Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic

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Intracytoplasmic sperm injection (ICSI) is a successful treatment option for severe male infertility, although the aetiology of the disorder remains unclear in most cases. Recently, microdeletions in the AZF region of the Y chromosome have been detected in men with azoospermia or severe oligozoospermia. In this study we investigated the prevalence of microdeletions in the AZF region of the Y chromosome in a population of men undergoing ICSI, and looked for clinical characteristics of men with and without this deletion. Blood was drawn from 164 men, who were on the waiting list for ICSI treatment: 19 were azoospermic, 111 oligozoospermic and 34 normozoospermic (after previous total fertilization failure). A total of 100 men with proven fertility served as a control. Microdeletions in the AZFc region were present in seven of the 111 oligozoospermic men (6.3%). Compared with oligozoospermic men without microdeletions, men with microdeletions had a lower concentration of follicle stimulating hormone (FSH), a lower number of motile spermatozoa and a lower frequency of abnormal findings at andrological history or examination. No microdeletions were found in the azoospermic, normozoospermic and control groups. In conclusion, microdeletions in the AZFc region are relatively frequently found in men with severe unexplained oligozoospermia. In the ICSI era this finding has an important impact because this form of male infertility is now potentially hereditary. Therefore we recommend DNA screening (and genetic counselling) before ICSI, especially in men with normal FSH, severe oligozoospermia and no abnormal clinical andrological findings.

Key words: AZF region microdeletion/ICSI/oligozoospermia/Y chromosome

Introduction

The introduction of intracytoplasmic sperm injection (ICSI) caused a revolution in the treatment of severe male infertility, with pregnancy rates up to 35% per cycle (Van Steirteghem et al., 1993). Unfortunately, this rapid development in therapeutic possibilities has not been accompanied by a rapid increase of knowledge on the aetiology of male infertility. In particular, little is known about the contribution of genetic factors. Since ICSI circumvents the underlying defects, transmission of genetic factors associated with oligo- or azoospermia to the offspring is possible (Bhasin et al., 1994).

More than two decades ago Tiepolo and Zuffardi (1976) reported six azoospermic men with gross deletions of the Y chromosome and postulated the existence of a factor, located on the Y chromosome, that is necessary for normal spermatogenesis. Later this factor was named ‘azoospermia factor’ (AZF). More recently, small interstitial deletions (microdeletions) could be detected by using molecular techniques, and several groups found microdeletions of Yq11 in azoospermic men (Ma et al., 1992; Reijo et al., 1995; Qureshi et al., 1996; Vogt et al., 1996). Reijo et al. (1996) and Qureshi et al. (1996) showed that these microdeletions are also present on the Y chromosome of oligozoospermic men. Although little is known on the relationship between the microdeletions and the clinical phenotype, the obvious consequence of this observation is that some forms of male infertility become hereditary if ICSI is used.

We investigated the prevalence of microdeletions in the AZF region of the Y chromosome and studied the clinical characteristics of men with and without these microdeletions.

Materials and methods

Annually at our centre, about 200 ICSI treatments are performed. There is waiting list of ~1 year. Indications for ICSI are severe oligozoospermia (<1×10⁶ spermatozoa/ml with progressive motility per ejaculate), azoospermia (in combination with surgical sperm retrieval) and total fertilization failure (TFF) in two previous IVF treatments.

At intake, each man was asked for specific andrological problems and underwent a thorough andrological examination. Special attention was paid to a history of cryptorchidism, male adenitis, genital surgery, sexual function, medication, intoxications and diseases like malignancies or colitis. Furthermore, the testis volume was measured by a Prader orchidometer and the presence of the vas deferens, varicoceles, signs of epididymal obstruction and gynaecomastia were assessed. The concentrations of follicle stimulating hormone (FSH), lutinizing hormone (LH) and testosterone were measured by commercially available immunoradiometric assays (FSH, LH) and radioimmunoassay (testosterone) (Medgenix, Fleurus, Belgium). Semen analysis was performed according to the guidelines of the World Health Organization (WHO, 1992). The first sample was used for further analysis. The ‘total number of motile spermatozoa per sample’ is the sum of spermatozoa with progressive motility and spermatozoa...
with non-progressive motility (WHO classification a–c). No testis biopsies were performed.

In May 1996 we sent a letter to all patients who were on the waiting list for ICSI and informed them about the possibility to screen for microdeletions on the Y chromosome. In September 1996, 173 men had responded and had given blood for chromosome and DNA analysis (111 oligozoospermic men, 28 azoospermic men and 34 normozoospermic men after TFF). Nine of the 28 azoospermic men had a failed re-anastomosis after previous vasectomy and were excluded from further analysis. Men with abnormal test results were counselled by a clinical geneticist. If microdeletions were found, the diagnosis was confirmed in the spermatozoa; the father or brothers of the men were asked to give blood for DNA analysis.

To screen for microdeletions in the AZF region of the Y chromosome by polymerase chain reaction (PCR), genomic DNA was prepared from peripheral blood samples. Each man was analysed for the presence of sequence tagged sites (STS) in respectively the AZFa, b and c regions (Vogt et al., 1996) using two different multiplex PCR assays. The STS probes used were DYS 148 and DYS 273 (AZFa), DYS 218 and DYS 222 (AZFb) (Vogt et al., 1996), sY254 and sY255 (AZFc) and DYS 266 and DYS 242 (control) (Reijo et al., 1995). PCR products were analysed on a 4% agarose gel. We only used PCR assays that gave a reliable result in the control sample and we did not record a deletion from a patient unless at least two successive PCR amplifications of two blood samples taken separately yielded negative results. Karyotyping was done using a standard protocol (Rooney and Czepulkowski, 1992).

As a control, the blood of 100 men with proven fertility was analysed for the presence of microdeletions. These men were healthy family members of patients who counselled the clinical geneticist for other reasons. All had one or more children. No semen or hormone parameters were available.

The clinical data, semen parameters and hormone measurements were coupled to the genetic data. Differences between the clinical data of men with and without microdeletions were statistically tested using Student’s t-test.

Results

Microdeletions

Microdeletions in the AZFc region were found in seven of the 111 oligozoospermic men (6.3%) and in none of the 19 azoospermic men and 34 normozoospermic men. No microdeletions in the AZFa or AZFb regions were found at all. The presence of microdeletions in the AZFc region was confirmed in the spermatozoa of all seven men. Three men agreed with our proposal to screen one of the male family members. One father and two brothers were tested and no microdeletions in the AZF region were found. Both brothers had children.

No microdeletions in the AZF region were found in the 100 fertile men.

Hormones

The hormonal data of the 164 men are summarized in Table I. The mean FSH concentrations in oligozoospermic and azoospermic men were significantly higher than in normozoospermic men (9.5 ± 0.7 IU/l and 16.8 ± 3.4 IU/l versus 4.6 ± 0.5 IU/l; P <0.01). There were no significant differences in LH and testosterone concentrations between the three groups. Looking at the oligozoospermic men with and without deletions, we found that the men with the deletion had significantly lower FSH and LH than the men without the deletion (FSH: 5.6 ± 1.3 IU/l versus 9.8 ± 0.8 IU/l, P <0.05; LH: 3.6 ± 0.3 IU/l versus 4.9 ± 0.4 IU/l, P <0.05).

Chromosomes

We detected four chromosome abnormalities in the 164 men (2.4%): two (1.8%) in the oligozoospermic group: a translocation (46,XY, t (4;16) (q31.1;q22)) and a mosaic Klinefelter (46,XY/47,XXY (12/18)), and two (10.5%) in the azoospermic group: a Klinefelter (47,XXY) and a mosaic Klinefelter (46,XY/47,XXY (30/4)).

Semen

The semen parameters of the 164 men are summarized in Table II. Concentration and total number of motile spermatozoa per sample in oligozoospermic men with the deletion were significantly lower than the concentration and total number of motile spermatozoa in oligozoospermic men without the deletion (concentration: 0.5 ± 0.2 x 10⁹/ml versus 4.3 ± 0.1 x 10⁹/ml, P <0.001; total number of motile spermatozoa: 0.4 ± 0.2 x 10⁶/sample versus 1.1 ± 0.2 x 10⁶/sample, P <0.01). The volume of the ejaculate in oligozoospermic and azoospermic men was significantly lower than in the normozoospermic men (3.5 ± 0.1 ml and 2.8 ± 0.2 ml versus 4.2 ± 0.3 ml; P <0.05).

Andrological history and examination

The results of the andrological history and physical investigation are summarized in Tables II and III. As expected the testis volume of the oligozoospermic and azoospermic men was significantly lower than the testis volume in the normozoospermic men (15.2 ± 0.5 ml and 14.8 ± 1.3 ml versus 19.1 ± 0.7 ml; P <0.01).

In nine of the 19 azoospermic men (47%) abnormal andrological findings were detected; in most cases obstruction was present [congenital bilateral absence of the vas deferens (CBAVD)]. In 61 of the 111 oligozoospermic men (55%) and in five of the 34 normozoospermic men (15%) abnormal andrological findings were detected.

Looking at the oligozoospermic men with and without microdeletions, the frequency of abnormal andrological findings was significantly lower in the men with the deletion as compared with the men without the deletion (1/7 versus 44/104; P <0.05).

Microdeletions in subgroups

As already stated, the prevalence of microdeletions in the AZFc region was 7/164 (4.3%) in the whole study group and 7/111 (6.7%) in the oligozoospermic group. Because of the significant differences between the oligozoospermic men with and without deletions, we also calculated the prevalence of the microdeletions in subgroups. The prevalence in oligozoospermic men with normal FSH (<10 IU/l) was 7/74 (10.3%), the prevalence in oligozoospermic men with a very low sperm count (total number of motile spermatozoa <1 x 10⁶) was 6/81 (7.5%) and the prevalence in oligozoospermic men with no specific findings at andrological history was 6/58 (10.3%). There were 25 oligozoospermic men who complied with all
Table I. Hormonal data of 164 men undergoing intracytoplasmic sperm injection (mean ± SE)

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>FSH (IU/l)</th>
<th>LH (IU/l)</th>
<th>Testosterone (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligozoospermia with deletion</td>
<td>111</td>
<td>9.5 ± 0.7**</td>
<td>4.8 ± 0.4</td>
<td>17 ± 0.6</td>
</tr>
<tr>
<td>Oligozoospermia without deletion</td>
<td>104</td>
<td>5.6 ± 1.3**</td>
<td>3.6 ± 0.3**</td>
<td>16.2 ± 1.7</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>19</td>
<td>6.8 ± 3.4*</td>
<td>5.2 ± 0.1</td>
<td>19.2 ± 1.3</td>
</tr>
<tr>
<td>Normozoospermia</td>
<td>34</td>
<td>4.6 ± 0.5</td>
<td>3.6 ± 0.3</td>
<td>17.8 ± 0.2</td>
</tr>
</tbody>
</table>

*Significantly higher than in normozoospermic men (P <0.01).
**Significantly lower than in oligozoospermic men without the deletion (P <0.05).

FSH = follicle stimulating hormone; LH = luteinizing hormone.

Table II. Semen parameters and testis volume of 164 men undergoing intracytoplasmic sperm injection (mean ± SE)

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Volume (ml)</th>
<th>pH</th>
<th>Concentration (X 10^6/ml)</th>
<th>Motility (%)</th>
<th>Total no. motile sperm cells per sample (X10^6)</th>
<th>Morphology (% abnormal)</th>
<th>Testis volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligozoospermia with deletion</td>
<td>111</td>
<td>3.5 ± 0.1**</td>
<td>7.7 ± 0.1</td>
<td>4.1 ± 1.0</td>
<td>15.7 ± 1.6</td>
<td>1.1 ± 0.2</td>
<td>64.5 ± 1.5</td>
<td>15.2 ± 0.5**</td>
</tr>
<tr>
<td>Oligozoospermia without deletion</td>
<td>7</td>
<td>3.9 ± 0.9</td>
<td>7.7 ± 0.1</td>
<td>0.5 ± 0.2*</td>
<td>13.9 ± 5.7</td>
<td>0.4 ± 0.2*</td>
<td>86.5 ± 6.5</td>
<td>15.6 ± 2.0</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>19</td>
<td>2.8 ± 0.2**</td>
<td>7.6 ± 0.1</td>
<td>0</td>
<td>15.8 ± 1.7</td>
<td>1.1 ± 0.2</td>
<td>63.8 ± 1.5</td>
<td>15.2 ± 0.5</td>
</tr>
<tr>
<td>Normozoospermia</td>
<td>34</td>
<td>4.2 ± 0.3</td>
<td>7.8 ± 0.1</td>
<td>25.0 ± 3.6</td>
<td>32.8 ± 2.5</td>
<td>36.2 ± 7.4</td>
<td>56.1 ± 2.8</td>
<td>19.1 ± 0.7</td>
</tr>
</tbody>
</table>

*Including rapid linear progression, slow or non-linear progression and non-progressive motility (WHO classification a, b and c)
**Significantly lower than in oligozoospermic men without the deletion (P <0.001),
^Significantly lower than in normozoospermic men (P <0.05).

Table III. Summary of findings during andrological history and examination in 164 men undergoing intracytoplasmic sperm injection

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Cryptorchism</th>
<th>Inguinal hernia</th>
<th>Varicocele</th>
<th>CBAVD</th>
<th>Torsion testis</th>
<th>Male adnexitis</th>
<th>Ejaculation problems</th>
<th>Radiotherapy</th>
<th>Miscellaneous*</th>
<th>No findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligozoospermia with deletion</td>
<td>111</td>
<td>21</td>
<td>7</td>
<td>17</td>
<td>–</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>Oligozoospermia without deletion</td>
<td>7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>19</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>44</td>
</tr>
<tr>
<td>Normozoospermia</td>
<td>34</td>
<td>–</td>
<td>2</td>
<td>4</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>29</td>
</tr>
</tbody>
</table>

CBAVD = congenital bilateral absence of the vas deferens.
*Including gynaecomastia (n = 1), lymph oedema (n = 1), colitis without medication (n = 2), intrauterine DES exposition (n = 3).

Discussion

The studies regarding the relationship between male infertility and Y deletions started with the publication of Tiepolo and Zuffardi in 1976. They found gross deletions in azoospermic men and postulated the existence of a factor located on the long arm of the Y chromosome, later named AZF. Two 'candidate' genes for AZF have been suggested: RBM (RNA binding motif) by the group of Chandley (Ma et al., 1992) and DAZ (deleted in azoospermia) by the group of Page (Reijo et al., 1995). Both genes are expressed in testis and encode a presumed RNA-binding protein of which the precise function remains to be determined. RBM (initially labelled YRRM; Y located RNA recognition motif) seems to be a gene family and multiple copies are distributed along the Y chromosome.

The DAZ gene is equivalent to AZFc, as has been suggested by Vogt et al. (1996). They proposed in their recent paper the presence of three spermatogenesis loci in Yq11 and designated them as AZFa, AZFb and AZFc.

Recently, Reijo et al. (1996) showed that microdeletions in the AZF region are not limited to azoospermic men, but may also be detected in men with severe oligozoospermia. They found microdeletions in two out of 35 men (6%) who presented with severe oligozoospermia. We also found microdeletions in oligozoospermic men and therefore the names AZF (azoospermia factor) and DAZ (deleted in azoospermia) are no longer correct.

The prevalence of microdeletions in infertile men ranges in the literature from 3% (12/370; Vogt et al., 1996), 8% (8/100; Qureshi et al., 1996), 9% (11/117; Hargreave et al., 1996), 13% (12/89; Reijo et al., 1995) to 19% (10/53; Kobayashi et al., 1994). There are several problems with the interpretation of these figures. Firstly, the study groups are not the same these three criteria and five of them had the microdeletion (20%).
since the inclusion criteria differ from oligozoospermia (Reijo et al., 1996), azoospermia (Kobayashi et al., 1994) or both (Reijo et al., 1995; Hargreve et al., 1996; Qureshi et al., 1996; Vogt et al., 1996). Secondly, different regions (RBM, DAZ, AZFa, b or c) have been studied with different techniques and, thirdly, information on clinical characteristics, such as semen parameters, hormone concentrations and andrological history, is scarce.

For the majority of the men, ICSI is the last resort to obtain offspring. There is discussion in the literature about the safety of ICSI (Meschede et al., 1995). Although data about short-term effects (e.g. major and minor congenital anomalies) are reassuring (Bonduelle et al., 1995), questions about genetic risks and long-term effects of ICSI remain to be answered. In the light of this discussion it is important to have more information on the prevalence of genetic defects in men undergoing ICSI and their clinical characteristics.

The percentages of abnormal karyotypes in the present study (1.8% in oligozoospermic men and 10.5% in azoospermic men) are comparable with data in the literature (Relief et al., 1984); as expected the most frequent abnormality was the Klinefelter syndrome.

The 4.3% prevalence of microdeletions in the AZFc region, in our diverse ICSI population, suggests that microdeletions in the Y chromosome are a major factor in `ICSI-men`. As expected, no microdeletions in normozoospermic men were found. Moreover, no deletions in the azoospermic group were found, probably because of the low number of azoospermic men in this population and the relatively high proportion of those azoospermic men with clear cause of the azoospermia (R/19). So, these data are not representative for all azoospermic men. The relatively low number of men with idiopathic non-obstructive azoospermia in our population may also explain the absence of microdeletions in the AZFa and AZFb region in our study, since these deletions have been described mainly in non-obstructive azoospermia (Vogt et al., 1996).

Our data show that oligozoospermic men with microdeletions have significantly lower FSH and LH concentrations in comparison with men without microdeletions. Furthermore, the FSH and LH concentrations of men with microdeletions were comparable to those in normozoospermic men. This suggests that the endocrine system, in contrast with the spermatogenic system, is intact. Moreover, the fact that the ejaculates of men with microdeletions contain a significantly lower total number of motile spermatozoa, provides evidence for a severe spermatogenic defect. Finally, the presence of microdeletions is associated with the absence of abnormal findings at andrological history and examination: one in seven men with a microdeletion had a varicocele, a prevalence comparable with that in normozoospermic men. Using these clinical parameters, it is possible to define a subgroup of men who are at risk for having a microdeletion: in this study, men with severe oligozoospermia, normal FSH and normal clinical andrological findings have a risk of 20% of having microdeletions in the AZFc region. Because of the relatively low numbers, care must be taken with the extrapolation of these percentages to oligozoospermic men in general.

For several reasons it is tempting to speculate that the percentage of male infertility due to genetic defects is even higher. Firstly, we did not screen the complete Y chromosome for the presence of microdeletions. Secondly, by performing PCR analysis, mosaicism of microdeletions could be missed. Recently, Kent-First et al. (1996) suggested that the existence of mosaicism may explain the finding that two of 32 oligozoospermic men without microdeletions (tested by PCR analysis) fathered `ICSI-sons` with microdeletions. Thirdly, it is possible that in addition to the known deletions other unknown mutations in the AZF genes could be present. Finally, it may be expected that in the near future other spermatogenesis genes (and their mutations) will be discovered.

One could imagine that microdeletions of the Y chromosome will be transmitted to male offspring and that these sons will have a risk of having the same fertility problems. Recently, it has been shown that microdeletions are indeed transmitted to the male offspring via ICSI (Kent-First et al., 1996). However, the expected fertility problems in the sons remain to be proven, since Vogt et al. (1996) described a man with a microdeletion in the AZFc region who fathered a son with the same deletion. Despite this uncertainty, we recommend screening for microdeletions in the AZF region before ICSI, especially in those men with severe oligozoospermia (or azoospermia), normal FSH and normal clinical andrological findings. The individual man with a microdeletion should be counselled extensively. Subsequently, it is up to the couple to make the final decision about further treatment.

In conclusion, the results of the present study show that microdeletions in the AZFc region are frequently found in men with severe and unexplained oligozoospermia. This finding has a great impact, because severe male infertility has now become a hereditary disorder that can be transmitted by ICSI.

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