CASE REPORT

Natural transmission of a partial AZFb deletion of the Y chromosome over three generations

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The natural transmission of microdeletions of the Y chromosome is occasionally reported in the literature. Here we describe the natural transmission of a partial AZFb deletion over three generations. PCR amplification of several sequence tagged site markers in the three AZF regions of the Y chromosome was carried out in a patient with oligoasthenoteratozoospermia, his father and his naturally conceived son. The deletion was confirmed by Southern blotting. The propositum, his father and his son showed a probably identical, partial deletion of the distal part of the AZFb region, involving sY130 and sY143. The deletion was confirmed by Southern blotting using the sY130 probe. Partial AZFb microdeletions can be associated with moderate oligozoospermia allowing natural conception and therefore natural transmission of this genetic anomaly. Further studies are needed to define the pathogenetic significance of microdeletions involving sY130 and sY143.

Key words: AZF/fertility/microdeletion/Y chromosome

Introduction

Genes in the azoospermia factor (AZF) region on the long arm of the Y chromosome are involved in the control of human spermatogenesis. At least three different AZF regions (AZFa, b and c) exist on the Y chromosome. Microdeletions in these gene loci may result in azoospermia or severe oligozoospermia (Simoni et al., 1998; Vogt, 1998; Krausz and McElreavey, 1999; Krausz et al., 2000; Ma et al., 2000; Foresta et al., 2001). In general, testis histology of patients with complete deletions of the AZFa region and/or the AZFb region reveals a complete Sertoli cell-only syndrome (SCOS), while testis histology of patients with partial AZFa and AZFb deletions or with AZFc deletions can result in a broad array of testicular phenotypes ranging from complete SCOS or spermatogenetic arrest to hypospermatogenesis (Vogt et al., 1996; Brandell et al., 1998; Krausz et al., 2000). According to the literature, at the present time >3000 infertile men have been screened for Y chromosomal microdeletions. On average, microdeletions were found in ~7.3% of infertile men; the vast majority of deletions were found in azoospermic men where deletion frequency was up to 15%. In men with sperm concentrations >5×10⁶/ml, microdeletions were found only sporadically (Simoni et al., 1998; Foresta et al., 2001). In this paper, we describe a case of natural transmission of a partial deletion of the distal part of the AZFb region over three generations.

Case report

In July 1998, a 31-year-old male and his 31-year-old wife with a 2 year history of primary infertility attended our clinic. Female medical history, including basal body temperature, hormone profile and laparoscopy revealed no abnormalities. Three homologous inseminations were performed, but no pregnancy was achieved.

Male medical history revealed asthma bronchiale since 1995 (treated with sympathicomimetics and glucocorticoid aerosoles) and a ligation of a left-sided varicocele in 1984. Post-surgery antibiotics were given because of an infection (probably epididymitis). In a first semen analysis performed in 1996 by an outside urologist, mild oligoteratozoospermia with a sperm concentration of 19×10⁶/ml was found. In four further semen analyses a moderate decline in sperm concentration to 6×10⁶/ml was found. Oral testosterone undecanoate (Andriol 160 mg/d) was prescribed for 4 weeks. Results of physical and genital examination were normal. Testes volume was 17 ml bilaterally. Ultrasonographic examinations of the testes showed homogenous architecture with single echodense areas in both testes.

Semen analysis performed in our institute in July 1998 according to World Health Organization guidelines (World Health Organization, 1992) showed oligoasthenoteratozoospermia [sperm concentration 2.4×10⁶/ml; 37% progressive motility (World Health Organization grade a and b); 11% normal morphology]. FSH, LH and testosterone were in the normal
range. Markers for the function of epididymis, seminal vesicles and prostate were in the normal range. We recommended termination of testosterone undecanoate medication, since no positive effects on male infertility had been found in controlled studies (Kamischke and Nieschlag, 1999).

After antibiotic treatment for an infection of the accessory glands in April 1999, semen analysis showed moderate oligoasthenoteratozoospermia (sperm concentration 8.3 x 10⁹/ml; 48% progressive motility; 13% normal morphology). A spontaneous pregnancy occurred and a healthy boy without evidence of minor or major malformations was born in April 1999. A control semen analysis performed in February 2002 confirmed moderate oligoasthenoteratozoospermia (sperm concentration: 7x10⁹/ml; 40% progressive motility; 18% normal morphology) with no signs of further deterioration of spermatogenesis.

The patient had two sisters who were 7 and 4 years older than himself. Both sisters had children and no cases of infertility were reported in his family. At the time of the patient’s birth, his mother was 29 years old and his father 35 years old. Further information about their reproductive state was not available. However, as the younger sister was 4 years older than the patient, and assuming no active contraception, a maximum time to pregnancy of ~3 years can be estimated, indicating that (at this timepoint) no severe fertility problems were present. The patient’s father was unwilling to deliver a semen sample when attending our clinic; however, slightly elevated FSH serum levels (7.6 IU/l, normal range for young men 1–7 IU/l) were found, while LH, testosterone, estradiol, prolactin and sex hormone-binding globulin serum levels were in the normal range.

Because the first semen sample at our institute showed sperm concentrations <5x10⁹/ml and because ICSI treatment was anticipated, we analysed the Y chromosome for microdeletions (Simoni et al., 1998). After we found a deletion in AZFb and when the spontaneous pregnancy occurred, we investigated whether the microdeletion was also found in the patient’s son and father.

Materials and methods

Genomic DNA was obtained from peripheral leukocytes of the patient and his father as well as from buccal epithelia of the patient’s newborn son using the Nucleon Kit II® (Scotlab, Wiesloch, Germany). In the first screening analysis, PCR amplification of genomic DNA was performed using primer pairs sY84 in the AZFa region, sY130 and sY109, Y66H34pr, sY117, Y6Phc54pr, sY153, Fr15-Ilpr, Y6HP52pr, sY157 and sY255.

The deletion of sY130 in the propositum was further analysed by Southern blotting. Genomic DNA (8 µg) was digested with either EcoRI or BamH1. The restricted DNA was separated by electrophoresis in 0.8% agarose gels and transferred to nylon membranes (Hybond-N; Amersham Pharmacia, Freiburg, Germany) by capillary blot. The filters were baked for 2 h at 80°C to fix the DNA. The blot was prehybridized using ExpressHyb (Clontech, Heidelberg, Germany) for 30 min and hybridized for 1 h 30 min at 60°C. Several washing steps were performed according to the recommendations of the manufacturers and the blot was exposed to Phosphoimager cassettes. The sY130 sequence tagged site (STS) was subcloned into the pGEM-Teasy vector (Promega, Heidelberg, Germany) and its identity was confirmed by DNA sequencing. For labelling purposes the sY130 fragment was removed from the vector by EcoR1 digestion and subsequently labelled with 3²dCTP by random priming.

Results

The patient, his father and his son were found to be carriers of a presumably identical partial microdeletion in the AZFb region involving the sY130 and sY143 loci (Figure 1). The deletion of sY130 was confirmed by performing a temperature gradient amplification at different annealing temperatures (55–60°C) in the patient and his son (not shown). The deletion in the propositum was confirmed by Southern blot analysis of sY130 (Figure 2). This analysis indicated cross-hybridization to DNA bands common to females and males, which might be caused by non-Y chromosomal DNA. In addition, specific male presumably Y chromosomal bands could be detected in the fertile man, which were completely lacking in the patient.

The remainder of the AZFb region as well as the AZFa and the AZFc regions appeared normal (Figure 3). Using more STS from the AZFb region confirmed the presence of all of them in all the family members (Figure 3), indicating that the observed deletions did not grossly expand over the generations; however, a minor extension of the deletion not covered by the STS used for AZFb cannot be excluded.

Discussion

The majority of deletions of the Y chromosome are believed to arise de novo. However, in some cases a deletion in the AZF region has been transmitted from the (fertile) father to the infertile patient (Stuppa et al., 1996; Vogt et al., 1996; Pryor et al., 1997; Chang et al., 1999; Saut et al., 2000). In other published cases the deletion was transmitted through ICSI treatment (Kent-First et al., 1996; Mulhall et al., 1997; Jiang et al., 1999; Kamischke et al., 1999; Kleiman et al., 1999; Page et al., 1999). One patient with a deletion in the DAZ region and severe oligozoospermia was able to induce two pregnancies earlier in life; these ended in abortion in the third month (Simoni et al., 1997).

Chang et al. reported a family in which the father transmitted a deletion in the DAZ region to his four sons (Chang et al., 1999). At the time of analysis the father, who had formerly proven his fertility, was azoospermic; the four sons were...
infertile with azoospermia or severe oligozoospermia. After testicular sperm extraction one son transmitted the deletion to his male newborn. Phenotypic differences of identical mutations in one family and an age-dependent decline of fertility may be causative. The case reported in this paper, however, is the first in which a patient who had inherited a partial deletion in the AZFb region from his father transmitted the deletion to his son spontaneously. We do not know whether the patient’s parents used contraceptives. As the two sisters were 7 and 4 years older than the patient, a maximum time to pregnancy in the parents of ~2 and 3 years respectively can be estimated, indicating only moderate subfertility at that timepoint. When the patient was born the father was 35 years and the mother 29 years old. Therefore in this case no rapid decline of fertility can be assumed. However, we do not have any information about the reproductive functions of the father to date, as he was not willing to provide a semen sample. At the actual age of 66 years, his serum FSH level was only moderately elevated, therefore severe testicular damage may be considered unlikely.

In one case reported the deletion found in the father was smaller than that of the son (Stuppia et al., 1996), suggesting that some deletions do not necessarily lead to infertility, but that these deletions make the Y chromosome more liable to a second deletion which leads to infertility. In agreement with other data reported previously (Kent-First et al., 1996; Vogt et al., 1996; Pryor et al., 1997; Chang et al., 1999; Kamischke et al., 1999; Kleiman et al., 1999), in the case presented here a probably identical deletion was transmitted over three generations. However, since the region of the Y chromosome in which the deletion is located contains repetitive sequences and the method employed in the present study allows only a rough determination of the boundaries, a minor extension over generations of the deletion not covered by the STS used for AZFb cannot be excluded.

The finding of AZFb deletions in fertile men is a contradictory issue. Pryor et al. found small microdeletions in the AZFb region (deletion site sY207 and sY272) in four of 200 fertile healthy men (Pryor et al., 1997). In addition, one father of an infertile patient with a mutation in the AZFb region had an identical deletion in this region. Today sY207 and sY272 are considered polymorphic markers. Our patient, his father and his son presented a partial microdeletion in the distal part of the AZFb region showing that the loss of this genomic region is compatible with natural fertility. In contrast, Brandell et al. found that the presence of an AZFb deletion is a severely adverse prognostic factor for ICSI based on sperm obtained by TESE (Brandell et al., 1998) and the analysis of the literature has confirmed that complete AZFb deletions are associated with the absence of mature sperm in the testis.
(Krausz et al., 2000; Kleiman et al., 2001). In our case the partial deletion in the AZFb region was spontaneously transmitted over three generations, indicating that the deletion is compatible with at least some degree of fertility and natural paternity. However, we cannot exclude the possibility that the moderate decrease in sperm count over the years was genetically determined. The medical history reported varicocele treatment and an infection of the accessory glands. In April 1999, after antibiotic treatment, an improvement of semen parameters could be observed, indicating that the reduced semen parameters in this patient could be due to partial obstruction of the efferent ducts. Semen parameters were practically unchanged in February 2002, raising the possibility that the moderate oligoasthenoteratozoospermia in the propositum is not related to his partial AZFb deletion. It is therefore likely that sY130 and sY143 are polymorphic markers with no effect on sperm production. However, without a semen analysis of the father of the propositus, the pathogenetic role of the deletion cannot be evaluated more precisely.

This case suggests that sons of men with deletions in the AZF region should undergo andrological examination in puberty and, possibly, cryopreservation of semen in early adulthood, since the AZF region should be tested for AZF deletions, even when they are not visible on the Y chromosome map. However, without a semen analysis of the father, the pathogenetic role of the deletion cannot be evaluated more precisely.

This case suggests that sons of men with deletions in the AZF region should be tested for AZF deletions, even when pregnancy has been achieved spontaneously. Moreover, they should undergo andrological examination in puberty and, possibly, cryopreservation of semen in early adulthood, since we cannot exclude an age-dependent, gradual worsening of spermatogenesis due to the deletion.

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**References**

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**Figure 3.** Y chromosome map and microdeletion in the AZFb region of the Y chromosome long arm in the patient, his father and son. Sequence tagged sites marked with asterisks were only investigated in the patient.
AZFb deletion and spontaneous fertility


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