CASE REPORT

Transmission of a Y chromosomal deletion involving the deleted in azoospermia (DAZ) and chromodomain (CDY1) genes from father to son through intracytoplasmic sperm injection

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The transmission of a deleted in azoospermia (DAZ) deletion from a severely oligozoospermic patient to his son following intracytoplasmic sperm injection (ICSI) treatment is reported. The case report highlights the fertilizing capacity of spermatozoa carrying Y chromosome deletions in patients treated with ICSI and stresses the importance of genetic counselling in severe male infertility.

Key words: DAZ/CDY1/ICSI/microdeletions/Y chromosome

Introduction

Although the function of most genes on the Y chromosome is not known, evidence exists that microdeletions of the Y chromosome play a causal role in male infertility (Vogt, 1998). In particular, fertility candidate genes such as deleted in azoospermia (DAZ) are believed to have an important function in spermatogenesis since DAZ is frequently deleted in azoospermic and severely oligozoospermic men. The DAZ gene belongs to a multigene family clustering in the AZFc region of the Y chromosome (Vogt et al., 1996). The chromodomain gene (CDY1), a member of another multigene family, maps to this region, in closest proximity to the DAZ gene cluster (Lahn and Page, 1997). No specific function has been described yet for all genes identified so far. However, the high frequency of microdeletions in patients with azoospermia or severe oligozoospermia led to the introduction of polymerase chain reaction (PCR) screening of the Y chromosome in the diagnostic work-up of infertile men (for review see Simoni et al., 1998).

Apart from sporadic cases of natural transmission of similar or identical Y chromosome microdeletions (Vogt et al., 1996; Pryor et al., 1997), the majority of deletions are believed to be de novo, i.e. somatic events arising randomly in some germ cells of fertile men (Edwards and Bishop, 1997) who thereby produce an infertile son with a Y chromosomal defect in his genome. Such an individual would normally be infertile and further transmission of the genetic defect would not occur. However, intracytoplasmic sperm injection (ICSI) now offers an effective therapeutic option for these men and is believed to allow transmission of genetically determined infertility to the male offspring. Accordingly, analysis of microdeletions should be performed in all patients who are candidates for ICSI. When deletions are found, patients should be counselled about the possibility of producing an infertile son bearing the same deletion (Meschede et al., 1998).

Notwithstanding, some patients with severe oligozoospermia could be carriers of mosaicisms, with the Y chromosome being deleted in leukocytes but normal in the germ cell lineage (Kent-First et al., 1996). If these subjects undergo ICSI, they produce healthy male offspring with a normal Y chromosome. Here we report the ICSI-mediated transmission of a deletion involving the DAZ and the CDY1 genes by a man with severe oligozoospermia.

Case report

In November 1996 a 28 year old male and his 31 year old wife, having experienced primary infertility of 1.5 years duration, presented at our infertility clinic. Female medical history and reproductive function revealed hypothyroidism under treatment with L-thyroxin (50 µg/day) and endometriosis at American Fertility Society (AFS) stage III, successfully resolved by laparoscopy.

Male medical history revealed acute pancreatitis in 1993 and two resolved, acute episodes of Crohn’s disease in 1987 and 1993. Although unlikely, it cannot be excluded that the previous Crohn’s disease or its therapy (which was completed 4 years before the ICSI treatment) had an influence on the current patient’s spermatogenesis or epididymal function. Apart from a first-degree varicocele, genital examination was normal. Combined testes volume was 33 ml with slightly decreased hypodense homogeneous architecture shown by ultrasound examination. Initial semen analysis (WHO, 1992) showed hypergonadotrophic [serum follicle stimulating hormone (FSH): 12.1 U/l] azoospermia with decreased glucosidase concentrations (a marker of epididymal function) and normal markers for the seminal vesicles (fructose) and prostate (zinc).

Because of the decreased glucosidase concentrations and the detection of increased leukocytes in the ejaculate (2.5 × 10⁶/ml), a genital tract infection was suspected and the patient was treated with tetracyclines for 10 days. Upon completion of antibiotic treatment repeated semen analyses revealed resolution of the genital tract infection with sperm concentrations now ranging between <0.1 × 10⁶/ml and 0.1 × 10⁶/ml (Table I), whereas serum FSH values remained constantly elevated. Following genetic counselling (Meschede...
Y chromosomal deletion involving DAZ and CDY genes

Table I. Semen parameters of the reported patient

<table>
<thead>
<tr>
<th>Date</th>
<th>31 October 1996</th>
<th>6 February 1997</th>
<th>3 April 1997</th>
<th>1 October 1997</th>
<th>3 November 1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstinence time (days)</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Ejaculate volume (ml)</td>
<td>2.9</td>
<td>2.7</td>
<td>2.7</td>
<td>2.6</td>
<td>4</td>
</tr>
<tr>
<td>Sperm motility (% WHO grade a+b)</td>
<td>68</td>
<td>11</td>
<td>49</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Sperm concentration (×10^9/ml)</td>
<td>0</td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Normal sperm morphology (%)</td>
<td>21</td>
<td>14</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

et al., 1998), ICSI was performed (Koppers et al., 1998). After injection of 16 oocytes, three embryos were transferred resulting in a singleton pregnancy. A normal pregnancy led to delivery of a son without evidence of minor or major malformations.

DNA from the father of the patient was not available as the patient’s parents were unaware that the patient’s son was conceived with medical help. Apart from a sister, the patient has no siblings.

Materials and methods

Genomic DNA was obtained from the peripheral leukocytes of the patient and from buccal epithelia of his son using the Nucleon Kit II® (Scotlab, Wiesloch, Germany). PCR amplification of genomic DNA was performed using primer pairs sY84 in the AZFa region, sY130 and sY143 in the AZFb region, sY147, sY149, sY254 and sY255 in the DAZ gene located in the AZFc region and primer pair CDY1, amplifying the CDY1 gene located distal to DAZ (Vollrath et al., 1992; Gromoll et al., 1996; Vogt et al., 1996; Lahn and Page, 1999). In addition, a part of exon 10 of the autosomal FSH receptor (Gromoll et al., 1996) was amplified to control the quality of the DNA preparation and to exclude PCR failure. A DNA sample from a proven father was used as positive control. As negative control, every PCR reaction included one sample of female genomic DNA (data not shown). The PCR reactions were carried out as previously described (Simoni et al., 1997).

Results

Both the father and his ICSI son were found to be carriers of a presumably identical, large deletion in the AZFc region involving both the DAZ and the CDY1 genes (Figure 1). The two more proximal AZFa and AZFb regions appeared normal.

Discussion

The existing evidence shows that deletions of the Y chromosome result in male infertility. As additional anomalies have been reported in adult males with large deletions of Yq (Salo et al., 1995) and as lengthening of deletions from father to son has been observed as well (Kobayashi et al., 1994; Stuppia et al., 1996), concerns have been expressed regarding the potential for adverse genetic consequences for sons generated by assisted reproduction techniques and possibly having larger Y chromosome deletions than their fathers. The case presented in this report shows the transmission of an apparently identical AZFc deletion from a severely oligozoospermic father to his otherwise healthy son, in agreement with data reported previously (Kent-First et al., 1996; Vogt et al., 1996; Pryor et al., 1997). However, since the precise breakpoints were not determined, we cannot exclude that minor extensions of the deletion might have occurred. Although larger deletions in the next generation might be observed in some cases, probably due to the inherent instability of the Y chromosome (Graves, 1995), the present case lends support to the increasing evidence that Y chromosome deletions found in peripheral leukocytes reflect the Y chromosome deletion in the germ line (Reijo et al., 1996). More cases of microdeletions after inherited ICSI should be analysed in the future in order to verify whether the genomic deletion can be significantly extended through residual spermatogenic activity in subjects with severe oligozoospermia.

In agreement with previous reports, this case demonstrates the fertilizing capacity of spermatozoa from patients with AZFc deletions resulting in both spontaneous (Kobayashi et al.,
1994; Stuppia et al., 1996; Vogt et al., 1996) and ICSI-induced (Kent-First et al., 1996) pregnancies. This suggests that although some gene(s) in the AZFc region might be necessary for normal spermatogenesis, they are not relevant for the fertilization process.

The occurrence of spontaneous pregnancies induced by men with deletions involving candidate AZF genes during their youth (Kobayashi et al., 1994; Stuppia et al., 1996; Vogt et al., 1996; Simoni et al., 1997) suggests the possibility of a gradual worsening of spermatogenesis due to the genetic defect. Unfortunately, in most studies fathers carrying the same deletion as their infertile sons were identified only after diagnosis in the sons, i.e. long after the time of procreation, when semen parameters were not available. Semen parameters of the fathers around the time of procreation are known in only two studies (Kent-First et al., 1996; Mullhall et al., 1997). Moreover, both azoospermic and oligozoospermic phenotypes and histology can occur in the presence of the same deletion, a finding difficult to reconcile with the concept of deletion-specific histological lesions (Vogt et al., 1996). Taken together, these data support the idea of a progression from oligozoospermia to azoospermia over time (see patient Mü2v in Vogt et al., 1996; patient no. 9 in Pryor et al., 1997; patient nos 3 and 5 in Simoni et al., 1997).

The prognosis resulting from the inherited Y chromosome microdeletion for the fertility of ICSI sons is presently unknown. It is, therefore, important to advise such patients to undergo andrological examination, including semen analysis, soon after puberty. If further evidence of progression from oligozoospermia to azoospermia accumulates, the ICSI sons should consider inducing a pregnancy early in life and/or cryopreserving their semen before they become azoospermic. Moreover, prior knowledge that natural conception could be problematic and that assisted reproduction techniques might be necessary could prevent years of frustrating search for help by the infertile couple (Kent-First et al., 1996). We recommend that all sons of Y chromosome-deleted fathers should be tested for AZF deletions early in life.

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References

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