AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test

Roy A.Brandell1,2, Anna Mielnik2, Deborah Liotta3, Zhen Ye3, Lucinda L.Veek3, Gianpiero D.Palermo3 and Peter N.Schlegel1,2,4

1James Buchanan Brady Foundation, Department of Urology, The New York Hospital–Cornell Medical Center, 525 East 68th Street. 2The Population Council, Center for Biomedical Research and 3The Center for Reproductive Medicine and Infertility, The New York Hospital–Cornell Medical Center, New York, NY 10021, USA
4To whom correspondence should be addressed

Introduction

Approximately 7% of all men presenting for infertility evaluation will be found to have microdeletions of the Y-chromosome (Pryor et al., 1997). The frequency of detection of Y-chromosomal abnormalities is dependent to some extent on the severity of the defect in sperm production (Girardi et al., 1997). For men with azoospermia or severe oligozoospermia, up to 18% will have detectable Y chromosomal abnormalities using polymerase chain reaction (PCR)-based analysis (Najmabadi et al., 1996). The region of the Y-chromosome that appears most important to spermatogenesis is the euchromatic portion of the long arm (Yq11). Cytogenetic analysis of the Y-chromosome performed by Tiepolo and Zuffardi (1976) revealed large terminal deletions in a small percentage of azoospermic men. They postulated the presence of an ‘azoospermia factor’ (AZF) containing one or more genes essential to spermatogenesis.

Reijo et al. (1995) used a PCR-based analysis to detect smaller, ‘submicroscopic’ deletions in the Y-chromosome and identified a novel, multicopy gene referred to as ‘deleted in azoospermia’ (DAZ). They suggested that DAZ might, in fact, be the elusive AZF. However, it was quickly observed that patients harbouring deletions incorporating DAZ did not have a consistent phenotype. Testicular biopsy results in men with deletions involving the DAZ region ranged from the complete absence of germ cells (Sertoli cell-only syndrome) to meiotic arrest with occasional production of mature, condensed spermatids. Subsequent analysis of additional patients even revealed that some DAZ-deleted men had sufficient spermatogenesis to have spermatozoa in the ejaculate (Reijo et al., 1996).

Vogt et al. (1996) conducted a large collaborative screening study of 370 men with azoospermia and severe oligozoospermia. Their analysis detected a distinct pattern of Y chromosomal deletions in 13 patients that mapped to three different subregions in Yq11. The most distal subregion coincided with the DAZ gene cluster described by Reijo et al. (1995) and was termed AZFc. The other two regions mapped proximal to AZFc on the Y-chromosome (Figure 1). Although the number of patients in each category was small, the testicular histology appeared to correlate with the particular Yq11 subregion deleted. They proposed using the terms AZFa, AZFb and AZFc to identify three distinct areas along the Y-chromosome that are frequently deleted in men with severe infertility (see Figure 1). The identification of various Y-chromosomal anomalies associated with severe male factor infertility has made the concept of a single ‘azoospermic factor’ unlikely (Yoshida et al., 1997). Instead, it appears that multiple factors may be responsible for defective spermatogenesis, including deletions of Y chromosomal segments (Lahn and Page, 1997).

Genetic abnormalities, including partial deletions of the Y-chromosome, are commonly detectable in men with non-obstructive azoospermia (NOA). NOA can be treated using testicular sperm extraction (TESE) with intracytoplasmic sperm injection (ICSI). Recent studies have shown that the presence of deletions involving the AZFc region do not appear to affect the chance of retrieving spermatozoa or have a significant impact on fertilization or pregnancy rates with ICSI. We investigated the effect of Y-chromosomal partial deletions on the chance of sperm retrieval with TESE. Eighty attempts at sperm retrieval were performed using TESE on men who were previously evaluated for Y-chromosome partial deletions.

Y-chromosome analysis was performed using a polymerase chain reaction (PCR)-based technique with 35 sequence-tagged-sites. Of the 80 men, nine (11%) had partial Y-chromosome deletions detected. Two azoospermic men with AZFc deletions had successful sperm retrieval, ICSI and a subsequent clinical pregnancy. Seven men had deletions involving the AZFb region (three men had isolated AZFb deletions, one had AZFa, AZFb and AZFc deleted, and three had AZFb and AZFc deleted). None of the seven men had spermatozoa extracted by TESE, a result that is significantly different from the overall 64% (47/73) sperm retrieval rate achieved at our centre (P = 0.001). Two men with AZFb deletions had cells consistent with round spermatids identified and injected into oocytes without effecting any normal fertilizations. Although preliminary, these results suggest that the presence of an AZFb deletion is a significantly adverse prognostic finding for TESE. Men with AZFb deletions should be apprised of these results before attempting TESE–ICSI. Alternatives such as donor insemination or adoption should be considered or therapy delayed until improved results with round spermatid injections are likely.

Key words: azoospermia/genetics/male infertility/micro-deletion/Y-chromosome
Men with severe infertility may now be treated with intracytoplasmic sperm injection (ICSI) if spermatozoa are present in the ejaculate or testicular sperm extraction (TESE) coupled with ICSI if azoospermic. The ability to extract spermatozoa from men with AZFc deletions using TESE was reported recently (Mulhall et al., 1997). TESE–ICSI treatment resulted in a term pregnancy and delivery. Although the specific AZF deletion may predict findings on diagnostic testicular biopsy, it is clear that diagnostic testicular biopsy histology does not always correlate with findings at the time of TESE. Some patients with a Sertoli cell-only pattern in their biopsy specimen may still have successful sperm procurement with TESE (Tournaye et al., 1996; Schlegel et al., 1997). At our centre, we have screened all azoospermic men for submicroscopic deletions of the Y-chromosome prior to attempting TESE–ICSI. We attempted to determine whether a patient’s Y deletion status impacted on the chances of successful sperm procurement using TESE.

Materials and methods

Patient selection and clinical evaluation

All men presenting to our centre for evaluation of male factor infertility undergo a thorough history and comprehensive physical examination. Initial laboratory evaluation included repeated semen analysis and hormonal profile. In addition, we offered Y-chromosome microdeletion screening to all men with azoospermia or severe oligozoospermia (<10 x 10⁶ spermatozoa/ml). The diagnosis of non-obstructive azoospermia was confirmed by formal diagnostic biopsy in which an additional testicular sample was cryopreserved for possible ICSI. Signed informed consent in accordance with Helsinki guidelines was obtained from all patients undergoing genetic analysis, as well as all patients who were candidates for diagnostic or therapeutic procedures. Referral for genetic counselling was provided for any patients found to have a genetic abnormality.

Testicular sperm extraction technique

All TESE procedures were performed by one surgeon (P.N.S.), who also reviewed the diagnostic biopsy pathology. All biopsies were preceded by analysis of a fresh semen specimen, obtained on the day of planned oocyte and sperm retrieval, and processed with an extensive evaluation for the presence of viable spermatozoa. TESE procedures were performed with either multiple biopsies as described by Devroey et al. (1995), or as single large incisions with multiple samples (Schlegel et al., 1997). The procedures were performed in conjunction with planned in-vitro fertilization (IVF) cycles with ICSI for the female partner.

Y-chromosome analysis

The techniques for Y-chromosome microdeletion screening applied to men in this study have been described in detail previously (Girardi et al., 1997). Briefly, DNA was extracted from peripheral leukocytes by two different methods using a Stratagene DNA Extraction Kit (Stratagene, La Jolla, CA, USA) and a Genomic DNA Purification Kit (Promega, Madison, WI, USA). A series of 35 sequence-tagged sites (STS) on Yq were used for detection of submicroscopic deletions. Primers were produced on an automated DNA synthesizer using previously published sequence tagged sites (Henegariu et al., 1993; Reijo et al., 1995, 1996). The technique of multiplex PCR was used for rapid molecular screening of these STS. Whenever failure of amplification was detected for a primer pair, subsequent PCR analysis using single primer pairs was performed and repeated, for a total of three evaluations with appropriate positive and negative controls to confirm the absence of each STS. All products of amplification were subjected to agarose gel electrophoresis.

Statistical analysis

Fisher’s exact test was used to assess statistical significance of differences between treatment outcomes (Microsoft Excel, Redmond, Washington, USA).

Results

Of the 286 men with severe male factor infertility screened for Y-chromosome microdeletions at our centre, deletions have been detected in 22 (7.7%). Of the 80 azoospermic men who were candidates for and subsequently underwent an attempted TESE procedure, 11% (9/80) had microdeletions detected. The clinical characteristics of these nine patients are summarized in Table I.

<table>
<thead>
<tr>
<th>AZFb deletions predict sperm extraction failure</th>
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<tr>
<td>two men with AZFc deletions, both had spermatozoa extracted with TESE and three embryos were transferred. One ongoing pregnancy has been achieved. All seven patients with deletions involving the AZFb region failed to have spermatozoa found despite aggressive TESE attempts. These seven attempts included men with isolated AZFc deletions as well as those with AZFb deleted as part of a larger deletion. Two of the</td>
</tr>
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Table I. Clinical characteristics of TESE patients with Y-chromosome microdeletions

<table>
<thead>
<tr>
<th>Patient</th>
<th>AZF region deleted</th>
<th>Age</th>
<th>FSHa</th>
<th>Semen analysis</th>
<th>Diagnostic biopsy resultsb</th>
<th>TESE result</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMG 135</td>
<td>a,b,c</td>
<td>30</td>
<td>26</td>
<td>azoo</td>
<td>SCO</td>
<td>Failed</td>
</tr>
<tr>
<td>SMG 32</td>
<td>b</td>
<td>NA</td>
<td>NA</td>
<td>azoo</td>
<td>NA</td>
<td>Failed</td>
</tr>
<tr>
<td>SMG 126</td>
<td>b</td>
<td>36</td>
<td>8</td>
<td>azoo</td>
<td>SpcA</td>
<td>Failed</td>
</tr>
<tr>
<td>SMG 190</td>
<td>b</td>
<td>49</td>
<td>11</td>
<td>azoo</td>
<td>SpcA</td>
<td>Failed</td>
</tr>
<tr>
<td>SMG 62</td>
<td>b,c</td>
<td>39</td>
<td>12</td>
<td>azoo</td>
<td>SpcA</td>
<td>Failed</td>
</tr>
<tr>
<td>SMG 99</td>
<td>b,c</td>
<td>32</td>
<td>17</td>
<td>azoo</td>
<td>SpcA</td>
<td>Failed</td>
</tr>
<tr>
<td>SMG 208</td>
<td>b,c</td>
<td>44</td>
<td>24</td>
<td>azoo</td>
<td>Hypo</td>
<td>Spermatozoa</td>
</tr>
<tr>
<td>SMG 79</td>
<td>c</td>
<td>26</td>
<td>16</td>
<td>&lt;1×10⁶(d)</td>
<td>Hypo</td>
<td>Spermatozoa</td>
</tr>
<tr>
<td>SMG 229</td>
<td>c</td>
<td>33</td>
<td>7</td>
<td>azoo</td>
<td>Hypo</td>
<td>Spermatozoa</td>
</tr>
</tbody>
</table>

Hypo = hypospermatogenesis; azoo = azoospermia; SpcA = spermatocytic arrest; SCO = Sertoli cell only; NA = not available.

aNormal FSH 0.4–8 mIU/ml.
bResults represent the most advanced stage of spermatogenesis seen.

cRound cells identified as presumed spermatids based on morphological criteria for oocyte injection.

dPatient known to have spermatozoa in ejaculate by history, but he became azoospermic prior to planned assisted reproduction procedure, requiring sperm extraction with TESE.

Discussion

Over the last several years there has been increasing interest in potential genetic causes of infertility. This interest stems from the success of ICSI, new sperm procurement techniques such as microscopic epididymal sperm aspiration (MESA) and TESE, and the identification of genetic abnormalities in a significant proportion of men with azoospermia who require sperm retrieval. Men with genetic abnormalities that would have rendered them sterile in the past are now capable of having their own biological offspring. Understandably, considerable concern has arisen over using immature or even ‘defective’ genetic material for assisted reproduction (Health Council of the Netherlands, 1996). Although Y-chromosome abnormalities are unlikely to affect somatic function adversely to a significant degree, long-term health effects (including fertility status) for children born after ICSI have yet to be evaluated. We have noted that detection of genetic abnormalities may alter a couple’s intention to proceed with assisted reproductive treatments (Rucker et al., 1998), but others have questioned whether pre-treatment genetic screening is worthwhile (van der Ven et al., 1997).

In this study, we report an additional advantage to Y-chromosome deletion screening for azoospermic men prior to TESE. The results of such testing may provide important prognostic information regarding the chances of obtaining spermatozoa by TESE for subsequent use with assisted reproduction. We have observed that men with deletions involving the AZFb region have invariably been found to have no extractable spermatozoa despite an extensive TESE procedure. Cells morphologically consistent with round spermatids have been identified from two men, but no fertilizations resulted using these immature cells.

As previously noted by Reijo et al. (1996), we have found that many men with AZFc deletions have spermatozoa in the ejaculate and do not require TESE for fertility treatment, only ICSI. Two patients with partial deletions of the Y-chromosome had spermatozoa successfully obtained by TESE. These individuals had isolated AZFc deletions. Of interest, one man had previously been documented to have spermatozoa in the ejaculate but subsequently became azoospermic.

Many reports have focused primarily on the AZFc region, possibly because of the early identification of the DAZ gene within this region by Reijo et al. (1995). The DAZ gene encodes an RNA binding protein expressed only in the testis. Large deletions of the Y-chromosome that encompass the DAZ gene cluster are clearly associated with impaired spermatogenesis. However, more subtle genomic abnormalities such as mutations of the DAZ gene have not been identified (Vereb et al., 1997). The fact that many men with isolated deletions of the AZFc region deletions is warranted. Given the prognostic significance of AZFc region deletions for success of TESE, continued attention to detection of AZFc region deletions is warranted.

Therefore, it appears that other regions of the Y-chromosome separate from, and proximal to the DAZ gene cluster may be equally or more important for completion of spermatogenesis. Deletions involving AZFb are commonly found in men who are TESE candidates. Our preliminary results indicate that the
presence of Y-chromosome deletions incorporating the AZFb region portend a poor prognosis for successful sperm procurement. In these cases, we present the poor prognosis for successful TESE and encourage couples to (i) consider the use of donor spermatozoa if TESE fails, (ii) delay a TESE attempt until techniques for round spermatid injection are more successful or (iii) consider alternative approaches to parenting (e.g. adoption, donor insemination). Our findings emphasize the importance of Y-chromosome microdeletion screening and appropriate counselling for all men found to have genetic anomalies.

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References


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