Validation of a simple Yq deletion screening programme in an ICSI candidate population

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This study reports on the validation of a diagnostic screening programme for Yq deletions in a population of infertile men. First, an unselected group of 402 intracytoplasmic sperm injection (ICSI) candidate patients was screened prospectively by means of three polymerase chain reactions (PCR) each with one marker in the region AZFa, AZFb or AZFc. With this screening strategy, eight males (2.2%) were found to carry a deletion in Yq11. Secondly, a subgroup of males were further analysed by multiplex PCR with 27 sequence-tagged sites. In this group of 229 cytogenetically normal males with azoospermia, cryptozoospermia or extreme oligozoospermia, including some patients with varicocele or a history of cryptorchidism, only one additional microdeleted patient was found with the multiplex PCR. Hence we obtained a frequency of 2.2% (9/402) or 4% (9/229) in the unselected and selected patient groups respectively. We conclude that in a diagnostic programme for Yq deletions in ICSI candidates it might be sufficient to use only four markers representing the three AZF regions and a more distal region in AZFc. In this way, it is possible to detect most, if not all, Yq deletions which might be the causal factor in the patient’s infertility.

Key words: AZF/male infertility/multiplex PCR/unexplained azoospermia/Yq deletions

Introduction

Infertility and subfertility have become a major problem in our society and 13% of couples experience fertility problems when attempting to conceive their first child (Greenhall and Vessey, 1990). In ~20% of such couples, the infertility is related to a male factor only, such as azoospermia or oligozoospermia. After an extended andrological examination, an explanation can be found for the husband's reduced sperm count in up to 60% of the cases (de Kretser, 1997). Unfortunately, there are still many patients in whom it is not clear what the underlying factor in their infertility is.

The first statement of the genetic origin of male infertility was made in 1976 (Tiepolo and Zuffardi, 1976). These authors cytogenetically analysed the Y chromosomes of subfertile, but otherwise healthy, males and identified microscopic deletions of the distal euchromatic and heterochromatic part of the Y chromosome in six men. They proposed that the infertility in these males was due to the absence of an azoospermia factor (AZF), a gene or gene complex located on Yq11, that controlled spermatogenesis.

As sequence-tagged site (STS) maps and polymerase chain reaction (PCR) technology became available, it later became possible to detect small, interstitial deletions in Yq11 invisible by karyotyping. Many studies followed in which such ‘microdeletions’ were found in azoospermic and oligozoospermic individuals (Ma et al., 1992; Kobayashi et al., 1994; Reijo et al., 1995; Vogt et al., 1996; Pryor et al., 1997; Foresta et al., 1998; Oliva et al., 1998). One team (Vogt et al., 1996) subdivided Yq11 into three different subregions defined as AZFa, AZFb and AZFc, on the basis of the detection of microdeletions in three non-overlapping regions along intervals 5 and 6 (Vollrath et al., 1992).

The DAZ gene family (Reijo et al., 1995), located in AZFc, and the RBM gene family, located in AZFb (Ma et al., 1993), have been isolated and proposed as candidate AZF genes. More recently, Lahn and Page (1997) identified 12 new genes in the euchromatic region of the Y chromosome and seven of these are specifically expressed in the testis (CDY, BPY1 and 2, XKRY, PRY and TTY1 and 2). However, no point mutations have been identified in any of the isolated genes resulting in azoospermia or oligozoospermia. The real function of these genes in the process of spermatogenesis is therefore still unclear.

According to the literature, a mean percentage of 12% and 6% microdeletions have been found in idiopathic azoospermic and oligozoospermic patients respectively, with a large variation among the different studies, probably due to differences in selection criteria and patient population.

The purpose of this study was to develop a standardized and efficient screening protocol for Yq microdeletions and thus to determine the frequency of microdeletions in our patient population of subfertile men.

First, we screened prospectively an unselected group of intracytoplasmic sperm injection (ICSI) candidate patients (n = 402) using only three single markers representing the three AZF regions. We detected a frequency of 2% microdeletions, which was lower than the mean percentage of 5.5% microdele-
tions described in other studies in which ICSI candidates were screened (Kent-First et al., 1996; Kremer et al., 1997; Pryor et al., 1997; Oliva et al., 1998).

In order to be sure that this screening strategy was adequate to detect all microdeletions, we decided on a second approach to submit the screened patient group to selection criteria based on clinical findings and semen analysis and to analyse the Y chromosomes of the selected patients with a higher number of Y-specific markers (n = 27) in a multiplex PCR system.

It was also our aim to make a comparison between these two screening strategies with different sets of primer pairs in order to develop a standardized protocol in which as few markers as possible are used but with a high efficiency in detecting Y deletions. Whenever a deletion was found, further DNA analysis with additional markers was performed to reveal the true limits of the microdeletion in order to gain information about the possible association with the patient’s reduced fertility status. Sperm characteristics, clinical parameters and histological findings of testis biopsies were also obtained.

Materials and methods

Selection and evaluation of patients

A large number (n = 402) of male patients presenting at the infertility clinic were screened for Yq microdeletions with three single primer pairs representing the three AZF regions. In this group of assisted reproductive technology candidate patients, a large variety of males with infertility and subfertility were included since semen parameters and clinical diagnosis were variable within this patient group. In a later period of the screening programme we decided to analyse retrospectively the Y chromosomes of a selected group (n = 229) of these patients on the basis of a larger set of primer pairs.

Most patients had anamnensis and physical examination and most azoospermic subjects underwent testicular biopsy. Histological examination of testicular tissues was performed in the pathology department of our university hospital. Patients were categorized according to the World Health Organization classification (WHO, 1993).

Semen samples were analysed according to WHO (1992) criteria except for sperm morphology which was assessed using the strict Kruger criteria (Kruger et al., 1988).

Serum FSH concentrations were measured using standard radioimmunoassay procedures and karyotyping was performed for all patients.

Male infertility patients with a normal karyotype and unexplained azoospermia (n = 71), cryptozoospermia (sperm concentration <0.1×10^6/ml) (n = 29) or severe oligozoospermia (sperm concentration 0.1–5×10^6/ml) (n = 50) were selected for multiplex DNA analysis. In addition, men with a varicocele (n = 25) or a history of cryptorchidism (n = 54) together with sperm concentrations <5×10^6/ml were also included in the study. It has already been demonstrated that microdeletions can be found in such patients (Pryor et al., 1997; Simoni et al., 1997).

Patients who fulfilled the criteria of unexplained infertility or subfertility (n = 37), presence of a varicocele (n = 5) or cryptorchidism (n = 1) but with a sperm concentration of >5×10^6/ml were not analysed by means of the multiplex PCR. Other patients with explained infertility and thus not selected for further DNA analysis are listed in Table I. These included patients with an abnormal karyotype (n = 17), congenital bilateral absence of the vas deferens (CBAVD) (n = 22), failed vas reversal (n = 25) and other forms of obstructions (n = 21), acquired testicular damage (n = 10), male accessory gland infection (MAGI) (n = 19) and endocrine (n = 2), systemic (n = 4) or iatrogenic (n = 4) causes of infertility. Similarly, three patients with sexual dysfunction and anejaculation, two patients with necrozoospermia and one patient with normozoospermia involving an immunological factor were not screened by multiplex PCR. This adds up to a final number of 173 ICSI candidates who were not included in the second part of the study.

Isolation of DNA and STS analysis

DNA was isolated from peripheral blood using the ‘QIamp Blood Maxi Kit Protocol’ from Qiagen.

In the first part of the study, 402 patients were screened using only three sequence-tagged-sites in a single primer PCR, each representing one AZF region of the Y chromosome: sY84 for AZFa, sY132 for AZFb and sY254 (DAZ) for AZFc.

In the second part, the Y chromosomes of the selected patient group (n = 229) were analysed using five multiplex PCR sets first described by Henegariu et al. (1993) composed of the following primer mixes: MixI (sY84, sY134, sY117, sY102, sY151, sY94, sY88), MixII (sY143, sY157, sY81, sY182, sY147), MixIII (sY86, sY105, sY82, Y6PH54pr, sY153, sY97), MixIV (sY14, sY95, sY127, sY109, sY149) and MixV (Y6BalH34pr, Fr-15-Iipr, Y6HP52pr, Y6D14pr). A 2 μl volume of DNA (100 ng/μl) was added to a 23 μl reaction mix containing 5 μl of 10X buffer (100 mmol/l Tris–HCl pH 8.3, 500 mmol/l KCl), 3 μl of MgCl₂ (25 mmol/l), 4 μl of dNTP mix (215 μmol/l each dNTP), 1.3 μl dimethylsulphoxide, 0.2 μl primer mix I, II, III, IV or V (each mix is prepared from a 500 pmol/μl primer stock solution), 0.4 μl Taq polymerase (5 U/μl).

Female negative and fertile-male positive PCR controls were included in each PCR experiment. When a negative PCR result was obtained, the reaction was repeated twice with a single primer pair, to confirm the deletion.

In the addition, the Y chromosomes of the microdeleted patients were further analysed with the following 17 markers: sY83 and sY85 (AZFa); sY122 (AZFb); sY272 (between AZFb and AZFc); sY152, sY262, sY232, sY239, sY204, sY255, sY267, sY202, sY254 (AZFc); sY159 and sY160 (Yq12).

Results

A group of 402 males presenting at the infertility clinic of the Dutch-speaking Brussels Free University was screened prospectively for Yq microdeletions by using three markers each located in one of the three AZF regions. Using this strategy, eight (2%) patients with a microdeletion were identified. In the patients carrying a deletion, it was mainly the DAZ region that remained unamplified.

Out of the eight patients found to have a microdeletion, four had idiopathic azoospermia, two had idiopathic cryptozoospermia, one was cryptozoospermic and had a varicocele and another one had idiopathic oligozoospermia.

In order to determine whether we might have missed some patients with a Y deletion by analysing only three markers, we decided to set up a multiplex PCR experiment.

On the basis of the above criteria for patient selection, 229 patients were analysed by multiplex DNA analysis. No Yq deletions were found in the unscreened 173 patients described above (see Materials and methods) when performing the first screening strategy with the three primer pairs.

When the Y chromosomes of the 229 selected subjects were
Validation of Yq deletion screening

Table I. Classification of patients with explained infertility or subfertility who were not selected for further multiplex DNA analysis

<table>
<thead>
<tr>
<th>WHO (1993) classification</th>
<th>Azoospermia</th>
<th>Cryptozoospermia</th>
<th>Severe oligozoospermia (&lt;5×10⁶/ml)</th>
<th>Mild oligozoospermia (5–20×10⁶/ml)</th>
<th>Asthenoteratozoospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBVD</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Failed vas reversal</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Other obstructions</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>MAGI</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Acquired testicular damage</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Abnormal karyotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47,XXY mosaic</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>47,XXY non-mosaic</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>47,XXY translocation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47,XXY inversion</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Systemic Abnormality</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Iatrogenic Abnormality</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Endocrine Abnormality</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

WHO = World Health Organization; CBVD = congenital bilateral absence of the vas deferens; MAGI = male accessory gland infection.

Table II. Prevalence of Y chromosomal microdeletions in the different subgroups of the 229 selected patients

<table>
<thead>
<tr>
<th>Subgroup with WHO (1993) classification</th>
<th>No. of patients</th>
<th>No. of microdeletions</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretory azoospermia</td>
<td>108</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>Unexplained</td>
<td>71</td>
<td>4</td>
<td>5.6</td>
</tr>
<tr>
<td>Varicocele</td>
<td>12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>25</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cryptozoospermic (&lt;0.1×10⁶/ml)</td>
<td>50</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>Unexplained</td>
<td>29</td>
<td>2</td>
<td>7.0</td>
</tr>
<tr>
<td>Varicocele</td>
<td>4</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>17</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oligozoospermic (&gt;0.1 and &lt;5×10⁶/ml)</td>
<td>71</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Unexplained</td>
<td>50</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Varicocele</td>
<td>9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
<td>9</td>
<td>3.9</td>
</tr>
</tbody>
</table>

WHO = World Health Organization.

Analysed with a larger set of 27 sequence-tagged sites in five multiplex PCR amplifications, only one further patient with a microdeletion was found. This male was cryptozoospermic and showed the presence of a varicocele. This makes a final deletion frequency of 4% (9/229) in the selected group or 2.2% (9/402) in the general patient group.

The prevalence of microdeletions found in the different subgroups of the 229 patients is shown in Table II. In the azoospermic males, a frequency of 3.7% (4/108) was found and in all four patients the azoospermia was unexplained. This led to a higher prevalence of 5.6% (4/71) in the unexplained group than in the general group of azoospermic patients. Cryptozoospermic subjects revealed a higher frequency of 8.0% (4/50) than azoospermic men. Two cryptozoospermic patients were included in the idiopathic group and another two had varicoceles. A lower prevalence of microdeletions was found in severe oligozoospermic males, where only one out of 71 patients carried a Y deletion. No deletions (0/54) were identified in azoospermic, cryptozoospermic and severe oligozoospermic patients who were known to have a history of cryptorchidism, while in the three different sperm categories, 8% (2/25) of men with a varicocele were Y-deleted.

Sperm concentrations (Table III) of eight patients were ≤0.1×10⁶/ml and the highest concentration found in these Y-deleted males was 0.25×10⁶/ml. Other semen characteristics (Table IV) indicated severely reduced progressive motility and poor morphology of the few spermatozoa present in the ejaculate. In all semen samples, where it was possible to assess morphology (patients 5, 6 and 9), amorphous heads were the main abnormality.

A schematic overview of the deletion patterns is given in Figure 1. Eight deletions were interstitial (patients 1–8) and one was terminal (patient 9). Seven out of eight interstitial deletions were exclusively located in AZFc (patients 1–7). Moreover, a similar region of unamplified markers was detected in these patients, spanning a region from sY153 to sY158 (interval 6C–6D). Patient 8 had an additional deletion in AZFb (Y6D14pr-sY143) but the marker sY272 representing a region between AZFb and AZFc was present. On the Y chromosome of patient 9 the terminal deletion started only from sY267 in AZFc and all markers that map within the DAZ cluster were therefore amplified in this patient. No deletions of AZFa were found in our patient population.

In Table III, clinical characteristics of the nine patients with Yq deletions are presented. In seven patients, the infertility was described as unexplained since no clinical factor that might affect semen parameters was detected. In two patients, the presence of a varicocele was diagnosed. Five patients were of Arabic origin, one was Latino and three males were Caucasians.

Testicular volumes were measured in seven cases. In six individuals testicular volumes were low (patients 1, 2, 3, 5, 8 and 9). Testicular histology varied from hypospermatogenesis to maturation arrest and Sertoli cell-only syndrome. There was...
even a spermatogenetic difference between left and right testicles in patients 3 and 6.

Serum concentrations of FSH were measured in six patients and ranged from normal to high values. No difference was demonstrated when the FSH concentrations of azoospermic males carrying a deletion (mean 21.83 ± 12.00) were compared to those of azoospermic males without a deletion (mean 21.92 ± 12.55). When the same comparison was made in a group of severe oligozoospermic patients with semen concentrations <1 × 10^6/ml, the mean FSH value measured in males with a deletion was 8.30 ± 4.44, while in men without a deletion a higher value of 14.65 ± 10.15 was detected. However, extreme variations in FSH values (1.7–53.7) were observed in the patient group without a deletion and only a small number of patients with a deletion were included. Thus, no strong conclusions can be drawn from these data.

**Discussion**

In this study, we report the set-up of a diagnostic programme for Yq deletions in our population of ICSI candidate patients. In the literature, a variable prevalence of microdeletions is respectively. In the present study, a low frequency of 2% (8/402) microdeletions was demonstrated in a group of unselected ICSI candidate patients, on the basis of PCR with the three markers sY84, sY132 and sY254 in AZFa, AZFb and AZFc respectively. In most patients, it was only the sY254 marker, which marks the DAZ locus, that remained unamplified. Patients who were microdeleted were shown to be azoospermic, cryptozoospermic or severely oligozoospermic subjects, in whom no demonstrable cause of the infertility was present, except for one subject who exhibited a varicocele. As expected, no deletions were detected in males with explained infertility, unexplained

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Ethnic background</th>
<th>Sperm concentration (×10^6/ml)</th>
<th>Clinical diagnosis</th>
<th>FSH (IU/l)</th>
<th>Testis volume (ml: R/L)</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>Arab</td>
<td>0</td>
<td>Unexplained</td>
<td>21.5</td>
<td>7/6</td>
<td>MA (spd)</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>Arab</td>
<td>0</td>
<td>Unexplained</td>
<td>NA</td>
<td>12/12</td>
<td>HS</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>Caucasian</td>
<td>0</td>
<td>Unexplained</td>
<td>34</td>
<td>7/6</td>
<td>L: SCO</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>Latino</td>
<td>0.0011</td>
<td>Varicocele</td>
<td>11.8</td>
<td>15/15</td>
<td>MA</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>Caucasian</td>
<td>0.25</td>
<td>Unexplained</td>
<td>9.4</td>
<td>NA/20</td>
<td>Not biopsied</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>Arab</td>
<td>0–0.1</td>
<td>Unexplained</td>
<td>3.3</td>
<td>NA</td>
<td>L: MA (20% spd)</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>Arab</td>
<td>0–0.00001</td>
<td>Unexplained</td>
<td>10</td>
<td>12/12</td>
<td>SCO</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>Caucasian</td>
<td>0</td>
<td>Unexplained</td>
<td>20</td>
<td>20/atrophic</td>
<td>L: SCO</td>
</tr>
<tr>
<td>9</td>
<td>42</td>
<td>Arab</td>
<td>0–0.07</td>
<td>Varicocele</td>
<td>5</td>
<td>Amorphous heads; small or absent acrosomes</td>
<td></td>
</tr>
</tbody>
</table>

**Table III. Clinical characteristics of nine patients with a microdeletion of the Y chromosome**

**Table IV. Sperm parameters of four patients carrying a Y chromosomal microdeletion where enough spermatozoa were present to evaluate motility and morphology**

**Table IV. Sperm parameters of four patients carrying a Y chromosomal microdeletion where enough spermatozoa were present to evaluate motility and morphology**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Concentration (×10^6/ml)</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Motility (%: A/B/C)^a</th>
<th>Morphology (%)</th>
<th>Dominant morphological abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.0011</td>
<td>3.2</td>
<td>7.9</td>
<td>0/14/14</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>2.5</td>
<td>7.5</td>
<td>0/3/12</td>
<td>0</td>
<td>Amorphous heads; small or absent acrosomes</td>
</tr>
<tr>
<td>6</td>
<td>0–0.1</td>
<td>4.3</td>
<td>7.9</td>
<td>0/4/9</td>
<td>5</td>
<td>Amorphous heads</td>
</tr>
<tr>
<td>7</td>
<td>0–0.07</td>
<td>2.9</td>
<td>7.2</td>
<td>0/2/8</td>
<td>0</td>
<td>Amorphous heads</td>
</tr>
</tbody>
</table>

^aSclerotic testes, no exact volumes were measured.

^b20% of seminiferous tubules showed presence of spermatids.

R = right; L = left; MA (spd) = maturation arrest up to the spermatid stage; NA = not available; HS = hypospermatogenesis; SCO = Sertoli cell-only syndrome.

In the literature, a variable prevalence of microdeletions is respectively. In the present study, a low frequency of 2% (8/402) microdeletions was demonstrated in a group of unselected ICSI candidate patients, on the basis of PCR with the three markers sY84, sY132 and sY254 in AZFa, AZFb and AZFc respectively. In most patients, it was only the sY254 marker, which marks the DAZ locus, that remained unamplified. Patients who were microdeleted were shown to be azoospermic, cryptozoospermic or severely oligozoospermic subjects, in whom no demonstrable cause of the infertility was present, except for one subject who exhibited a varicocele. As expected, no deletions were detected in males with explained infertility, unexplained
Validation of Yq deletion screening

Figure 1. Schematic overview of the Yq11 microdeletions detected in nine patients. Positions of sequence-tagged sites (STS) in intervals of Yq11 and their location in AZFa, AZFb and AZFc are shown. The three STS used in the first screening strategy are written in bold italics and those of the multiplex polymerase chain reaction are underlined. Blank spaces indicate deletion of an STS, solid boxes indicate presence of a marker. It should be noted that marker sY272 is considered to be polymorphic.

mild oligozoospermia or in patients with higher sperm concentrations.

No blood samples of male relatives were available at the time of screening and thus the possibility of polymorphisms, although highly unlikely since large deletions were detected, cannot be excluded.

The next step was to check whether we could find more deletions by selecting out a group of patients, based on clinical information and semen parameters, in whom it was more likely to detect additional microdeletions and to analyse these with a higher number of markers in a multiplex PCR. Only males with a normal karyotype and a sperm concentration of $<5 \times 10^6$/ml with unexplained infertility, a history of cryptorchidism or a varicocele were selected for further analysis. The concentration of $5 \times 10^6$ spermatozoa/ml ejaculate was taken as an arbitrary limit because this was the highest sperm concentration analysed in a subject with a large microdeletion (multiple sites deleted) of the Y chromosome (Girardi et al., 1997). Other investigators also reported Yq microdeletions in patients with moderately reduced sperm counts or even in normozoospermic subjects, but mostly it was only a single or at most three STS that were absent and these could therefore have reflected polymorphisms (Pryor et al., 1997; van der Ven et al., 1997). Patients with varicoceles and cryptorchidism were included because microdeletions have been detected in this patient group (Pryor et al., 1997; Simoni et al., 1997).

In screening the selected patient group with 27 markers instead of three markers, only one further patient was found to carry a Yq deletion. A terminal deletion located more distally from DAZ was present in this subject. According to these findings, it would probably be enough to analyse the Y chromosomes of Yq deletion candidates with the three STS used in the first part of the study together with an additional marker to amplify a region in the distal part of AZFc (interval
6E–6F) in order to detect most, if not all, Y chromosomal deletions. For a routine screening method, four primer pairs may be combined in one multiplex PCR to provide a rapid and cost-effective Yq deletion screening in ICSI candidate patients. Whenever a deletion is found with this rapid detection method, further molecular analysis should be performed with additional primer pairs to reveal the size of the deletion and its relevance for the patients infertility. Also genotype/phenotype correlations can be postulated in this way.

The highest rate of microdeletions was found in the cryptozoospermic patient group (4/50, 8.0%), followed by the azoospermic patients (4/108, 3.7%) and males with oligozoospermia (1/71, 1.4%).

These percentages are below the high numbers of microdeletions found in non-obstructive azoospermic men in several other studies (Reijo et al., 1995; Najmabadi et al., 1996; Vereb et al., 1997; Brandell et al., 1998; Foresta et al., 1998), but are comparable with the results from Girardi et al. (1997), Simoni et al. (1997) and Liow et al. (1998). Taking the groups of azoospermic and severe oligozoospermic males together, our study reported a similar frequency of microdeletions (4%) to that given in other reports (3%: Simoni et al., 1997; 4.6%: Vogt et al., 1997; 4%: Oliva et al., 1998).

It is interesting to know that two of our microdeleted azoospermic patients (patients 1 and 3) had previously been cryptozoospermic and had evolved to azoospermic males with a complete absence of spermatozoa in their testicular tissue in the last treatment cycle.

No microdeletions were demonstrated in a large group of 54 patients with cryptorchidism. Similar findings were reported by Kremer et al. (1997), who failed to detect microdeletions in a group of 22 males with cryptorchidism and concluded that the presence of microdeletions is associated with the absence of abnormal andrological findings. They also reported that one out of seven microdeleted subjects had a varicocele, a prevalence that was comparable to the frequency of varicoceles in normozoospermic men. The present study reveals a slightly higher prevalence of this pathology, since two out of nine males with a microdeletion had a varicocele. In the recent publication by Kleiman et al. (1999), no Yq deletions were detected in males with cryptorchidism or in males with a varicocele, but only small groups of seven and six men respectively were analysed.

We observed that serum concentrations of FSH in oligozoospermic men with a deletion (<1×10⁹/ml) were within normal values whereas oligozoospermic men without a deletion had a higher mean concentration of serum FSH. However, no strong conclusions can be drawn from these values because there are extreme variations in FSH concentrations in the latter group and a small number of patients in the first group. Kremer et al. (1997) reported that FSH concentrations of seven men with severe oligozoospermia and a microdeletion on the Y chromosome were comparable to those for normozoospermic men and differed significantly from those for oligozoospermic men without a deletion.

We also compared the FSH values of the azoospermic males with and without a deletion, but here the two groups showed similar results.

When the Y chromosomes of the nine deleted patients were analysed with 46 STS, the deletion pattern of eight patients showed interstitial deletions and in one patient a terminal deletion was demonstrated. Seven males had similar deletions located in AZFc spanning a region from the marker sY153 to sY158. This deletion pattern is very frequently observed, as is indicated in several other studies (Qureshi et al., 1996; Reijo et al., 1996; Girardi et al., 1997; Liow et al., 1998; Oliva et al., 1998). The histopathological findings in these patients varied from hypospermatogenesis to maturation arrest and incomplete Sertoli cell-only syndrome. It is interesting to note that in all of these men spermatozoa had previously been found in their ejaculates or testicular biopsies. Brandell et al. (1998) examined the possibility of using the deletion pattern as a predictive factor for the presence or absence of spermatozoa in testicular biopsies. In a group of non-obstructive azoospermic males carrying a Yq deletion, no spermatozoa were retrieved in the seven patients where an AZFb deletion was detected, either with or without the presence of an additional deletion in AZFa or AZFc. In two other cases of non-obstructive azoospermia in which only the AZFc region remained unamplified, spermatozoa were found in the biopsies and were used for ICSI. In our patient with a deletion in AZFb and AZFc, multiple diagnostic biopsies were taken before starting an ICSI treatment cycle. It was a clear case of complete Sertoli cell-only syndrome and we were therefore unable to find sperm cells, which correlates to the observations of Brandell et al. (1998).

With regard to our patient in whom a terminal deletion was found by the multiplex PCR, all DAZ markers were present (sY152, sY147, sY232, sY149, sY254, sY255). As discussed in the review by Kostiner et al. (1998), in the case of the DAZ gene family, positive PCR results do not indicate that all members are present. It is therefore possible that in this patient too some copies were present and others were missing while a positive result was still demonstrated in all STS. In this patient, an additional discontinuous deletion was detected at locus sY204, a marker that does not map within DAZ. The significance of this deletion is very doubtful and probably indicates the need to re-order this marker on the deletion map, as suggested previously (Kostiner et al., 1998). In such cases, further research can be performed in addition to the general screening programme.

From this study we can conclude that although screening with a higher number of STS in a more selective group of ICSI patients revealed one extra patient to be deleted in Yq, no great increase in the frequency of Yq deletions was obtained by this strategy. Patients who were especially at risk of having a microdeletion were azoospermic and cryptozoospermic males with unexplained infertility. However, patients with a varicocele should not be excluded from the screening programme since microdeletions were detected in these subjects. Although we did not find any, it is even possible that some patients with debatable conditions such as male accessory gland infection may carry a Yq deletion. In these patients, we cannot exclude that another detrimental factor (such as a Yq deletion) could have caused the reduced sperm concentration, before the patient became infected.
Because the Yq deletion can be inherited through ICSI, it is advisable to provide adequate counselling to the infertile couple. It has to be explained to the couple that their ICSI-derived son will carry the same deletion as his father and consequently may encounter infertility or subfertility problems. Preimplantation genetic diagnosis should be offered but it is up to the couple to decide whether they opt to avoid possible future problems in this way.

Acknowledgements

The authors acknowledge the skilful assistance of the clinical, laboratory and paramedical staff of the Centres for Reproductive Medicine and Medical Genetics. Frank Winter of the Language Education Centre reviewed the language of the manuscript. The work was supported by grants from the Fund for Medical Research (FWO – Vlaanderen).

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Received on July 19, 1999; accepted on January 20, 2000