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Case Report: Natural transmission of an AZFc Y-chromosomal microdeletion from father to his sons

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Y-chromosomal microdeletions, associated with oligozoospermia or azoospermia, are usually *de novo* deletions in the affected patients. We report here the rare case of an affected father who transmitted a Y-chromosomal microdeletion to at least two of his three sons naturally and who also fathered a daughter. The extent of the deletion, which was determined with new STS-primers and covers 3.5 Mb, was identical in the father and his azoospermic sons. To determine any possibly modifying influence of other genes involved in spermatogenesis, we analysed two polymorphisms of the *DAZL* gene, the autosomal homologue of the deleted *DAZ* gene. *DAZL* and *DAZ* might be functionally related to each other. However, we found identical polymorphisms in exon 2 and 3 of the *DAZL* gene, in both father and his sons, corresponding to the most prevalent genotype in fertile men. Thus, other genes or environmental factors must modify spermatogenesis in men with identical Y-chromosomal microdeletions.

Key words: AZFc Y-chromosomal microdeletion/*DAZL* gene polymorphism/natural transmission/spermatogenesis

Introduction

Microdeletions in the AZF (Azoospermia factor) regions a, b and c on the long arm of the Y-chromosome represent the most common form of genetically defined infertility (for review, see Krausz *et al*., 2003). AZFc deletions are known to be associated with a heterogeneous histological profile, varying from SCO-syndrome to spermatogenic arrest and hypospermatogenesis (Luetjens *et al*., 2002) and the seminal parameters range between azoospermia and oligozoospermia. Fathers of affected patients usually do not reveal any microdeletion, so that microdeletions of the Y-chromosome are regarded as *de novo* events (Foresta *et al*., 2001). Here we describe the exceptional case of a Kurdish family in which the affected father spontaneously induced four pregnancies and transmitted the microdeletion to at least two of his three infertile sons. We defined the extent of their deletions exactly and analysed two known single-nucleotide polymorphisms of the *DAZL* gene, an autosomal homologue of the *DAZ* gene on chromosome 3p24. Originally detected in the Taiwanese population, these polymorphisms with an A to G transition at nucleotide position 260 in exon 2 and at position 386 in exon 3 result in a threonine to alanine substitution (Teng *et al*., 2002). The latter was found at a higher prevalence in Taiwanese patients with severe oligozoospermia and nonobstructive azoospermia, compared to a control group.

Case report

The index patient, born in 1973, came to our clinic for the first time in 1999 with a history of barrenness for three and a half years. He mentioned a history of previous drug-treated hyperprolactinemia and a mesangioproliferative glomerulonephritis type IgA not requiring treatment. His older brother had a 16-year period of barrenness, his younger brother suffered from slight mental handicap and had no partner, whereas a 35 year-old sister had six children. There was no history of male-factor infertility (Figure 1). The clinical examination and genital ultrasound of the proband were normal. Semen analysis and hormone analysis are summarized in Table I. Yq deletion screening was performed according to the standards of the European Academy of Andrology (Simoni *et al*., 1999). After detection of an AZFc deletion the extent of the deletion was analyzed with STS

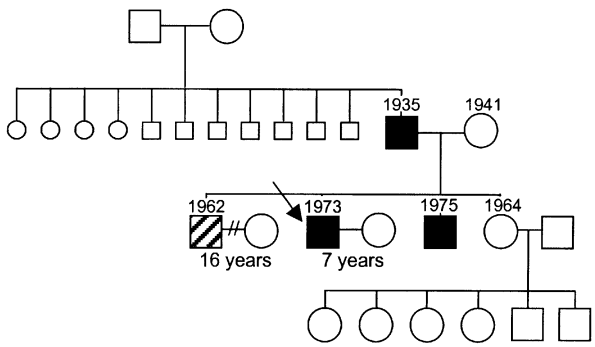

Figure 1. Pedigree of the AZFc deleted family. Black boxes indicate that an AZFc-microdeletion was detected. Dashed box indicates that an AZFc-microdeletion is expected. The arrow points at the index patient. 'Years' correspond to the time of barrenness.

primers known to define the proximal or distal borderlines of the AZFc region (Kuroda-Kawaguchi *et al*., 2001; Luetjens *et al*., 2002). Chromosome analysis revealed a normal karyotype. A testicular biopsy demonstrated a Sertoli cell-only pattern in most of the tubuli with single spermatogonia and spermatocytes in a few tubuli. The proband was re-examined in 2002 and azoospermia was confirmed. Hormone analysis and Y chromosome deletion screening were also performed in the younger brother and in the father (Table I and Figure 2). The younger brother was azoospermic; the 67-year old father refused semen analysis. The older brother was excluded from the examinations at the request of the index patient. His 16-year period of barrenness indicates impaired spermatogenesis and, in conjunction with the results of the other male family members, a microdeletion of the Y chromosome has to be assumed.

Analysis of the single-nucleotide polymorphisms (SNP) at nucleotide position 260 in exon 2 and at position 386 in exon 3 of the *DAZL* gene was performed according to the protocol of Teng *et al*. (2002) in the proband, his younger brother and the father. All three men had the same allele (AA) at position 260 and 386. The AA genotype is the most prevalent genotype in fertile men, thereby excluding any contribution of the *DAZL* SNPs to the infertility of the affected family. In a control group of Caucasian men 16% displayed a heterozygous nucleotide variant (AG) for the 260 SNP and the remaining 84% were homozygous for the A allele. For the 386 SNP we found the AA variant in 100% of Caucasian men (unpublished data).

Discussion

Microdeletions of the Y chromosome are associated with infertility of the affected patients since they cause azoospermia or oligozoospermia. Notwithstanding, microdeletions do not exclude pregnancy induction and in rare cases spontaneous pregnancies occur (Chang *et al*., 1999; Saut *et al*., 2000; Calogero *et al*., 2002; Gatta *et al*., 2002). Moreover, microdeletions can be transmitted by ICSI (Kamischke *et al*., 1999).

Table I. Semen parameters and hormone levels of relevant family members

	Age (years)	Semen analysis	FSH (1–7 IU)	Inhibin B (>100 pg/ml)	Testosterone (>12 nmol/l)
Patient	30	azoospermia	26	34.9	20
Brother	28	azoospermia	36.6	<30	15.4
Father	67	not investigated	21.1	70.6	12.3

Here we report the uncommon case of an AZFc deleted patient who spontaneously fathered four children. At least two of his three sons are azoospermic, reflecting a phenotypic heterogeneity despite identical microdeletions in both generations. Even if the seminal parameters of the father are not known, he must have had spermatozoa for a period of at least 12 years. The difference between the phenotypes of the two generations is also reflected by FSH and inhibin B levels. They are not in the normal range, neither for the father nor for the sons, but they are less affected in the father, suggesting that spermatogenesis of the father was less impaired compared to his sons. Nevertheless, the father's fertility should not be regarded as normal as it took several years to father four children, and as his wife was only 34 years old when the last child was born.

Undoubtedly, the phenotype of this deletion deteriorated in the sons, who were azoospermic. It has been suggested that the worsening of the phenotype by transmission of the microdeletion from one generation to the next might be due to a widening of the Yq microdeletion, as reported in two cases: in one case the microdeletion involved the *DAZ* gene (Calogero *et al*., 2002), in the other case the microdeletion was distal to the *DAZ* gene (Stuppia *et al*., 1996). In our case, however, we demonstrate that the extent of the deletion in the father and his infertile azoospermic sons was identical. Other authors who found identically deleted Y chromosomes in father and sons used conventional primers and obtained a lower level of resolution (Chang *et al*., 1999; Saut *et al*., 2000; Gatta *et al*., 2002). These authors also report a spontaneous vertical transmission of a *DAZ* deletion to multiple offspring. From these data we conclude that the occurrence of the higher penetrance of the phenotype 'infertility' in the second generation is not due to an extension of the microdeletion, in accordance with the current view of the deletion mechanism (Repping *et al*., 2003).

In some patients with a Y-chromosomal microdeletion, a decrease of spermatogenesis with age has been observed (Simoni *et al*., 1997), whereas Oates *et al*. (2002) found a fluctuation in sperm concentration but no decrease over a time of maximal 7 years in four AZFc-deleted oligozoospermic men. In the family described here, the father induced the last pregnancy at the age of 40, whereas azoospermia was detected in the sons in their 25th and 27th years of life. In the other families with vertical transmission to multiple offspring the time course is comparable (Chang *et al*., 1999; Saut *et al*., 2000) so that different degrees of infertility in both generations are not due to age-dependent alterations of spermatogenesis.

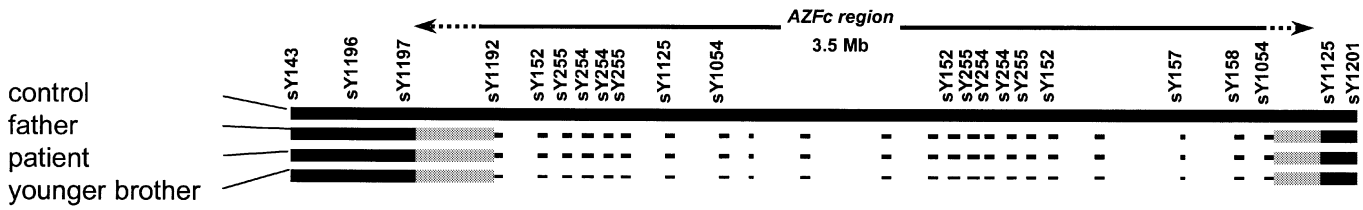

Figure 2. AZFc-region of the Y chromosome with the used STS (sequence tagged sites) primers and present (black boxes) and deleted (dashed box) DNA-portions.

Thus, this case supports previous observations that identical AZFc microdeletions may be associated with a different impairment of spermatogenesis not excluding natural conception. A deterioration of the phenotype may be associated with the transmission to the following generation, whereby genes or environmental factors which modify spermatogenesis still have to be identified.

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