DEBATE—continued

Prognostic value of Y deletion analysis

The role of current methods

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Y chromosome microdeletions represent the most frequent genetic alteration in azoospermic and severely oligozoospermic men, and screening for microdeletions in AZFa, b and c are routinely performed in the major andrology and infertility centres. Since patients with Y microdeletions often require intracytoplasmic sperm injection (ICSI), the question of whether the type of the microdeletion present could have prognostic value for the presence of spermatozoa in the ejaculate or in the testes [by testicular sperm extraction (TESE)] is an interesting one. The review of the literature on this topic showed that there is still no clear genotype–phenotype relationship, i.e. similar testicular alterations may be caused by different types of microdeletions, and apparently identical microdeletions may be associated with diverse tubular damage. Even in azoospermic men, the localization of the microdeletion cannot be used as a valid prognostic parameter before TESE–ICSI to identify patients with spermatozoa in their testes. The only finding with absolute negative prognostic value is the presence of complete AZFa–c deletions, which are invariably associated with an absence of spermatozoa. Microdeletions in AZFa or AZFb seem to have promising prognostic value, but more data and gene–specific deletions have to be provided to draw clear conclusions. The absence of a clear genotype–phenotype relationship, and therefore of a prognostic value of Y deletion analysis, is probably due to the current methods used for the screening of the microdeletions. In fact, to date most centres do not use gene-specific markers but instead use anonymous primers that contribute little information to the pathogenic role of the microdeletions.

Key words: azoospermia/ICSI/pathogenesis/TESE/Y chromosome deletions

Microdeletions of the Y chromosome long arm (Yq) have assumed in the last few years a fundamental relevance in the management of the infertile male, since it is now clear that they represent an important cause of spermatogenic alteration and the most frequent genetic aetiology of severe testiculopathy. Basic research in the field of the genetics of male infertility has been of primary importance to drive the current clinical approach of the infertile patient, and this is exemplified by Y chromosome screening that is now performed in major andrology and infertility centres. The explosive growth in the use of intracytoplasmic spermatozoon injection (ICSI) and related techniques has further contributed to the research of possible genetic aetiologies of male infertility, due to the risk of transmission of existing genetic abnormalities to the offspring. These concerns are of particular interest for male infertility related to Y chromosome microdeletions since many of these patients require ICSI. In fact, microdeletions cause severe oligozoospermia or azoospermia although spermatozoa are frequently found in the testes. The prevalence of deletions of the Y chromosome in such patients is 10–15% (Hargreave, 1999; Foresta et al., 2000a; Ma et al., 2000).

Indeed, the term ‘microdeletion of the Y chromosome’ does not represent a well-defined genetic diagnosis at the molecular level. It defines only the absence of segments of the Y chromosome and it includes many different conditions depending on the localization and the extent of the deletion. Furthermore, microdeletions have to be distinguished from more limited gene deletions and even gene mutations.

A recent review of the literature (Foresta et al., 2001) showed that nearly 5000 infertile men have been analysed for the presence of Y microdeletions and the results published from 1992. Taken together, these data show that the overall prevalence of microdeletions in infertile patients is 8%, but remarkable differences exist among the various studies, ranging from 1% (van der Ven et al., 1997) to 35% (Ferlin et al., 1999). One hypothesis to explain such differences is related to the different patient selection criteria (Foresta et al., 2001), strengthening the concept that male infertility is not a homogenous disorder. For example, the prevalence of microdeletions increases with more strict patient selection and the higher percentages are found in patients affected by idiopathic severe oligozoospermia (prevalence of 14%) and in idiopathic non-obstructive azoospermia (16%). Microdeletions most frequently involve the AZFc (azoospermia factor c) region (60%), less frequently the AZFb region (16%) and only rarely the AZFa interval (5%). Larger microdeletions involving two or three AZF regions are diagnosed in 14% of cases. Notably, in the remaining 5% of cases the microdeletions are located in regions not overlapping AZFa, b or c.

The most intriguing data resulting from the analysis of the literature are related to the so-called genotype–phenotype
conditions, which can be diagnosed only by diagnostic biopsy of testicular spermatozoa. AZFa et al. Sertoli cell-only syndrome (SCOS) to hypospermatogenesis, prognostic parameter in order to identify patients with sperm for several reasons: it is well known that azoospermia could deletions, in azoospermic men requiring ICSI the localization of microdeletions. AZFb to produce azoospermia more frequently than severe oligozoospermia (Table I). Furthermore, it should be kept in mind that a microdeletion of microdeletions may be associated both with azoospermia have prognostic signiﬁcance (Reijo et al., 1995; Sargent et al., 2000b). Patients with deletions in the AZFb region could have variable defects and in the majority of cases a spermatogenic arrest is observed (Elliott, 2000; Foresta et al. 2001). However, it seems that men with complete deletions of the AZFb region have almost no chance of sperm retrieval with ICSI (Brandell et al., 1998), but this finding has to be conﬁrmed since only low numbers of patients have been studied and reported. The phenotype associated with deletions in the AZFa region was initially considered to be SCOS, but more recent evidence demonstrated that in this case no genotype–phenotype relation exists (Foresta et al., 2000c). Two genes have been clearly mapped to the AZFa interval, although the presence of other genes cannot be deﬁnitively excluded (Sargent et al., 1999; Sun et al., 1999; Foresta et al., 2000b). Infertile patients with speciﬁc deletions of AZFa-genes have been described (Sun et al., 1999; Foresta et al., 2000b), as well as one patient with a point mutation in USP9Y (Sun et al., 1999). A more precise genotype–phenotype relationship could therefore be attempted in this case (Foresta et al., 2000c) (Table I), looking at single gene deletions. Deletions or even point mutations in USP9Y may result in severe hypospermatogenesis (Sun et al., 1999; Foresta et al., 2000b), whereas the loss of DBY may be associated both with SCOS and severe hypospermatogenesis (Foresta et al., 2000b). Patients with deletion of both USP9Y and DBY (and therefore lacking the entire AZFa region) are invariably azoospermic with a testicular histology of SCOS. Therefore this type of deletion seems to have prognostic signiﬁcance, but the small number of patients reported does not allow the drawing of clear conclusions (Table I). Furthermore, it should be kept in mind that a diagnosis of SCOS based on a biopsy of a random site within the testis does not necessary preclude the finding of a small amount of sperm at TESE, since focal spermatogenesis could be present. As previously shown with the seminal pattern, a strict association between genotype and phenotype is clearly evident only in patients with larger deletions involving more than one AZF locus, and this is exempliﬁed by the invariable finding of SCOS in patients with deletions of AZFa-c, and the total absence of testicular spermatozoa at TESE when deletions extending to the AZFc region (AZFb-c or AZFa-c) are present (Silber et al., 1998). In conclusion, apart from these very large deletions, in azoospermic men requiring ICSI the localization of the microdeletion cannot at present be used as a valid prognostic parameter in order to identify patients with sperm in their testes. Promising adverse prognostic ﬁndings for TESE are the presence of a complete AZFa or AZFb deletions, while speciﬁc deletions removing USP9Y seem to predict the presence of testicular spermatozoa.

The second and most important question is whether we could predict the presence of spermatozoa in the testis in order to undergo TESE–ICSI in azoospermic patients. This is crucial for several reasons: it is well known that azoospermia could be associated with different tubular pictures, ranging from Sertoli cell-only syndrome (SCOS) to hypospermatogenesis, spermatogenic arrest and obstructive forms (Foresta et al., 1995). At present no clinical parameter has been clearly demonstrated to be able to distinguish among these different conditions, which can be diagnosed only by diagnostic biopsy or ﬁne needle aspiration cytology. It would be very important if the type of the deletion could be used in azoospermic men as a predictor of sperm recovery at TESE. However, in this case the review of the literature did not demonstrate a clear relationship between the localization of the microdeletion and the testicular phenotype. In fact, the testicular histology in patients with deletions limited to AZFc may vary from SCOS to spermatogenic arrest and hypospermatogenesis (Foresta et al., 2001). Furthermore, this type of microdeletion does not predict the presence of spermatozoa in the testes at TESE (Silber et al., 1998). Patients with deletions in the AZFb region could have variable defects and in the majority of cases a spermatogenic arrest is observed (Elliott, 2000; Foresta et al. 2001). However, it seems that men with complete deletions of the AZFb region have almost no chance of sperm retrieval with ICSI (Brandell et al., 1998), but this finding has to be conﬁrmed since only low numbers of patients have been studied and reported. The phenotype associated with deletions in the AZFa region was initially considered to be SCOS, but more recent evidence demonstrated that in this case no genotype–phenotype relation exists (Foresta et al., 2000c). Two genes have been clearly mapped to the AZFa interval, although the presence of other genes cannot be deﬁnitively excluded (Sargent et al., 1999; Sun et al., 1999; Foresta et al., 2000b). Infertile patients with speciﬁc deletions of AZFa-genes have been described (Sun et al., 1999; Foresta et al., 2000b), as well as one patient with a point mutation in USP9Y (Sun et al., 1999). A more precise genotype–phenotype relationship could therefore be attempted in this case (Foresta et al., 2000c) (Table I), looking at single gene deletions. Deletions or even point mutations in USP9Y may result in severe hypospermatogenesis (Sun et al., 1999; Foresta et al., 2000b), whereas the loss of DBY may be associated both with SCOS and severe hypospermatogenesis (Foresta et al., 2000b). Patients with deletion of both USP9Y and DBY (and therefore lacking the entire AZFa region) are invariably azoospermic with a testicular histology of SCOS. Therefore this type of deletion seems to have prognostic signiﬁcance, but the small number of patients reported does not allow the drawing of clear conclusions (Table I). Furthermore, it should be kept in mind that a diagnosis of SCOS based on a biopsy of a random site within the testis does not necessary preclude the finding of a small amount of sperm at TESE, since focal spermatogenesis could be present. As previously shown with the seminal pattern, a strict association between genotype and phenotype is clearly evident only in patients with larger deletions involving more than one AZF locus, and this is exempliﬁed by the invariable finding of SCOS in patients with deletions of AZFa-c, and the total absence of testicular spermatozoa at TESE when deletions extending to the AZFc region (AZFb-c or AZFa-c) are present (Silber et al., 1998). In conclusion, apart from these very large deletions, in azoospermic men requiring ICSI the localization of the microdeletion cannot at present be used as a valid prognostic parameter in order to identify patients with sperm in their testes. Promising adverse prognostic ﬁndings for TESE are the presence of a complete AZFa or AZFb deletions, while speciﬁc deletions removing USP9Y seem to predict the presence of testicular spermatozoa.

Figure 1. Seminal pattern in patients with AZF deletions, based on analysis of homogeneous studies reporting clear semen analysis and patient classiﬁcation (Reijo et al., 1995, 1996; Stuppia et al., 1996; Vogt et al., 1996; Girardi et al., 1997; Hoefsloot et al., 1997; Kremer et al., 1997; Mulhall et al., 1997; Pryor et al., 1997; Simoni et al., 1997; van der Ven et al., 1997; Vereb et al., 1997; Brandell et al., 1998; Grimaldi et al., 1998; Liow et al., 1998; Rucker et al., 1998; Silber et al., 1998; Ferlin et al., 1999; Kim et al., 1999; Krausz et al., 1999a; Sargent et al., 1999).
The unclear genotype–phenotype relationship probably reflects our current knowledge of the AZF regions. In fact, the structure of these intervals, their gene content, and a detailed analysis of selected infertile patients are not yet available. AZF regions are very large since they are megabases in length and they include various genes (other than the ‘historical’ AZF-candidates such as DAZ and RBMY) whose function is still unknown (Lahn and Page, 1997; Vogt et al., 1997; Yen, 1998; Wong et al., 1999; Foresta et al., 2001). Only when all these genes are characterized in terms of structure, expression and loss of function, might we theoretically be able to have proper diagnostic and prognostic parameters. In fact, Y chromosome microdeletions are generally assessed for diagnostic purposes using a set of anonymous sequence tagged sites (STS) that frequently are not specific for the genes that map the AZF intervals. For example, most laboratories utilize two or three STSs for each AZF region and they include in their screening only the DAZ gene. This screening protocol is also suggested by the European Academy of Andrology (Simoni et al., 1999).

On the other hand, the use of an excessive number of anonymous STSs may lead to the identification of insignificant small deletions that actually represent normal polymorphisms without any pathogenic role (Pryor et al., 1997). Therefore, in the future, the general trend may be to study the Y chromosome using gene-specific markers (together with anonymous STSs). In this way the diagnosis will be more accurate and the study of a greater number of infertile patients with this protocol will probably allow us to better understand the biology of spermatogenesis and to clarify the genotype–phenotype relationships, a prerequisite for any prognostic purpose. Nevertheless, if we exclude the AZF-candidate genes DAZ, RBMY, USP9Y and DBY, little is known about the other genes mapping the AZF regions and only future studies will clarify if these are involved in human spermatogenesis and male infertility and if they should be included in the screening protocol. Furthermore, as previously noted, 5% of the microdeletions reported in the literature do not fall into any AZF region and it is possible that they include genes with still unknown functions in spermatogenesis. Obviously, no prognostic information can be made in such cases. With the current knowledge of this pathology, at least DAZ, RBMY, USP9Y and DBY should be included in the screening of Y microdeletions and research is needed in order to find deletions of single genes and point mutations with pathogenic role. In this light we have reported the characterization of deletions involving only DBY (Foresta et al., 2000b) and the first evidence of a deletion involving all but one of the DAZ copies and associated with severe hypospermatogenesis (Moro et al., 2000b), and Page’s group reported the identification of a point mutation in the USP9Y gene (Sun et al., 1999). Obviously, with the current proposed commercial ‘kits’ for the screening of Y microdeletions all these findings would not be possible.

Another issue concerning male genetic infertility is the question of who should undergo Y chromosome microdeletion testing. It is now clear that all infertile patients with a severe testiculopathy should be analysed for Y microdeletions, regardless of their clinical presentations (idiopathic versus non-idiopathic infertility), hormonal parameters or testicular volumes (Krausz et al., 1999a; Foresta et al., 2000a). For example, we reported a high prevalence of microdeletions in infertile patients affected by unilateral ex-cryptorchidism or left varicocele and presenting with a severe bilateral testiculopathy (Foresta et al., 1999; Foresta et al., 2000a; Moro et al., 2000a). These studies demonstrated that the phenotype associated with a Y chromosome microdeletion might also be linked with cryptorchidism and varicocele, other than idiopathic infertility. The bilateral testicular damage observed in these patients is probably related to the deletion of the Y chromosome and not to the abnormal location of the testis or to varicocele itself, although these conditions may worsen the testicular alteration. These observations clarified that all infertile patients with a sperm count <5×10⁶/ml sustained by a severe bilateral testiculopathy should be screened for microdeletions, despite the presence of other concomitant causes of testicular damage.

Neither the hormonal parameters nor testicular volumes can be used to trace the relationship with Y chromosome microdeletions, and they do not allow us to distinguish between patients at risk for microdeletions. In all patients these parameters indicate a severe testiculopathy involving only the spermatogenic system, since testes are reduced in size, FSH concentrations are high, and LH and testosterone are within the normal ranges. However, one study reported that FSH concentrations in patients with microdeletions, although higher than controls, were lower than in patients with similar tubular alterations but without microdeletions (Kremer et al., 1997). Another study showed that Y-deleted patients had normal FSH concentrations (Liow et al., 1998). However, the most important point is that these hormonal parameters do not allow

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<th>Type of deletions</th>
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<td>Azoospermia</td>
<td>Severe oligozoospermia</td>
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<td>USP9Y</td>
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<td>DBY + USP9Y</td>
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SCOS = sertoli cell-only syndrome.

Table I. Genotype–phenotype relation in patients with deletion of AZFa-genes. Only the studies where the single genes were analysed and where testicular histology were available have been considered (Sargent et al., 1999; Sun et al., 1999; Foresta et al., 2000b)

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us to distinguish which specific region of the Y chromosome is deleted. This is not surprising considering that the testicular phenotype associated with the different localization of Y microdeletions may be very similar or identical. Furthermore, it is well known, for example, that in azoospermic patients FSH values and testicular volumes do not allow the clinician to distinguish between SCOS and severe hypospermatogenesis, or between spermatid arrest and obstructive forms (Foresta et al., 1995). Again, the only method to distinguish the specific tubular alteration present in azoospermic men is to directly analyse the testicular structure by diagnostic open biopsy or fine needle aspiration.

In conclusion, in our opinion the prognostic value of Y microdeletion analysis is very limited since our current knowledge of this pathology is still partial. In fact, the methods used to diagnose the microdeletions are not yet validated and a diagnosis could be possible only when a genotype–phenotype relationship can be demonstrated. This, in turn, will be the result of a detailed analysis of the genes contained in the AZF regions. To date, the prognosis as to whether an azoospermic man with Y microdeletion has spermatozoa in the testes that can be used for TESE–ICSI cannot be made on the basis of the localization of the deletion (apart from cases with deletions of the entire AZFa–c). The promising prognostic value of AZFa and AZFb deletion still awaits confirmation from the study of a larger number of patients. Furthermore, neither the clinical presentation (idiopathic versus non idiopathic) nor clinical parameters (testicular volumes, hormonal concentrations) support the clinician in his decision.

These data further strengthen the usefulness of a proper diagnosis of male infertility, which should include, at least when the patient is a candidate for TESE–ICSI, a careful analysis of the testicular alteration underlying the azoospermia. Such modern techniques of assisted reproduction should impose a more accurate management of the fertile man, since a correct diagnosis is essential to avoid unnecessary, expensive and stressful therapies (above all for the female partner) such as those related to multiple follicular growth and oocyte retrieval.

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References


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