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INVITED LECTURE

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Y chromosome microdeletions are the most frequent genetic cause of severe oligozoospermia (<5 million spermatozoa ml^{-1}) and azoospermia (absence of spermatozoa in the ejaculate) (for review see Krausz *et al.*, 2003 and references therein). The Y microdeletions associated with infertility occur in specific regions of the long arm of the Y called azoospermia factor (AZF) regions (Tiepolo & Zuffardi, 1976). Originally, three types of AZF deletion (AZFa, AZFb and AZFc) have been described by Vogt *et al.* (1996); however, after the complete physical map and sequence of the AZFb and AZFc regions, it became evident that the AZFb and AZFc intervals are partially overlapping (Repping *et al.*, 2002). The Y chromosome is extremely rich of repetitive sequences organized in amplicons. Ampliconic sequences are characterized by sequence pairs showing nearly complete (>99.9%) nucleotide identity, organized in massive palindromes. These repeated sequences may undergo genetic exchange through gene conversion, i.e. nonreciprocal transfer of sequence information occurring between duplicated sequences within the chromosome, a process that could account for the >99.9% nucleotide identity between the arms of a palindrome. Although this mechanism may serve to preserve Y genes from the gradual accumulation of deleterious mutations and thus prolong their genetic fitness, this peculiar organization also provides the structural basis for deletions and rearrangements.

The complete Y chromosome sequence and its gene content has now been published in GenBank. The human Y chromosome contains mainly gene encoding factors, which are essential for male fertility. Although the majority of the AZF candidate genes have been identified years ago, their exact function and role in spermatogenesis remain unclear (for review see Skaletsky *et al.*, 2003 and references therein).

Despite this fact, after resolving many initial contradictory issues such as specificity of Y deletions, variability in deletion frequency, markers to be tested, genotype–phenotype correlation, the essential role of Y chromosome related factors in the spermatogenetic process, and thus the clinical significance of AZF deletions have been well established.

Clinical significance of Y deletions

Are Y deletions specific for spermatogenic failure?

The aetiopathogenetic role of Y deletions in male infertility has been questioned by reports describing Y microdeletions in 'proven fertile men', however, appropriate studies using normospermic men as controls and not 'fertile subjects with unknown sperm count', have highlighted that AZF deletions are specific for spermatogenic failure (see Krausz & McElreavey, 2001 and references therein).

What is the frequency of this genetic anomaly among infertile men?

The incidence of Y deletions varies enormously among studies from 1 to 55%. Many of the initial studies suffered from technical problems, and papers presenting a mosaic type of pattern of deletions, especially with deletions of single sequence tag sites (STS) without confirmation are of dubious significance. Apart from the lack of rigorous testing of negative results or inappropriate choice of markers, other factors such as differences in the composition of the study populations (clinical characteristics and ethnic background) have been evoked to explain this variability. We studied four different populations using a similar set of markers and similar clinical criteria for the definition of patients. It was demonstrated that the main factor influencing deletion frequency is the composition of the study population (highest frequency found in the two studies with the largest number of azoospermic men included) rather than the ethnic origin of a given population (Krausz *et al.*, 1999a,b, 2001a, 2003). The incidence of Y deletions is 10–15% in azoospermic men and 5–10% in oligospermic men.

Which category of patients has to be tested?

Y deletions have been found almost exclusively in patients with <1 million spermatozoa ml^{-1} and are extremely rare with a sperm concentration of >5 million spermatozoa ml^{-1} (approximately 0.7%). Deletions have also been

found (7%) as 'chance association' in the presence of other abnormal andrological findings such as varicocele, cryptorchidism, hypogonadotrophic hypogonadism, etc. (Krausz *et al.*, 1999b).

In two studies of the Danish population, complete hormonal analysis was available in all patients and serum inhibin B concentration was uniformly below the normal range in patients with microdeletions because of their severe spermatogenic impairment (Krausz *et al.*, 2001a; Frydelund-Larsen *et al.*, 2002). In a large study by Tomasi *et al.* (2003), inhibin B and follicle-stimulating hormone levels were undistinguishable in patients with idiopathic and microdeletion-associated oligo/azoospermia. These data do not support the hypothesis proposed by another group (Foresta *et al.*, 2002) that microdeleted patients have a less severe impairment of Sertoli cell function than patients with idiopathic oligo/azoospermia.

Therefore, indications for AZF microdeletion analysis are sperm concentrations of <5 million spermatozoa ml^{-1}.

Is there any genotype–phenotype correlation?

Deletions removing the entire AZFa or AZFb regions ('complete' deletions) are associated with Sertoli cell only syndrome (SCOS) (Kamp *et al.*, 2001) and spermatogenic arrest (Vogt *et al.*, 1996; Krausz *et al.*, 2000; Hopps *et al.*, 2003) respectively. Partial deletions of these regions or complete or partial AZFc deletions are associated with a variable phenotype ranging from hypospermatogenesis (oligozoospermia) to SCOS (azoospermia). 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. An alternative explanation for the variable phenotype is related to influences of the genetic background and environmental factors in different individuals.

Which markers have to be tested?

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%). For diagnostic purposes, it is important to use a relatively small number of well-chosen markers, which cover all the three AZF regions (Simoni *et al.*, 2004). It is still debated if gene-specific deletion screening, which theoretically is more appropriate, gives any advantage in the clinical management of the patients. The absence of isolated gene specific deletions in >1600 severe male factor patients are suggestive of no practical advantage (see for review Krausz *et al.*, 2003 and references therein; Silber *et al.*, 1998).

Once a deletion is found, it is important to define with a second set of primers, the extension of the deletion especially in case of AZFa and AZFb deletions (see below).

What is the clinical significance of Yq deletions?

The identification of Y deletions has a diagnostic, prognostic and preventive value as: (i) the presence of Y microdeletions explains the aetiology of infertility and thereby helps avoid unnecessary medical and surgical treatments (for e.g. correction of varicocele, Cayan *et al.*, 2001); (ii) 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; Hopps *et al.*, 2003); (iii) in patients presenting oligozoospermia who are at risk for a progressive decrease of sperm concentration over time, cryopreservation of spermatozoa could avoid future more invasive techniques such as testicular sperm extraction/intracytoplasmic sperm injection (TESE/ICSI) (Krausz & McElreavey, 1999).

Genetic counselling

Are spermatozoa bearing Y chromosome microdeletions fully fertile?

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.

What will be the phenotype of the son?

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.

Is there any other risk than infertility?

We have previously 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. 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. Until now, only 16 male and eight female ICSI babies born from fathers affected by Yq microdeletions have been reported. It appears that the children are phenotypically normal (except one son born with pulmonary atresia and a hypoplastic right ventricle, Page *et al.*, 1999) and no ambiguous genitalia or Turner's syndrome have been observed among them. Considering that embryos bearing a 45,XO karyoptye have a higher risk of spontaneous abortion, it would be important to know whether among the partners of Y deleted men there is a higher incidence of spontaneous abortion. If this is the case, pre-implantation diagnosis could be offered to the couple.

The gr/gr deletions

The classical AZFc deletion, which removes 3.5 Mb between the b2/b4 amplicons is the most frequent type of deletion. Considering the Y structure and the suggested deletion mechanism, a number of other possible partial deletions have been proposed both in the AZFb and AZFc regions. The frequency and the pathological significance of these partial deletions is not yet clear, although a partial deletion termed 'gr/gr' has been described specifically in infertile men with varying degrees of spermatogenic failure (Repping *et al.*, 2003). This deletion removes half the AZFc gene content including two copies of the major AZFc candidate gene called DAZ (deleted in azoospermia). Another deletion named 'b2/b3' (Repping *et al.*, 2004) or 'u3-gr/gr' (Machev *et al.*, 2004) or 'g1/g3' (Fernandes *et al.*, 2004), which removes a similar quantity of AZFc genes seems to have no effect on fertility status in association with a certain Y chromosome background commonly present in Northern Eurasian populations (Y haplogroup N). A similar conclusion can also be drawn for the 'gr/gr' deletion found in association with Hgr D2b, which is present in 20% of Japanese men. It is therefore possible that the type and number of missing gene copies or other unknown Y related factors (for example duplications or beneficial mutations in other parts of the Y chromosome) may also influence the phenotype.

We studied a large group of normospermic ($n = 189$) and oligo/azoospermic ($n = 150$) men using an STS plus/minus method. Our analysis showed that: (i) partial AZFc deletions are not specific for spermatogenic failure; (ii) 'gr/gr' deletion can be considered as a risk factor, as its frequency was significantly higher in the oligo/azoospermic group (5.3%) with respect to controls (0.5%) $P < 0.012$. Gene-specific analysis revealed three distinct deletion patterns, indicating that further combined studies based on gene copy and haplogroup analysis are likely to provide a means for the distinction between pathogenic and neutral (or compensated by other Y factors) deletion types (Giachini *et al.*, 2005).

Y chromosome and genetic susceptibility to infertility

For a number of years, different lineages of the human Y chromosome have been used extensively by human population geneticists to trace population movements and understand human origins and histories. Positive selection (such as increased fecundity) or negative selection (reduced fertility) on the Y chromosome can be determined by screening for associations between Y chromosome haplogroups and male-specific phenotypes such as infertility or germ cell cancer. It is now possible to use a wide variety of molecular tools to explore questions related to the Y chromosome and male reproductive fitness. We and others have applied Y chromosome haplogroup distributions in study and control populations to understand the relationship between different classes of Y chromosome and susceptibility to AZF microdeletions and reduced sperm counts. (i) Y chromosome microdeletions were found to occur on different Y chromosome backgrounds, and there was no significant difference between Y chromosome haplogroup distribution in the study and control groups. However, the number of AZFa deletions included in the studies was small and it remains to be seen if there is predisposing class of Y-chromosomes for this type of deletion (Paracchini *et al.*, 2000; Quintana-Murci *et al.*, 2001). (ii) The association between Y chromosome haplogroups and sperm counts has been explored in three populations, the Danish, Japanese and Italian. In Denmark, a study of men with $<20 \times 10^6$ spermatozoa ml^{-1} revealed a significant overrepresentation of haplogroup 26 (Krausz *et al.*, 2001b). In the Japanese and Italian populations, the results are mixed both the presence (Kuroki *et al.*, 1999) and absence (Paracchini *et al.*, 2002) of predisposing Y haplogroups have been reported.

Conclusions

The screening for Yq deletions became part of the routine diagnostic work-up of severe male factor infertility in many assisted reproductive technique and andrology clinics. Although the indications for testing and the markers to be used are well defined, many laboratories are producing unreliable results. If we exclude 'unusual deletions' found only in certain laboratories, the minimal set of primers proposed by the European Academy of Andrology (Simoni *et al.*, 2004) is able to detect almost 100% of clinically relevant deletions.

Future studies are addressed to the definition of the function of Y genes in spermatogenesis and the incidence of their intragenic mutations. An other challenging area with potential clinical value concerns the reduction of single copies of multicopy AZF genes and the definition of pathogenic gr/gr deletions.

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