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High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome

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Idiopathic Sertoli cell-only syndrome (SCOS) is characterized by azoospermia, small testes, absence of germ cells in the testes, elevated follicle stimulating hormone and normal testosterone concentrations. The Y-chromosome is involved in the regulation of spermatogenesis and in the pathogenesis of a fraction of idiopathic male infertility. An azoospermia factor (AZF) is present on the Y-chromosome long arm euchromatic region (Yq11) and two gene families (*DAZ* and *RBM*) have been identified within this region.

The aim of this study was to investigate whether a specific pattern of Yq11 microdeletions may be associated with idiopathic SCOS. Eighteen idiopathic subjects showing a testicular cytological picture of bilateral SCOS were selected and tested by polymerase chain reaction for a set of 29 Y-specific sequence-tagged sites (STS). We found Yq microdeletions in 10 out of 18 patients (55.5%) while the fathers or brothers of six out of 10 patients deleted for Yq were shown to carry an intact Y-chromosome. These deletions may therefore be considered as de-novo deletions and the cause of SCOS. The analysis of the microdeletions allowed us to identify two homogeneous regions that have a high incidence of deletion. The smallest deletion, common to all patients, is located in Yq interval 5. We therefore speculate that there is a relationship between specific, well-characterized Yq11 microdeletions and a testicular picture of SCOS, identifying an Y-related region frequently deleted in this syndrome.

In conclusion, the findings of this study demonstrate that a large percentage of idiopathic SCOS may be genetically determined and identify an Y-related region that seems to possess one or more still unknown genes essential for spermatogenesis.

Key words: fine needle aspiration/male infertility/Sertoli cell-only/Y-chromosome deletions

Introduction

Sertoli cell-only syndrome (SCOS) was first described by Del Castillo in 1947 and is suspected in the infertile man with idiopathic azoospermia, small testes, elevated follicle-stimulating hormone (FSH) and normal testosterone concentrations. The diagnosis can be made only with testicular biopsy or fine needle aspiration cytology (FNAC) (Foresta *et al.*, 1992) where only Sertoli cells with a complete absence of germ cells are found. Several pathological conditions, such as infections, toxic agents and radiation may also cause a loss of the testicular germinal population as a consequence of degenerative secondary effects. However, in these cases few germ cells may be observed in a small number of tubules. The prevalence of SCOS among non-obstructive azoospermic patients is ~30%, and 30% of these are idiopathic and can be considered as true SCOS (Foresta *et al.*, 1992, 1995). The pathogenesis of this condition remains unknown, and it has been postulated that the primary event occurs premeiotically before or during the proliferation phase of spermatogonia (Terada and Hatakeyama, 1991).

Recently, a genetic component has been proposed as the pathogenetic mechanism for a fraction of idiopathic severe oligozoospermia and azoospermia and various studies have focused on the possible role of the Y-chromosome in the regulation of spermatogenesis and in the pathogenesis of idiopathic male infertility (Bardoni *et al.*, 1991; Ma *et al.*, 1992, 1993; Vogt *et al.*, 1992, 1996; Chandley and Cooke, 1994; Reijo *et al.*, 1995, 1996; Kobayashi *et al.*, 1995; Najmabadi *et al.*, 1996; Stuppia *et al.*, 1996; Qureshi *et al.*, 1996; Kent-First *et al.*, 1996; Pryor *et al.*, 1997; Mulhall *et al.*, 1997; Foresta *et al.*, 1997). Tiepolo and Zuffardi (1976) first suggested the presence of a spermatogenesis locus (gene or gene complex) in the euchromatic region of the Y-chromosome long arm (Yq11), defined as azoospermia factor (AZF). Subsequent reports on infertile patients with small interstitial deletions (microdeletions) in this region confirmed these original findings and localized the AZF locus and possible genes for the regulation of male fertility in Yq11 interval 6 (Bardoni *et al.*, 1991; Ma *et al.*, 1992; Vogt *et al.*, 1992; Ma *et al.*, 1993; Chandley and Cooke, 1994; Reijo *et al.*, 1995, 1996; Kobayashi *et al.*, 1995; Najmabadi *et al.*, 1996; Stuppia *et al.*, 1996; Qureshi *et al.*, 1996; Mulhall *et al.*, 1997; Foresta *et al.*, 1997). Two gene families believed to play a role in male germ cell development and to be candidate genes for AZF have been identified: the *RBM* gene family (RNA-binding motif, formerly YRRM) (Ma *et al.*, 1993), and the *DAZ* gene cluster (deleted in azoospermia) (Reijo *et al.*, 1995). Both gene families have been isolated from Yq11.23, are expressed specifically in the testis and encode presumed RNA-binding protein (Ma *et al.*, 1993; Chandley and Cooke, 1994; Reijo *et al.*, 1995; Saxena *et al.*, 1996). However, they do not seem to be the only genes regulating spermatogenesis, since *RBM* or *DAZ* deletions may be detected both in oligozoospermic and in azoospermic subjects (Reijo *et al.*, 1996; Najmabadi

et al., 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996; Qureshi *et al.*, 1996; Kent-First *et al.*, 1996; Pryor *et al.*, 1997; Mulhall *et al.*, 1997; Foresta *et al.*, 1997) and microdeletions of the Y-chromosome other than at these gene loci seem to determine the same tubular alterations (Najmabadi *et al.*, 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996; Pryor *et al.*, 1997; Foresta *et al.*, 1997). Furthermore, there is recent evidence that regions of Yq11 outside interval 6 may play an important role in the regulation of spermatogenesis (Vogt *et al.*, 1996; Pryor *et al.*, 1997) and three distinct subregions, defined AZFa, AZFb and AZFc have been distinguished (Vogt *et al.*, 1996). The latter coincides with the AZF region in distal interval 6 containing the *DAZ* gene, while the first two could represent two further spermatogenesis loci in interval 5 and proximal interval 6. These authors have hypothesized a correlation between specific spermatogenic defects and a well-defined Yq11 microdeletion pattern, suggesting that deletions occurring in AZFa result in Sertoli cell-only syndrome and deletions in AZFb result in spermatogenic arrest. In other similar studies these associations were not evident; however, different patient selection criteria were used and frequently only seminal parameters were considered (Reijo *et al.*, 1995, 1996; Qureshi *et al.*, 1996; Pryor *et al.*, 1997; Foresta *et al.*, 1997).

Therefore, in this study we investigated Yq11 microdeletions by means of polymerase chain reaction (PCR) analysis in a large group of well-characterized idiopathic non-obstructive azoospermic subjects with SCOS diagnosed by FNAC.

Materials and methods

Patient selection

Our study was approved by the Hospital Ethical Committee, and informed consent was obtained from each patient. Among adult men referred to our infertility centre, we selected 59 azoospermic subjects in whom bilateral testicular FNAC showed a picture of SCOS, as described below.

Azoospermia was confirmed on two different semen analyses, separated by a 3 week interval, following a 3 day period of sexual abstinence, according to World Health Organization (WHO, 1992) guidelines for semen analysis. The diagnosis of azoospermia was established by pellet analysis after semen centrifugation (1000 *g*, 20 min). Subjects showing azoospermia on both occasions were recruited for this study.

All selected patients underwent ultrasound scanning of the testes to evaluate testicular size and rule out a subclinical varicocele and parenchymal lesions compatible with neoplasms (if found, these patients were excluded from the study and referred for further urological examination).

FSH and luteinizing hormone (LH) plasma concentrations were measured in each subject by radioimmunoassay using ^{125}I-labelled FSH and LH and a double monoclonal antibody (Ares-Serono, Milan, Italy). Testosterone plasma concentrations were determined using a double antibody radioimmunoassay.

Only patients with an apparently normal 46,XY karyotype, as shown by Giemsa–trypsin (G-banding) and Quinacrine fluorescent (Q-banding) were included in this study.

Thirty-five age-matched normozoospermic subjects ($n = 35$), whose characteristics were reported in our previous study (Foresta *et al.*, 1996), were considered as controls for seminal parameters, hormonal concentrations and testicular volumes.

Testicular fine needle aspiration and cytological quantification

The method has been described in detail previously (Foresta *et al.*, 1992). Briefly, bilateral FNAC was performed using 23 guage (0.6 mm) butterfly needles and aspirating with a 20 ml syringe. The cellular material was placed on two or more microscope slides for each testis, air- dried for 24 h, stained with May–Grünwald–Giemsa and examined under a light microscope at ×125, ×400 and ×1250 magnification. At least 200 spermatogenic cells were counted per smear. The following forms were identified and expressed as a percentage: spermatogonia (dark and pale), primary spermatocytes, secondary spermatocytes, early and late spermatids (corresponding to Sa–Sb and Sc–Sd steps of spermatogenesis, respectively), and spermatozoa. Sertoli cells are expressed as the Sertoli index (SEI, the number of Sertoli cells/100 spermatogenic cells), which has been found to be a reliable index of the tubular germ potential. The proportion of spermatozoa is expressed as the spermatic index (SI, the number of spermatozoa/100 spermatogenic cells).

As described in previous studies (Foresta *et al.*, 1992, 1995), cytological analysis in azoospermic subjects permits the identification of five different features: (i) SCOS; (ii) hypospermatogenesis; (iii) spermatogonial or spermatocytic arrest; (iv) spermatidic arrest; (v) normal germ line with an increased percentage of mature spermatozoa indicating an obstruction of the efferent ducts. Only patients with SCOS were included in this study. Diagnosis of SCOS was given when all the testicular material obtained from both testes contained only Sertoli cells without any germ cells (Foresta *et al.*, 1992, 1995).

Among azoospermic-SCOS patients only subjects showing no gonadal abnormalities, varicocele and without a history of cryptorchidism, post-mumps orchitis, cancer chemotherapy, irradiation, testicular trauma or other causes of possible testicular damage were selected, since in these cases SCOS can be considered idiopathic. According to these criteria we have recruited 18 idiopathic azoospermic subjects showing bilateral SCOS.

The results are given in the text as means ± SD. Statistical comparisons between groups were made by analysis of variance (ANOVA). $P < 0.05$ was regarded as being statistically significant.

Sequence-tagged sites (STS)–PCR and criteria used to define microdeletion

A set of 29 Y-specific STS, which span through the euchromatic region of Yq, was tested in each patient. All STS were previously described (Vollrath *et al.*, 1992; Ma *et al.*, 1993; Reijo *et al.*, 1995, 1996; Kobayashi *et al.*, 1995) and we used the order and the localization of the sequences proposed by Vollrath *et al.* (1992) and Foote *et al.* (1992), as reported by Reijo *et al.* (1995). Thus one STS was from subinterval 4B [sY78 (centromere)], two from 5A (sY79 and sY81), three from 5C (sY83, sY84 and sY86), two from 5D (sY87 and sY88), one from 5E (sY151), one from 5M (sY117), two from 5P (sY124 and sY125), one from 5Q (sY127), one from 6A (sY131), two from 6B (RBM1 and 2), two from 6C (sY153 and sY152), six from 6D [sY155, sY147, sY148, sY146, sY149, sY255 (DAZ)], two from 6E (sY236 and sY269), one from 6F (sY158) and two from 7 (sY159 and sY160).

PCR was carried out on 2 µl (50 ng/µl) of genomic DNA extracted from peripheral blood cells in 50 µl final reaction volume, including buffer ($MgCl_2$ 1.5 mmol), dNTP mix (0.2 mM), each primer (5 ng) and *Taq* DNA polymerase (0.04 U/µl). The PCR buffer, dNTP mix and *Taq* DNA polymerase were all obtained from Celbio (Pero, Milan, Italy). Thermocycling consisted of an initial denaturation of 10 min at 94°C and of 35 cycles of 1 min at 94°C (melting), 1 min at 60°C (annealing) and 1 min at 72°C (extension). PCR reaction products were eventually stored at 4°C and then separated on 2%

agarose gel by electrophoresis in TAE (Tris–acetic acid–EDTA) buffer at room temperature using a voltage gradient of 8 V/cm for 30–60 min.

A total of 20 normal healthy men of proven fertility and 10 normal women were included in the study as positive and negative controls, respectively. Precautions were taken to keep false-negative results to a minimum: we only included PCR assays that gave products of the expected size in the positive control DNA and that did not have products in the negative control DNA; patients were considered positive for a STS if the PCR product was of the expected size and negative only after three amplification failures.

Fathers or brothers in six out of 10 patients deleted for Yq (patients no. 1, 9, 12, 13, 27, 31) were also investigated under the same experimental conditions. No male relatives were available for the other four patients deleted for Yq.

Results

By means of bilateral testicular FNAC we have selected a group of idiopathic azoospermic patients showing bilateral SCOS. Figure 1 shows a typical testicular cytological appearance from a normozoospermic subject, as previously reported (Foresta *et al.*, 1992, 1995; Foresta and Varotto, 1992), and a SCOS from an azoospermic patient.

The STS used in this study were all analysed in 20 normal fertile men before their application to SCOS patients in order to ascertain that each of the STS produced a single amplification product of the expected size, thus excluding a normal polymorphism. The Y-specificity was tested in 10 normal women. Each of the 29 STS produced an amplification product of the expected size in all normal fertile men and failed to amplify in normal women. No deletions were detected in any of the 20 fertile men tested. Using the criteria listed above, 18 SCOS patients were studied and PCR analysis with this set of Y-DNA markers showed deletions of portions of Yq in 10 (55.5%) patients.

Table I summarizes the clinical, hormonal and cytological characteristics of the 18 SCOS patients compared with fertile controls. In all patients a reduction of testicular volume and an increase in FSH plasma concentrations were evident ($P < 0.05$ compared with controls). No differences in LH and testosterone plasma concentrations were observed in all subjects compared with controls. Furthermore, there was no difference in testicular volume and hormonal concentrations between patients with and without a deletion of Yq.

Figure 2 summarizes the STS-PCR data of the 10 SCOS patients deleted for Yq. Eight deletions were interstitial deletions whereas two were terminal deletions of the Yq euchromatin (patients no. 1 and 9, who failed to amplify 28 of the 29 Yq-specific STS). Eight out of 10 patients had deletions that overlapped with the *DAZ* gene and two out of 10 had deletions including the *RBM* gene. Two patients had deletions outside *DAZ* and *RBM*. Analysis of the microdeletions obtained in the eight patients with interstitial microdeletions allowed us to identify two homogeneous regions that have a high incidence of deletion. The first is localized in interval 5 in the proximal Yq and was deleted in all eight patients, while the second, overlapping the *DAZ* gene, is in the distal interval 6 and was deleted in six of the eight patients. Six deletions confined to interval 5 had the first proximal break point between sY78 and sY79, one between sY81 and sY83 and one between sY86 and sY87. This deleted region had the distal break point between sY125 and sY127 in six patients and between sY131 and *RBM1* in two patients. The deleted subregion in distal interval 6 had a proximal break point between sY148 and sY146 in six patients; its distal break point was between sY269 and sY158 in three patients and between sY158 and sY159 in three other patients. Only patient no. 111 presented a smaller and more proximal interval 6 deletion from sY147 to sY149 and excluded *DAZ*. The smallest deletion found in interval 5 was in patient no. 111 and it encompassed 6 Y-DNA loci from subinterval 5D to subinterval 5P. This deletion is contained in its entirety within each of the other deletions associated with SCOS, and thus it represents the smallest deletion common to all SCOS patients deleted for Yq.

The father or brothers of six patients deleted for Yq (no. 1, 9, 12, 13, 27, 31) were investigated in order to exclude a polymorphism of little or no functional consequence. In all cases the male relatives were found to carry an intact Y-chromosome. On this basis we concluded that these were de-novo deletions and may be implicated as the cause of the spermatogenic defect in these patients.

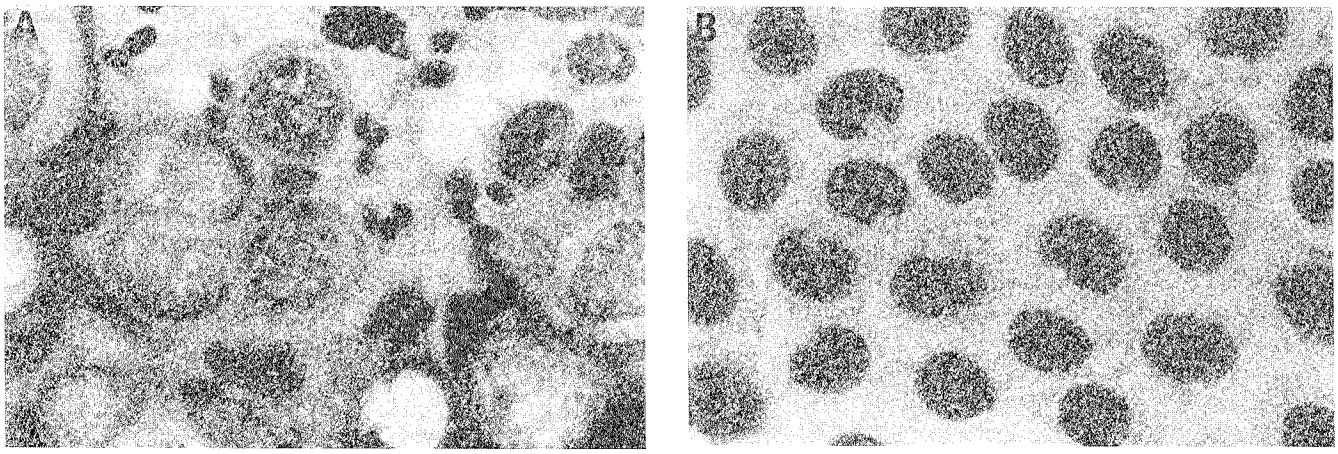

Figure 1. Representative appearances of testicular cytological findings. (A) Normozoospermic subject (control): all cell subtypes are seen in normal proportions. (B) Sertoli cell-only syndrome: only Sertoli with no germ cells are seen. (May–Grünwald–Giemsa stain, magnification ×1250).

Table I. Clinical, seminological, hormonal and cytological parameters of Sertoli cell-only syndrome patients with and without deletions, compared with controls

	n	Seminal pattern	Testicular cytological picture	Testicular volume (ml)	FSH (IU/l)	LH (IU/l)	Testosterone (nmol/l)
With Yq deletion	10	azoospermia	Sertoli cell-only syndrome	10.7 ± 2.1[a]	16.6 ± 4.7[a]	3.3 ± 0.7	15.6 ± 2.9
Without Yq deletion	8	azoospermia	Sertoli cell-only syndrome	10.8 ± 3.6[a]	17.1 ± 6.0[a]	3.5 ± 1.0	16.3 ± 3.1
Controls	35	45.8 ± 7.4	Normal spermatogenesis	14.3 ± 2.6	3.1 ± 0.9	2.8 ± 0.9	16.3 ± 3.1

[a]$P < 0.05$ compared with controls.
LH = luteinizing hormone.
FSH = follicle-stimulating hormone.

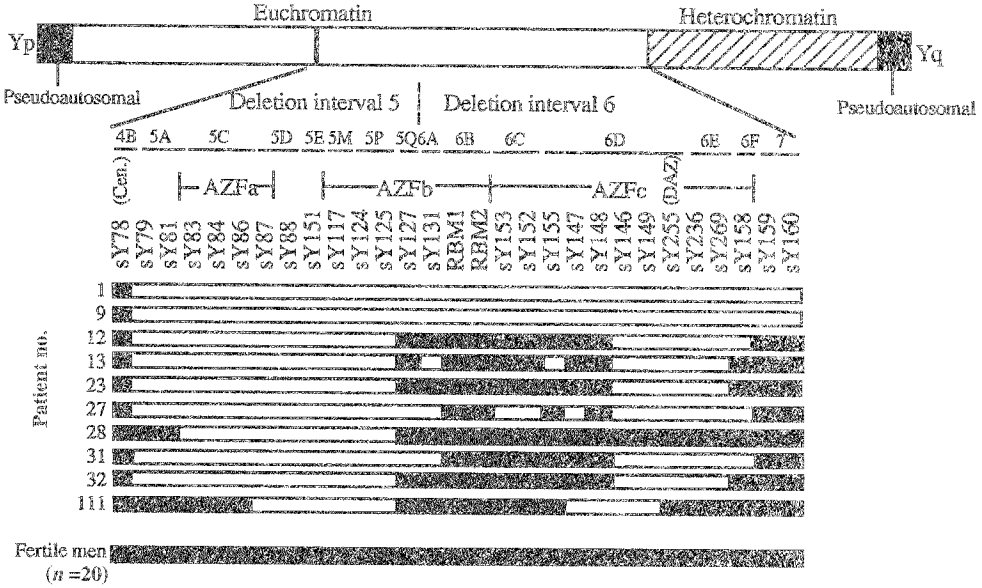

Figure 2. Sequence-tagged sites (STS)–polymerase chain reaction (PCR) data of the 10 Yq microdeletions detected in our Sertoli cell-only syndrome patients deleted for Yq. Deletion intervals and Y-chromosomal STS used are listed above. Results for fertile men are shown for comparison. Black boxes: STS present; white boxes: STS absent. Fathers or brothers of patients no. 1, 9, 12, 13, 27 and 31 were studied and shown to carry all markers listed.

Discussion

There is increasing evidence that gene families localized on the Y-chromosome long arm (Yq) direct spermatogenesis in man (Ma *et al.*, 1993; Chandley and Cooke, 1994; Reijo *et al.*, 1995, 1996; Kobayashi *et al.*, 1995; Najmabadi *et al.*, 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996; Qureshi *et al.*, 1996; Kent-First *et al.*, 1996; Pryor *et al.*, 1997; Foresta *et al.*, 1997; Simoni *et al.*, 1997; Vereb *et al.*, 1997). Since the initial observations by Tiepolo and Zuffardi (1976), a number of reports have shown microdeletions in Yq in 10–15% of idiopathic azoospermic and severe oligozoospermic subjects (Chandley and Cooke, 1994; Reijo *et al.*, 1995, 1996; Kobayashi *et al.*, 1995; Najmabadi *et al.*, 1996; Stuppia *et al.*, 1996; Qureshi *et al.*, 1996; Vogt *et al.*, 1996; Pryor *et al.*, 1997; Foresta *et al.*, 1997; Simoni *et al.*, 1997; Vereb *et al.*, 1997; Mulhall *et al.*, 1997). Although Yq microdeletion prevalence seems to be higher in idiopathic testiculopathies characterized by a complete lack or a severe depopulation of germ cells (Reijo *et al.*, 1995; Najmabadi *et al.*, 1996; Vogt *et al.*, 1996; Qureshi *et al.*, 1996; Pryor *et al.*, 1997; Foresta *et al.*, 1997; Vereb *et al.*, 1997), the actual relationship between genotype and phenotype related to Yq microdeletions is not yet defined, probably because the testicular structure observed in these patients is not homogeneous or is not defined at all (Reijo *et al.*, 1995, 1996; Najmabadi *et al.*, 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996; Qureshi *et al.*, 1996; Pryor *et al.*, 1997; Vereb *et al.*, 1997).

Idiopathic SCOS, characterized by a complete absence of germ cells in all seminiferous tubules, represents a well-defined extreme condition of testicular alteration and may have a genetic cause. Therefore, if Yq is involved in testiculopathies, a linkage between genotype and phenotype should be evident in such cases. In the present study the diagnosis of SCOS has been performed on the basis of bilateral testicular FNAC. This technique has proven to be a less painful and a minimally invasive diagnostic procedure in the evaluation of the status of several organs and in our previous studies the effectiveness of this procedure has been verified in large groups of azoospermic and oligozoospermic subjects (Foresta *et al.*, 1992, 1995; Foresta and Varotto, 1992). In these studies the cytological analysis performed on material obtained by testicular fine needle aspiration was proven to have high statistical reproducibility and strict agreement with histological findings.

By means of this procedure we have recruited 59 azoospermic patients showing bilateral SCOS. Among them we have selected 18 subjects whose SCOS can be considered idiopathic by means of history, clinical and hormonal aspects. In these patients PCR analysis of Yq intervals has been evaluated utilizing 29 Y-specific STS, spanning the entire euchromatic region from centromere to interval 7 (Yq11). A high prevalence of Yq11 microdeletions was detected (10/18, 55.5%). Such microdeletions were not found in fertile controls and in male relatives of six patients deleted for Yq, allowing us to exclude a polymorphism and to consider them as de-novo deletion and probably the cause of the testicular damage, even if no relatives were tested for four patients (no. 23, 28, 32 and 111).

Our data show that SCOS patients with microdeletions present the same clinical characteristics (phenotypic feature, hormonal parameters and testicular volume) as SCOS patients without microdeletions. This aspect is consistent with a primary testiculopathy involving only the spermatogenic system. Therefore, it is not possible to define a subgroup of patients at risk for having a microdeletion, in contrast to that reported by Kremer (1997) with regard to oligozoospermic patients.

Macroscopic deletions of the Y-chromosome long arm that are detectable by karyotyping (i.e. 46,X,Yq-) are known to cause SCOS (Hartung *et al.*, 1988); however, only a small portion of these patients show such large deletions (Tiepolo and Zuffardi, 1976; Bhasin *et al.*, 1994). The relevance of the present study is the focusing of Yq submicroscopic microdeletions in idiopathic apparently normal 46,XY azoospermic patients with SCOS. The high frequency of specific Yq microdeletions found in this study suggests a primary importance for these microdeletions in the pathogenesis of SCOS. Moreover, the actual role played by such genetic mechanism in this syndrome could be more frequent than that shown by our data. In fact the incidence of de-novo microdeletions may be underestimated since the PCR strategy is not able to detect smaller genetic damages, such as Yq point mutation.

The hypothesis that Yq microdeletions may have a role in the pathogenesis of SCOS arises also from the analysis of previous studies: Najmabadi *et al.* (1996) have detected SCOS in two out of four idiopathic azoospermic Yq-patients deleted for Yq in which a testicular biopsy was available; Vogt *et al.* (1996) detected SCOS in one of five Yq-deleted idiopathic azoospermic patients in which a testicular biopsy was performed; Pryor *et al.* (1997) have reported SCOS in two of four deleted idiopathic azoospermic patients with available testicular biopsy. The only study that looked for a relationship between SCOS and Yq microdeletions was performed by Reijo *et al.* (1995), who presented results from a large group of SCOS patients, showing Yq microdeletions in four out of 42 cases. Unfortunately this study cannot be considered conclusive since the recruited patients were affected by both non-idiopathic and idiopathic SCOS. However, considering that ~30% of SCOS are idiopathic (Foresta *et al.*, 1992, 1995), the Yq deletion prevalence in idiopathic SCOS is estimated to be far higher. Therefore different recruitment criteria of patients could explain the discrepancy between the Yq microdeletion frequencies found in the cited studies.

The Yq microdeletions observed in our patients are distributed in two well-defined regions. The first is localized in the proximal Yq interval 5 and was deleted in all patients; the second, including the *DAZ* gene and localized in the distal interval 6, was deleted in eight out of the 10 patients. *RBM* gene was deleted only in the two cases presenting the largest deletions. Therefore, as previously reported (Reijo *et al.*, 1995; Najmabadi *et al.*, 1996; Vogt *et al.*, 1996; Pryor *et al.*, 1997; Foresta *et al.*, 1997), one *RBM* gene does not seem to be associated specifically to SCOS and its role in spermatogenesis remains to be clarified. The prevalence of *DAZ* deletions in SCOS patients is very high, confirming the previous hypothesis that this gene may participate in the regulation of spermatogenesis. This gene actually represents the best candidate for AZF: it is specifically expressed in testicular germ cells (Menke *et al.*, 1997) and various previous studies have reported microdeletions overlapping this gene in different groups of infertile patients (Reijo *et al.*, 1995, 1996; Najmabadi *et al.*, 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996; Qureshi *et al.*, 1996; Pryor *et al.*, 1997; Foresta *et al.*, 1997; Simoni *et al.*, 1997; Vereb *et al.*, 1997). However, *DAZ* deletions seem not to be specific in determining the SCOS phenotype, since in our study *DAZ* was normally present in two of the 10 patients deleted for Yq. Furthermore, *DAZ* deletions are always associated with a concomitant deletion in interval 5, which may be considered the fundamental Y-chromosome region related to SCOS since it was deleted in all patients with Yq deletions. On the other hand, in SCOS patients with Yq microdeletions, the combination of *DAZ* deletion and interval 5 deletion was present in 80% of patients. The smallest deletion in interval 5, found in patient no. 111, is localized in subintervals 5D–5P and is not associated with a deletion in the *DAZ* region.

PCR analysis frequently showed the presence of non-contiguous microdeletions in Yq. As previously reported (Foresta *et al.*, 1997) this aspect could be explained by different hypotheses, none of which is yet confirmed: (i) the PCR observations may reflect separate microdeletions; (ii) the order of these STS may be incorrect, as discrepancies in the reported deletion maps do exist; (iii) some STS may be from repetitive sequences, and PCR products may reflect amplification from different sites; (iv) a complex rearrangement may be the cause.

The deletion in interval 5D–5P of patient no. 111 appears to measure 6–7 Mb (Affara *et al.*, 1996) and it is the sole region contained in its entirety within each of the other deletions. Therefore this deletion is the only one always associated with a testicular phenotype of bilateral idiopathic SCOS, and it may represent a 'spermatogenic locus' harbouring one or more genes required for male germ cell development. All patients deleted for Yq do not present clinical pathological conditions other than SCOS, and thus are otherwise healthy. The functions of this Yq region are not yet known, since no gene has been identified, but are likely to be restricted to spermatogenesis, thus representing a 'pure male sterile' locus. Vogt *et al.* have described two subregions in the interval 5 and proximal portion of the interval 6, denoted as AZFa and AZFb (Vogt *et al.*, 1996). On the basis of the testicular biopsies available, AZFa has been associated with SCOS and AZFb

with spermatocytic arrest. Our data show a deletion pattern that differs from this classification, and the smallest deletion we found (5D–5P) appears to encompass the final portion of AZFa to the proximal AZFb, while no deletions were limited to only one of these loci.

In conclusion, the findings of this study demonstrated that a large percentage of idiopathic SCOS may be due to a genetic cause related to Yq11 microdeletions. In addition a Y-related region related to a SCOS phenotype was identified, which seems to reflect the presence of one or more genes for spermatogenesis. The lack of this Yq deletion in about 45% of the studied subjects may reflect point mutations in this region not detectable by PCR method performed in this study, or alternatively may suggest that SCOS phenotype in these cases remains idiopathic and may be related to other pathogenetic causes.

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