognostic value for testicular sperm retrieval (Brandell *et al.*, 1998; Krausz *et al.*, 2000; S. Silber, personal comunication). In patients presenting oligozoospermia who are at risk for a progressive decrease of sperm concentration over time, cryoconservation of spermatozoa could avoid future invasive techniques such as TESE/ICSI (Krausz & McElreavey, 1999).

Genetic counselling

Spermatozoa from patients with Yq microdeletions have been found to be fully fertile both following IVF and ICSI procedures and even by natural conception. However, it is not clear if the fertilization rate and embryo development are comparable with that observed in men without deletions (Rossato *et al.*, 1998; Silber *et al.*, 1998; van Golde *et al.*, 2001). After conception, Y deletion is obligatory transmitted to the male offspring. The phenotype of son may vary substantially and the extent of spermatogenic failure cannot be predicted entirely because of different genetic background and the presence or absence of environmental factors with potential toxicity to reproductive function.

We have recently reported that a significant proportion of spermatozoa from men with Y microdeletion are nullisomic for sex chromosomes (Siffroi *et al.*, 2000). This result indicates a potential risk for the offspring to develop 45,X0 Turner's syndrome and other phenotypic anomalies associated with sex chromosome mosaicism, including ambiguous genitalia. The screening for Y chromosome microdeletions in patients bearing a mosaic 46XY/45X0 karyotype with sexual ambiguity and/or Turner stigmata has shown a relatively high incidence of AZFc deletions (33%; Patsalis *et al.*, 2002). These data suggest that some Yq microdeletions are associated with an overall Y chromosomal instability leading to the formation of 45,X0 cell lines. In order to avoid the transfer of embryos with sex chromosome mosaicism, pre-implantation diagnosis could be offered to the couple.

Genetic studies (caryotype and Y chromosome microdeletion screening) combined to long-term follow up of ICSI babies born from father at high risk to be carrier for genetic anomalies, i.e. severe male factor infertility, are needed.

Y chromosome background and sperm production

Although Y chromosome microdeletions are the most frequent genetic cause of oligo/azoospermia the majority of patients have a grossly intact Y chromosome. We hypothesize that some of these men may carry other rearrangements, undetected microdeletions, or sequence variants on the non-recombining region of the Y chromosome that are associated with reduced spermatogenesis. We have studied the association of the Y chromosome background with sperm counts

by defining Y chromosome haplogroups (hgr: are mono-phyletic groups of Y chromosomes which share common binary markers such as single nucleotide polymorphisms) in a group of Danish men with known sperm count (Krausz *et al.*, 2001a). This data was compared as the haplogroup distribution with that of the general Danish population. We found that one class of Y chromosomes, referred to as haplogroup 26+, was significantly over-represented (27.9%; $p < 0.001$) in the group of men with either idiopathic oligozoospermia or azoospermia compared with the general male population (4,6%; Krausz *et al.*, 2001a). The odds ratio of developing either azoospermia or oligozoospermia between Danish individuals with Y chromosomes belonging to hg 26+ compared with hg1 (the most frequent hgr in Denmark) was 8.92 (2.8–28.5; 95% confidence limits). Sperm count is declining in certain industrialized European countries by 2% per year and it seems that this decline began within the last 30–40 years. Our data indicate that a class of Y chromosome is over-represented in men with reduced sperm counts, 70% of men bearing hgr 26+ has <5 million spermatozoa/mL. Sperm counts within this range are associated with very poor reproductive success and, in the absence of assisted reproduction, these chromosomes will be rapidly eliminated from the population. These data, taken together suggest that the selection acting on this class of Y chromosomes is a recent phenomenon. Environmental pressure by as yet unknown factors that are active in Denmark, could explain negative selection on a rare haplotype that is associated with decreased reproductive fitness. Within hg 26+, rearrangements, polymorphisms in Y chromosome specific gene coding sequences or differences in gene copy numbers, all of which in theory may alter spermatogenic efficiency. Further studies are needed in order to determine the molecular mechanism of this susceptibility.

Conclusions and perspectives

Since 1995, thanks to a large number of studies, Y chromosome microdeletion screening has become part of the routine diagnostic work-up of severe male factor infertility. Many initial contradictory issues such as variability in deletion frequency, presence of deletions in 'fertile' men and the number of STS to be used to obtain high sensibility and accuracy have been resolved. It is clear that deletion frequency varies in different subgroups of patients according to sperm number (azoospermic vs. oligospermic men) and aetiology (idiopathic vs. non–idiopathic infertility). The absence of deletions in normospermic subjects indicates a specific association between deletions and spermatogenic failure. Moreover, it is clear that for diagnostic purposes it is more important a relatively small number of well-choosen

markers rather than their absolute number (Simoni *et al.*, 1998). The use of such minimal set of primers (Simoni *et al.*, 1999) allows the detection of >95% of clinically relevant deletions.

Despite progresses in the field, there are still many unresolved issues such as the definition of the function of AZF genes, the incidence of gene-specific deletions and mutations, the role of deletions of single or multiple copies of multicopy gene families, and the role of other structural anomalies such as inversions and duplications in the spermatogenic damage.

Although a strict genotype–phenotype correlation has been observed for the *complete* deletions of the AZFa and AZFb regions, the variable phenotype associated with *partial* AZFa and AZFb and all types of AZFc deletions indicates the importance of the genetic background (for example compensation by X and autosomal homologues) and eventually of environmental factors in the statement of certain gene defects. Future studies will be oriented on male fertility genes situated outside the Y chromosome. There are an increasing number of autosomic and X linked genes identified in animal models mainly using the knock-out strategy. The systematic screening for mutations in human homologues would be expensive and time consuming. A more appropriate approach is the search for candidate genes involved in spermato- and spermiogenesis using technologies which allow the analysis of changes in gene statement profile in testis tissue or sperm suspension with different type of anomalies. Another approach for the identification of non–Y fertility genes is the study of familial cases of infertility through linkage and association studies.

Technological progresses in molecular genetics and accumulation of data from animal models and from the human genome project represent an excellent background for a large-scale research in the field of genetics of male infertility.

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