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Three-Generation Evaluation of Y-Chromosome Microdeletion

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ABSTRACT: Sperm cells can be retrieved directly from the testis (testicular sperm extraction [TESE] procedure) and used for intracytoplasmic sperm injection (ICSI), circumventing underlying spermatogenetic defects. Thus, it is important that added information be available on the genetic defects in men undergoing TESE for the ICSI procedure and on the transmission of genetic factors associated with infertility to the offspring. We report a three-generation genetic analysis of a family with a case of male factor infertility. The proband, previously diagnosed as infertile, was physically examined and laboratory tested for gonadotrophic hormones, semen analysis, karyotype and Y-chromosome microdeletion screening in the blood and testis. The Y-chromosome microdeletion screening was performed by multiplex polymerase chain reaction with 20 Y-chromosome sequenced, tagged sites located at the Y chromosome. A microdeletion including the AZF-c region was detected in the azoospermic patient. His father, four brothers, and three offspring born after ICSI also underwent Y-chromosome microdeletion screening. The genetic analysis of the male members of the patient's family did not reveal similar microdeletions. The newborn male was found to bear a Y-chromosome microdeletion similar to that of his father. The fertilization capacity of the proband testicular microdeleted spermatozoa by the ICSI procedure is described. The transfer of the genetic defect raises the possibility that the son will have the same fertility problem as his father.

Key words: Deletion, DAZ, infertility, inherited microdeletion, azoospermia.

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Introduction of the intracytoplasmic sperm injection (ICSI) technique caused a revolution in the treatment of severe male infertility due to the high success rates, even in cases of severely-impaired spermatogenesis. However, development of therapeutic possibilities has not been accompanied by an appropriate increase in knowledge of male infertility etiology and the contribution of genetic factors. Since the ICSI procedure circumvents underlying spermatogenetic defects, transmission to the offspring of genetic factors associated with infertility has thoroughly been discussed and currently investigated (Kent-First et al, 1996; Van Assche et al, 1996; Kremer et al, 1997, van der Ven et al, 1997). In light of this issue, it is important that more information be available on the genetic defects in men undergoing testicular sperm extraction (TESE) for the ICSI procedure. Knowledge of their clinical characteristics and of the possible transmission of their defects to their offspring should also be obtained.

The incidence of chromosomal constitutional aberrations among azoospermic males has been described to be as high as 14–16% (Tiepolo and Zuffardi, 1976; Kleiman et al, 1998). Tiepolo and Zuffardi (1976) reported six azoospermic men with gross deletions in the Y-chromosome long arm and stated the existence of a factor necessary for normal spermatogenesis, termed "azoospermia factor" (AZF) (Ma et al, 1992). Recently, using Y-chromosome sequenced, tagged sites (STS), small interstitial deletions (microdeletions) were detected among infertile men at a frequency of 5–18% (Ma et al, 1992; Reijo et al, 1995; Qureshi et al, 1996). The microdeletions were identified in the AZF region located at Yq11.22–23 (Andersson et al, 1988). The microdeletions were mapped to at least three close subregions, labeled AZF-a, AZF-b, AZF-c (Vogt et al, 1996). Two candidate genes were first identified: the ribonucleic acid [RNA]-binding motif (RBM) mapped on the AZF-b region and the deleted-in-azoospermia (DAZ) genes identified in the AZF-c region (Ma et al, 1993; Reijo et al, 1995). Additional genes have been recently isolated in the AZF regions (Jones et al, 1996; Lahn and Page, 1997; Brown et al, 1998). Microdeletions in the AZF-c region, including the DAZ gene, have been identified not only in azoospermic males but also among those with oligozoospermia (Kent-First et al, 1996; Qureshi et al, 1996; Reijo et al, 1996; Pryor et al, 1997). The consequence of these observations is that some forms of male infertility may become hereditary once the ICSI procedure is used.

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We report a three-generation genetic analysis of a family with male factor infertility. The occurrence of a microdeletion is demonstrated in a second-generation male, and the transmission of the microdeleted Y chromosome to his male baby conceived by the ICSI technique using testicular sperm cells is demonstrated.

Methods

Patient Evaluation

The proband, a 38-year-old male who was previously diagnosed as infertile due to primary testicular failure, was referred to our institute for testicular sperm extraction. Physical examination revealed normal masculine appearance, no gynecomastia, and testicular volume of 15 ml (normal range, 15–25). Laboratory studies revealed elevated follicle-stimulating hormone levels of 23 IU/ml (normal range, 2–8) but normal values of luteinizing hormone (5.9 IU/ml; normal range, 1–6) and plasma testosterone (4.2 ng/ml; normal range, >3 ng/ml). The semen volume was 3.1 ml (normal range, 2–5 ml), pH 8.2 (normal pH range, 7.2–8.3), fructose concentration 2400 μg/ml (normal range, >1260). No spermatozoa was identified in the semen, even after high-speed centrifugation at 8000 × *g*.

After signing a detailed informed consent, the couple was enrolled in our *in vitro* fertilization (IVF) ICSI-TESE program, and detailed genetic surveillance was performed, comprising karyotype analysis and Y-chromosome microdeletion screening. As a result of the findings, his father and four brothers underwent Y-chromosome microdeletion screening, and the couple received genetic counseling.

Multiple testicular biopsies were performed from both testes as previously described (Hauser et al, 1998). Ovarian stimulation, retrieval of oocytes, and fertilization with testicular sperm (ICSI) were achieved as aforementioned (Yavetz et al, 1997). After successful triplet conception and birth, cord blood from the three offspring (two females used as a control and one male) were tested with all the Y-chromosome STS markers used in this study to identify Y-chromosome microdeletions.

DNA Isolation

Genomic DNA was extracted from blood and cord blood lymphocytes with the Master Pure® Genomic Purification Kit (Epicenter Technologies, Madison, Wisconsin). Genomic DNA from the testes was isolated after washing the mashed tissue with saline twice. Cells were incubated with lysis buffer including proteinase K at 55°C, and subsequent proteins were salted out with sodium chloride, followed by precipitation of DNA with ethanol.

Multiplex PCR Y-chromosome Analysis and Cytogenetic Evaluation

Eighteen STS on Yq spread over intervals 5 and 6 were used to identify submicroscopic deletions. In addition, two STS located within the gene SRY were used as internal control testing for the presence of the Y chromosome. A multiplex PCR technique in 25 μl on a PTC-200 thermal cycler (MJ Research, Inc., Watertown, Massachusetts) was used to evaluate the STS. The primer mix used in the multiplex PCR included the following: mix A (SRY 37, sY143, sY158, sY153), mix B (DAZ, sY14, sY134), mix C (sY14, sY84, sY127, sY81), mix D (sY254, sY136, sY255), mix E (sY86, sY160, sY121), and mix F (sY108, sY105, sY87, sY97). The STS primers have been previously published (except the DAZ primers) by Vollrath et al (1992) and Reijo et al (1995). The DAZ primers used were the following: left primer, ggAAgCTgCTTTggTAgATAC and right primer, TAggTTTCAgTgTTTggATTCCg. The DAZ PCR product is 1.3 kb. Whenever failure of amplification was detected, two additional PCRs (in the multiplex PCR mix and PCR of the STS alone) were performed to confirm the absence of the unamplified STS.

Chromosome analysis was performed on peripheral lymphocytes of the patient with G-banding staining. Twenty-seven metaphases were analyzed.

Table 1. *Intracytoplasmic sperm injection cycles*

IVF-ICSI cycle	Source of spermatozoa	No. of oocytes retrieved	No. of embryos transferred	No. of fetuses
I	First TESE (fresh)	4	3	none
II	First TESE (thawed)	9	2	none
III	Second TESE (fresh)	8	6	3

IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; TESE, testicular sperm extraction.

Results

Patient

The patient underwent scrotal exploration and multiple-sample TESE. The testes had a volume of 15 ml; epididymis and vas deferens were present. Bilateral histological examination of testicular material showed spermatocyte arrest and Leydig cell hyperplasia. Fortunately, after thorough shredding of the TESE samples, a few spermatozoa were found in one sample from the left testis and were used for two cycles of IVF-ICSI (Table 1), one with fresh and the other with frozen-thawed spermatozoa, but no pregnancy was achieved. An additional TESE procedure was then performed only in the left testis. Spermatozoa were isolated once again and used immediately for an additional cycle of IVF-ICSI. Three of six embryos were implanted, resulting in a triplet pregnancy. The remaining testicular sperm cells were frozen in four aliquots for future use. The pregnancy course was uneventful until the thirtieth week of gestation, when the mother gave birth to three healthy babies: two girls and a boy, weighing 1,100 g, 1,250 g, and 1,400 g, respectively.

Genetic Analysis

Microdeletion in the AZF-c region was detected in the azoospermic patient and included 5 of the 20 STS checked (sY153, sY254, sY255, DAZ, and sY158) (Fig.

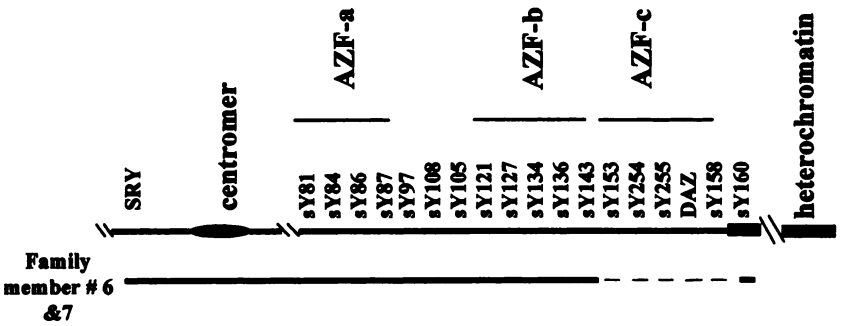

FIG. 1. Diagram mapping the deletion detected in the family. Solid black box indicate presence of the STS and minus sign indicate absence of it. The corresponding family member number is specified in Figure 2.

1). The microdeletion was confirmed in testicular DNA isolated from biopsy samples containing no spermatozoa. The karyotype was normal (46,XY). Further genetic analysis of the male members of the patient's family, including the father and four brothers, did not reveal microdeletions. The newborns were also examined, and the male was found to be bearing a Y-chromosome microdeletion similar to that of the patient; no Y-chromosome STS was found in the two newborn females that were used as control (Fig. 2).

Discussion

This paper reports on the genetic study performed on three generations of a family in which a microdeletion including the AZF-c region was detected in one of the five men of the second generation. The fertilization capacity of his testicular microdeleted spermatozoa by the ICSI procedure is described, and the transfer of the genetic defect to the third generation is demonstrated.

The microdeletion test was performed prior to the TESE-ICSI procedure, and the couple was counseled. Because of religious beliefs (Islam), they refused any use of donor sperm even as backup in the case of oocyte retrieval without sperm from the husband being available for insemination. They also rejected the option of preimplantation diagnosis for gender analysis and the transfer of female embryos only. They expressed the hope that in the future, advanced knowledge and experience would be available to treat their son if need be.

The possibility of genetic risks and long-term effects of TESE-ICSI is now being assessed. Accordingly, data concerning short-term effects of ICSI, such as major and minor congenital anomalies, as well as chromosomal anomalies, have been compiled (Bonduelle et al, 1995; Bowen et al, 1998). The microdeletion defect transmitted by the father to his male offspring raises the possibility that the son will have the same fertility problems. The fertilization capacity of testicular sperm extracted from AZF-c–deleted men was analyzed by Mulhall et al (1997). The embryonic development was also reported in this study; one pregnancy was achieved with testicular extracted spermatozoa from an AZF-c–deleted azoospermic male, and twin females were birthed. Recently, a transmission of a similar microdeletion defect from an oligozoospermic male to his "ICSI-son" was reported (Kent-First et al, 1996). Another two reports described the transmission of similar AZF-c microdeletions to two oligozoospermic patients from their fathers who fathered them normally (Ma et al, 1993; Vogt et al, 1996). In addition, Stuppia et al (1996) reported a case of a man with a microdeletion distal to AZF-c whose oligozoospermic son with an expanded deletion was fathered normally. Unfortunately, the semen characteristic values, such as sperm concentration, motility, or morphology, of the fathers with microdeletion were not reported (Stuppia et al, 1996; Vogt et al, 1996). Reijo reported two oligozoospermic men with deletion in the AZF region, but their fathers were found to carry intact Y chromosomes. To the best of our knowledge, an expansive three-generation analysis for Y-chromosome microdeletion and transmission of the microdeletion from second-generation males to male offspring by testicular-extracted spermatozoa has not been reported.

The aforementioned findings suggest the linkage between inherited, microdeleted Y-chromosomes and impairment of spermatogenesis. Indeed studies reporting deletions of one or two STS markers of the Y chromosome, such as polymorphic markers (like 207 and 272), are not proven to be linked to fertility (Pryor et al, 1997). However, in most cases, when a panel of contiguous STSs is deleted, we can safely assume that there will be consequences for the man's fertility since several studies have reported the loss of STSs in infertile but not fertile men (Kent-First et al, 1996; Reijo et al, 1996; Vogt et al, 1996; Kremer et al, 1997; Kleiman et al, 1998). Consequently, the most likely outcome is that the newborn will be infertile since no such deletions have been identified in fertile men, but the degree of severity of the spermatogenic

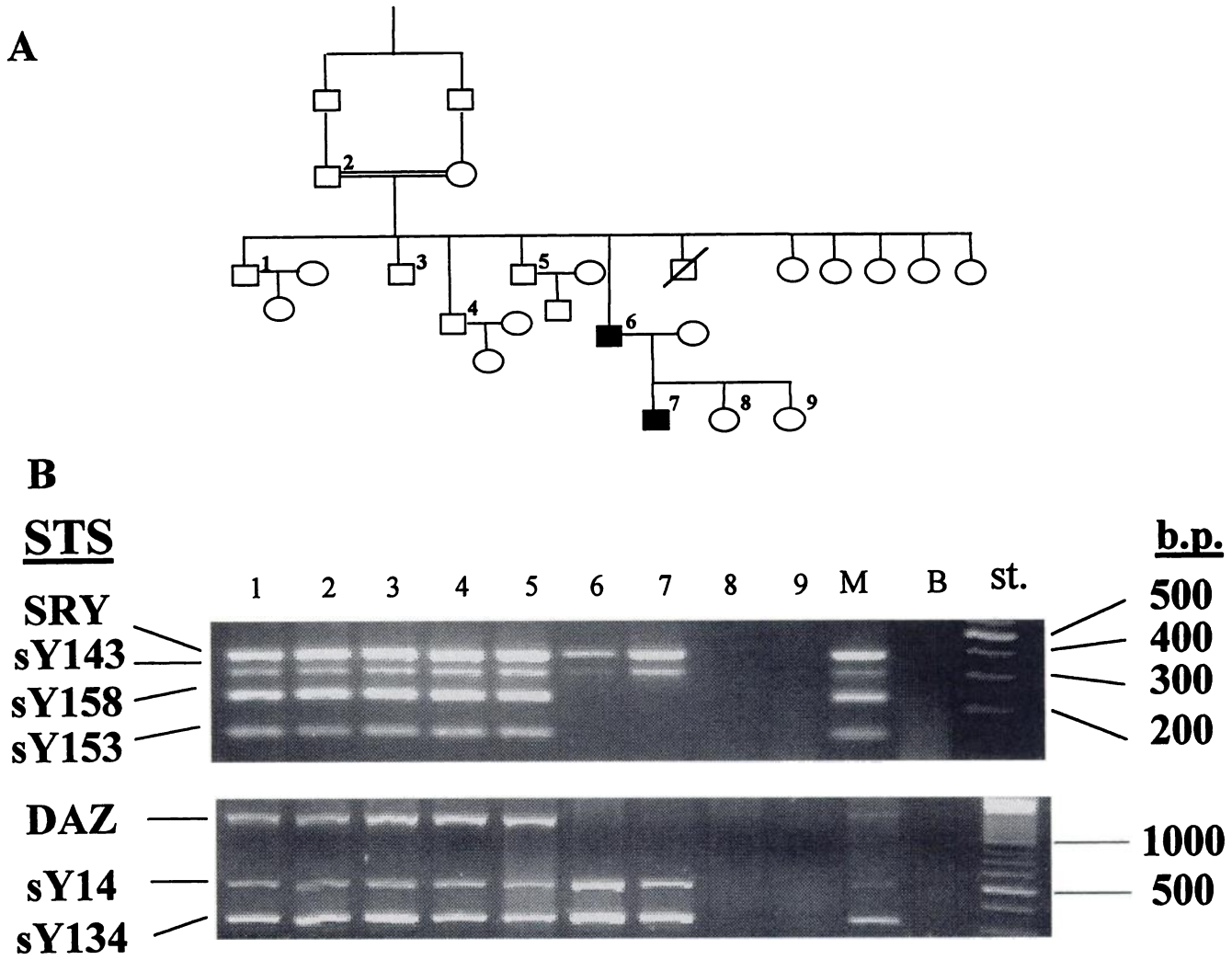

FIG. 2. Analysis of Y-chromosome microdeletion segregation over three generations. **Panel A**: Family segregation. Squares denote males; circles denote females; full squares denote males with Y-chromosome microdeletions. The individuals tested were numbered. **Panel B**: PCR results from two (mix **A** and mix **B**) of the six multiplex PCR mentioned for screening of microdeletions. DNA was extracted from peripheral or cord blood lymphocytes. After multiplex PCR amplification, the samples were run on 2% agarose gel. One to nine family members as specified in **Panel A**; st: 100-bp ladder standard; B: blank PCR control; M: male PCR control.

defect the son will display cannot be predicted at this time. Only when male babies who inherited microdeletions from their fathers reach the age of fertility will it be known whether they face similar or more-advanced impairment. Understanding the role of the gene(s) located at the AZF-c region will clarify why deletion at this particular region results in gradual reduction or complete depletion of spermatozoa. Genes mapped to this region (like DAZ, BPY2, or CDY) are multicopy and are clustered or dispersed along the Y chromosome, adding complexity to the study of their role. The possible existence of a dosage effect has not yet been investigated. For the time being, providing patients with the current knowledge concerning the issue of "inherited infertility" and assisting them with the appropriate genetic counseling is highly recommended.

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