CASE REPORT

Spontaneous regression over time of the germinal epithelium in a Y chromosome-microdeleted patient

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Azoospermia factor (AZF) region microdeletions, which account for about 10–15% of patients with oligoazoospermia, seem to lack a close genotype–testicular phenotype correlation. Although many genetic and non-genetic factors may contribute to this outcome, it was thought that a spontaneous regression of testicular germ cells might also play a relevant role. The opportunity for carrying out two different testicular biopsies one year apart in an AZFc-microdeleted patient enabled corroboration of this possibility. Indeed, the first biopsy showed a spermatocyte maturation arrest with mean Johnsen scores of 4 and 3.9 in the right and left testes respectively. One year later, the right testicular biopsy showed a picture of Sertoli cell-only syndrome in 90% of the tubules examined, and of spermatogonial maturation arrest in the remaining tubules, with a mean Johnsen score of 2.1. The almost complete absence of germinal cells was confirmed by four left testicular sperm aspirations (TESA), conducted at the same time as the biopsy during an intracytoplasmic sperm injection cycle, which showed the almost exclusive presence of Sertoli cells (85% of the whole cell population). No spermatozoa could be retrieved by TESA or testicular biopsy. To our knowledge, this is the first case of a spontaneous regression of the germinal cell epithelium over time in a patient with a Yq microdeletion without the apparent intervention of any cause known to affect the germinal epithelium.

Key words: azoospermia factor/germinal epithelium/oligoazoospermia/spermatogenesis/Y chromosome microdeletion

Introduction

Microdeletions of the so-called azoospermia factor (AZF) region of the Y chromosome long arm (Yq) are associated with spermatogenetic disruption, leading to severe oligozoospermia or azoospermia in humans. This suggests the presence of genes necessary for germ cell development in this region of the Y chromosome (Krausz and McElreavey, 1999). Accordingly, animal models with AZF region gene deletion show severely damaged testicular histology comparable with the histological picture found in oligozoospermic patients with Yq microdeletions (Vogel et al., 1999). These findings support a cause–effect relationship between Yq microdeletions and spermatogenetic impairment. However, the contribution of each AZF gene (Lahn and Page, 1997) to the various phenotypes described in patients with Yq microdeletions remains to be clarified.

In an evaluation of the testicular biopsies of a cohort of oligozoospermic patients carrying Yq microdeletions (Vogt et al., 1996), it was proposed that microdeletions were clustered in three different regions of the Yq, named AZFa, AZFb and AZFc, and that each of these related to a particular testicular histology. AZFa deletions are associated with Sertoli cell-only syndrome (SCOS), whereas deletions of the AZFb region are mostly associated with an arrest of spermatogenesis before or during meiosis. Both genotypes are generally detected in patients with azoospermia. On the other hand, the testicular pathology of men with microdeletions of the AZFc region could not be associated with a specific interruption phase of spermatogenesis. In these cases, most of the testicular tubules are depleted of germ cells (SCOS phenotype), whilst a more or less complete spermatogenesis is observed in other tubules and, therefore, AZFc deletions are compatible with the presence of spermatozoa in the ejaculate (Vogt et al., 1996). Nevertheless, results from different studies have questioned the existence of a direct link between testicular histology and the site of Yq microdeletions (Qureshi et al., 1996; Pryor et al., 1997;
Calogero et al., 1999). Thus, a clear relationship between genotype and phenotype does not seem to exist (for reviews see Simoni et al., 1998; Krausz and McElreavey, 1999). The reason for this is that spermatogenesis is a complex biological process which depends on a precise, sequential and well-coordinated activation of autosomal and Yq genes whose precise role is still far from clear. However, other factors may also contribute to the lack of a genotype–phenotype relationship; an important one may be germ cell regression over time, ultimately leading to complete SCOS. The decline of sperm count over time reported in Yq-microdeleter patients can be taken as indirect evidence of this phenomenon (Girardi et al., 1997; Simoni et al., 1997; A.E.Calogero et al., unpublished results). Here, we report the case of a patient with an AZFc microdeletion, who was severely oligozoospermic at the time of his first control, and became azoospermic one year later. Two testicular biopsies, obtained one year apart, demonstrated a tubular cellular regression from post-meiotic phase which depends on a precise, sequential and well-coordinated activation of autosomal and Yq genes whose precise role is still far from clear. However, other factors may also contribute to the lack of a genotype–phenotype relationship; an important one may be germ cell regression over time, ultimately leading to complete SCOS. The decline of sperm count over time reported in Yq-microdeleter patients can be taken as indirect evidence of this phenomenon (Girardi et al., 1997; Simoni et al., 1997; A.E.Calogero et al., unpublished results). Here, we report the case of a patient with an AZFc microdeletion, who was severely oligozoospermic at the time of his first control, and became azoospermic one year later. Two testicular biopsies, obtained one year apart, demonstrated a tubular cellular regression from post-meiotic germ cell arrest, at the time when he was oligozoospermic, to SCOS when he had become azoospermic.

Case report

A 25-year-old man and his 20-year-old wife consulted our Andrology and Reproductive Endocrinology Unit (AREU) in March 1998 about a primary infertility dating back 2 years. At the time of this first consultation, the patient was azoospermic. The male clinical history was negative for any major cause known to affect gonadal function, but the patient reported a past condition of severe oligozoospermia. Semen analyses were conducted after 3–5 days of abstinence, and sperm parameters were evaluated following WHO criteria (World Health Organization, 1992). Testicular trauma or exposure to gonadotoxins were ruled out following a thorough history was negative for any major cause known to affect gonadal function, but the patient reported a past condition of severe oligozoospermia. Semen analyses were conducted after 3–5 days of abstinence, and sperm parameters were evaluated following WHO criteria (World Health Organization, 1992). Testicular trauma or exposure to gonadotoxins were ruled out following a thorough investigation. At physical examination, the male patient was well androgenized with a reduced testicular volume which was confirmed by ultrasound (10 ml bilaterally). The testicular ecopattern was normal, and so were both epididymides. Echo-colour Doppler flusimetry showed good arterial blood supply bilaterally, and no varicocele. Serum concentrations of FSH, LH, testosterone and prolactin were within the normal range. Blood karyotype was normal.

In September 1998, because of his azoospermic status, the patient entered an intracytoplasmic sperm injection (ICSI) programme associated with testicular sperm recovery, but oocyte retrieval had to be cancelled, because spermatозoa could not be found even after four left testicular sperm aspirations (TESA) followed by a contralateral open testicular biopsy (testicular exploration and sperm extraction; TESE).

TESA and TESE were performed under local anesthesia, which was achieved by infiltrating the homolateral spermatic cord in several parts with 5–8 ml of 7% ropivacaine hydrochloride monoidrate (Naropina, Astra, Milan, Italy). TESA was conducted as follows: an insulin needle attached to a 10 ml syringe with a 19-gauge needle was passed through the sterilized skin into the testis. Once the needle was inserted, a strong negative pressure was exerted and maintained, while the needle was moved gently up and down until a small liquid aliquot appeared within the microtubing set. The needle was then slowly withdrawn and a core of attached tissue was cut off on withdrawal from the skin surface. The content of the needle was flushed into a 100 µl volume of IVF medium (Medicult, Copenhagen, Denmark) in a Petri dish. This dish was then examined at ×200 magnification under an inverted microscope in order to detect the presence of any spermatozoa.

TESE was conducted as follows: a 0.5 to 1 cm incision was made through the skin and the underlying layers. After incision of the albuginea, a gentle pressure was exerted on the testicular mass and a small portion of the extruding tissue was removed using curved scissors. The tissue was then fixed in Bouin’s solution and embedded in paraffin wax according to standard procedures. Sections (5–6 µm thick) were cut, stained with haematoxylin and eosin, and observed at ×100, ×200 and ×400 magnification under a light microscope. All cross-sectioned tubules present in a section of the testicular biopsy were evaluated systematically, and each was given a score from 1 to 10 following Johnsen’s criteria (Johnsen, 1970). Four different sections were analysed for each biopsy.

Yq intactness was evaluated on genomic DNA obtained from peripheral leukocytes using a set of 19 sequence-tagged site (STS) primers (Primml, Milan, Italy), spanning throughout intervals 5 and 6 which comprised the AZFa, AZFb and AZFc regions (see Table I for the list of STSs used). Briefly, DNA (100–200 ng) was amplified by PCR in a 25 µl final reaction volume, using: 2.5 µl of 10-fold PCR buffer (Celbio, Milan, Italy), 0.5 µl of dNTP mix (10 mmol/l) (Celbio), 1 µl of each primer (50 mmol/l), and 0.5 µl of Taq DNA polymerase (2 IU/µl) (Celbio). Thermocycling consisted of 35 cycles each at 94°C for 1 min (melting), at 61°C for 1 min (annealing), and at 72°C for 1 min (extension). The programme was preceded by a 3 min denaturing phase at 94°C and followed by a final extension phase at 72°C for 7 min. Reaction products were stored at 4°C until they were loaded onto a 2% agarose gel and the gradients separated by electrophoresis in TAE 1× (Tris–acetate–EDTA) buffer at room temperature using a voltage gradient of 9 V/cm for 50–60 min. An STS was recorded as absent after three amplification failures.

| Table 1. Sequence-tagged sites (STS) of Y chromosome and their presence or absence |
|----------------|----------------|----------------|
| STS            | PCR result     | STS            | PCR result     |
| SY84           | Present        | SY147          | Absent         |
| SY87           | Present        | SY149          | Absent         |
| SY128          | Present        | SY254          | Absent         |
| SY129          | Present        | SY277          | Absent         |
| SY130          | Present        | SY283          | Absent         |
| SY132          | Present        | SY255          | Absent         |
| SY134          | Present        | SY243          | Absent         |
| SY143          | Present        | SY236          | Absent         |
| SY152          | Absent         | SY158          | Absent         |
| SY220          | Absent         |                |                |

Results

PCR analysis showed intact AZFa and AZFb regions, whereas the DAZ gene cluster as well as other markers of the AZFc region were deleted (Table I). The first semen analysis, carried out in June 1996, showed a density of 0.1×10⁶ spermatozoa/ml, 0% progressive motility and 0% normal forms (Kruger’s criteria). All the other chemical sperm properties were normal. Two other sperm analyses, carried out in October 1997 and March 1998, showed a total absence of spermatozoa even after centrifugation. In October 1997, a diagnostic testicular biopsy, carried out in another institution but blindly re-evaluated by two different observers from our department (N.B. and E.V.), showed a picture of spermatocyte maturation arrest bilaterally.
Germinal epithelium involution and AZF deletion

Figure 1. (A) Representative photomicrographs of a right testicular biopsy carried out in 1997 (original magnification, ×312.5). The tubules contain Sertoli (sc) and the following germinal cells: spermatogonia (s pang), spermatocytes (spc) and one early spermatid (spd).

(B) Representative photomicrograph of a right testicular biopsy carried out in 1998 (original magnification, ×312.5). The tubules show the presence of Sertoli cells only.

Figure 2. Quantitative evaluation of the testicular histology following Johnsen’s criteria (Johnsen, 1970). There was a progressive germ cell depopulation over time. In 1997, the mean scores were 4 and 3.9 in the right (72 tubules examined) and left (106 tubules examined) testes, respectively. In 1998, the mean score was 2.1 (68 tubules examined). Johnsen score: 2 = no germ cells but Sertoli cells present; 3 = spermatogonia are the only germ cells present; 4 = only few spermatocytes (<5) and no spermatids or spermatozoa present; 5 = no spermatozoa, no spermatids but several or many spermatocytes present; 6 = no spermatids and only a few spermatids present.

Discussion

Although known to impair human spermatogenesis and to cause severe oligozoospermia or azoospermia, the presence of Yq microdeletions does not lead to the prediction of any specific testicular histological pattern. Therefore, a conclusive genotype–phenotype correlation in microdeleted oligoazoo-spermic patients is not apparent from the bulk of evidence gathered so far, since the initial attempt to establish such a relationship (Vogt et al., 1996) was questioned by the observations reported in subsequent studies (Qureshi et al., 1996; Pryor et al., 1997; Calogero et al., 1999). In addition, microdeletions of the AZFc region, the most frequently detected (Simoni et al., 1998), are associated with a variety of testicular histological patterns, ranging from hypospermatogenesis to germ cell aplasia (Silber et al., 1998).

Many factors, whether genetic or not, may weaken the possibility of establishing a genotype–phenotype relationship. For example, an approximate estimation of the microdeletion extension in the various patients may be a relevant factor since deletions considered similar may, instead, include important sequences within their boundaries that impair gene expression even further. Concomitant non-genetic factors capable of impairing spermatogenesis may also play a relevant role in the lack of a genotype–phenotype relationship. For example, if not carefully studied, varicocele, exposure to gonadotoxins, orchitis and/or other urogenital inflammatory processes may upset the phenotypic expression of Yq microdeletions by further damaging spermatogenesis.

The case of the microdeleted patient reported herein suggests that a lack of genotype–phenotype correlation may
also be due to a spontaneous progression of testicular lesion in the course of time. Sparse clinical evidence has suggested the occurrence of such a case in some Yq-microdeleted patients. Indeed, few microdeleted patients have been reported to have a decline in sperm output over time (Girardi et al., 1997; Simoni et al., 1997). According to a recent study, a patient with Yq microdeletions was able to father four children from the age of 25 to the age of 38 but later, at the time of the Yq microdeletion assessment, was found to be azoospermic (Chang et al., 1999). The case presented here fits in with these findings since, following an earlier sperm analysis, our patient proved to be cryptospermic and in less than two years—without the intervention of any other factors known to impair spermatogenesis—he became azoospermic. This clinical finding implied that depletion of the tubular germ cell line may account for this phenomenon. The possibility of carrying out two sequential testicular biopsies one year apart gave us the opportunity of ascertaining that there was indeed a progressive tubular depletion of germ cells, ultimately leading to an almost complete germ cell aplasia. Although a single biopsy has been claimed to be unrepresentative of the whole testicular picture because of the heterogeneity of the testicular histology of patients with non-obstructive azoospermia (Johnsen, 1970), there is some inconsistency in the literature concerning the testicular histology of such patients. Others (Silber et al., 1997) have detected a multi-focal distribution of spermatogenesis throughout the entire testicle, rather than a regional distribution. Moreover, biopsy samples obtained following fine-needle aspiration biopsy have been found to show a good correlation with conventional histology obtained by open biopsy (Mallidis and Baker, 1994; Craft et al., 1997). In our hands, TESA performed twice, 6–12 months apart, in the same patients undergoing ICSI resulted in the same cytological diagnosis in six out of seven cases. The fact that the majority of the cellular yield, following four TESA in different areas of the left testis of the microdeleted patient described in this article, consisted mainly of Sertoli cells strengthened the hypothesis of a worsening of the testicular histology, from the least severe picture of maturation arrest to the severest SCOS, without the apparent intervention of any cause known to affect germinal epithelium. This may support the argument whereby the same genetic alteration may show-up with a continuous spectrum of testicular phenotypes, depending on the time of observation, which contributes to the lack of a close relationship between genotype (position and length of the microdeletion) and phenotype (testicular biopsy and, consequently, sperm output). Although the case described herein seems to warrant such a conclusion, it is possible that this finding may not be a common feature of AZF-microdeleted patients; further investigations are therefore needed to support this hypothesis.

To our knowledge, this is the first case of spontaneous germinal cell epithelium regression in a patient with a Y chromosome microdeletion, which may in part account for the lack of a genotype–phenotype correlation observed in these patients.

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References


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