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Transmission of Y chromosomal microdeletions from father to son through intracytoplasmic sperm injection

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Abstract We conducted chromosomal analysis of three male infants fathered by severe oligozoospermic males with Y chromosomal microdeletions through intracytoplasmic sperm injection (ICSI). Two of the infants had the same Y chromosomal microdeletions as their fathers. The third infant also had a Y chromosomal microdeletion, which was longer than that found in his father. The results confirm that Y chromosomal microdeletions are transmitted from a father to a son via ICSI and also suggest that the microdeletions may be expanded during such transmission. Genetic counseling for infertile couples contemplating ICSI is important if the male carries Y chromosomal microdeletions.

Key words Microdeletion · Y chromosome · Azoospermia · Oligozoospermia · ICSI

Introduction

Infertility is a major problem affecting 10%–15% of couples seeking to have children, and a male factor can be identified in one third of these couples. Spermatogenic failure has been shown to be a major cause of male infertility (Thielemans et al. 1998).

Evidence exists that microdeletions in three regions of the Y chromosome, azoospermic factor (AZF)a, AZFb, and AZFc, play an important role in spermatogenesis and male infertility (Vogt et al. 1996), although the identity and function of the genes involved are not clear at present (Ma et al. 1993; Reijo et al. 1995; McElreavey and Krausz 1999; Foresta et al. 2001; Stouffs et al. 2001). Foresta et al. (2001) reported that 402 (8.2%) of 4868 infertile men examined from 1992 to 2000, including 10.5% of azoospermic and severe oligo-zoospermic men (sperm count $<5 \times 10^6$/ml) and 3.8% of intracytoplasmic sperm injection (ICSI) candidates, carried Y chromosomal microdeletions. Our studies have indicated that 7.6% of Japanese azoospermic and severe oligozoospermic men (sperm count $<1 \times 10^6$/ml) have microdeletions in the AZFc region of the Y chromosome (Kato et al. 2001). Others have also reported microdeletions of the Y chromosome in azoospermic and oligozoospermic males (Kobayashi et al. 1994; Simoni et al. 1997; Pryor et al. 1997; Kostiner et al. 1998; Vogt 1998; McElreavey and Krausz 1999; Simoni 2001; Kleiman et al. 2001).

With the advancement of assisted reproductive technology (ART), an increasing number of infertile couples have been able to have babies (Palermo et al. 1992; Van Steirteghem et al. 1993). In the case of male infertility caused by severe oligozoospermia, ICSI has been successfully used to fertilize the egg. However, this method may allow direct transmission of some genetic defects from father to son (Pryor et al. 1997; Vogt 1998; Thielemans et al. 1998). Several researchers have reported that male offspring born through ICSI carry the same microdeletions of the Y chromosome as their fathers (Jiang et al. 1999; Kamischke et al. 1999; Page et al. 1999).

In this study, we analyzed microdeletions in three male infants fathered by means of ICSI in Japanese infertile males with microdeletions. We report that the same or similar microdeletions were identified in all of these male offspring.

Subjects and methods

ICSI was performed using sperm from three oligozoospermic men with Y chromosomal microdeletions (Fig. 1). Their wives became pregnant and delivered male infants.

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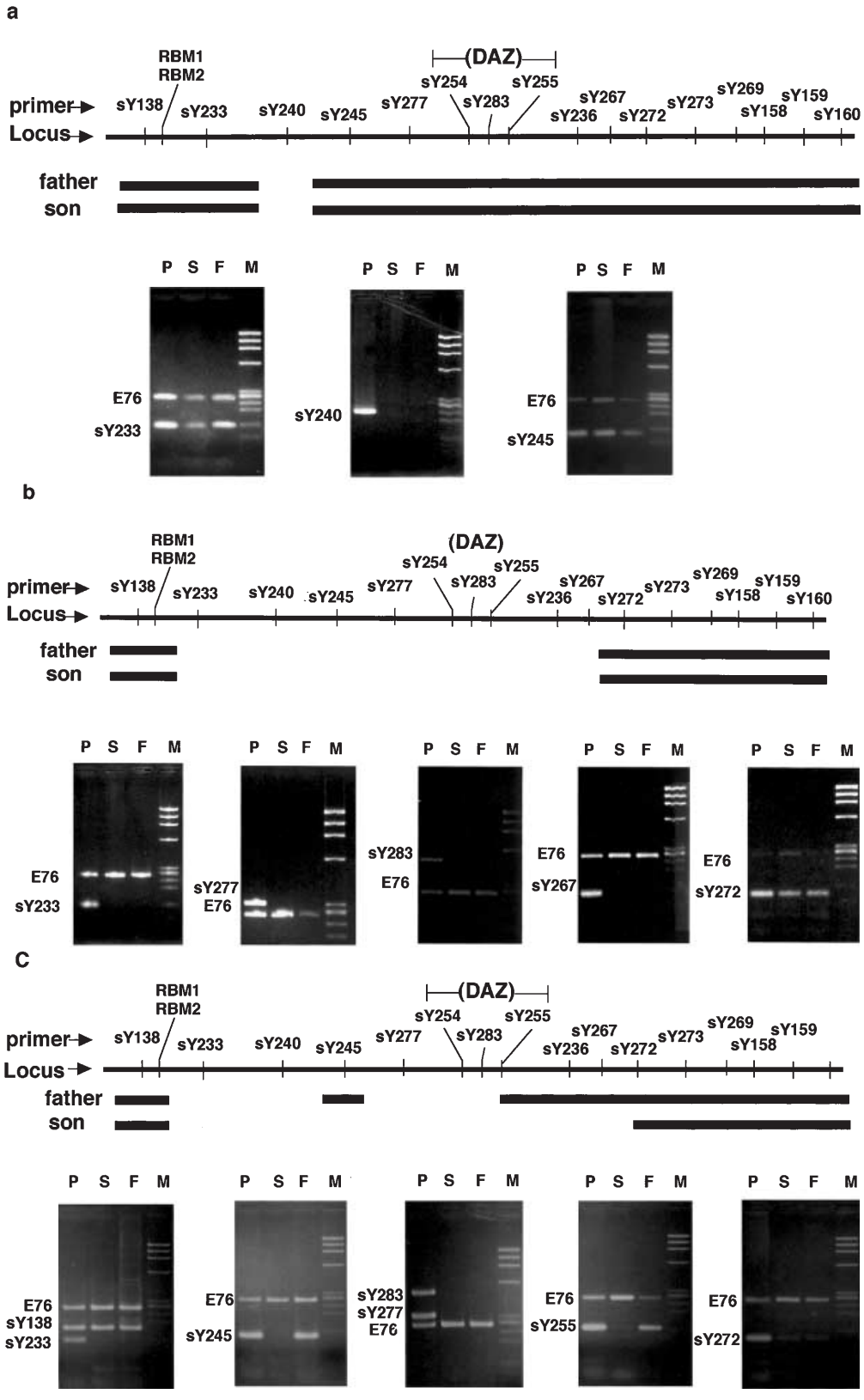

Fig. 1a–c. Analysis of Y chromosomal microdeletions in infertile men and their male offspring who were fathered using intracytoplasmic sperm injection. *P*, Positive control (normal male sample); *F*, father; *S*, son; *M*, molecular marker (*Hae* III digest of f174 DNA). *Solid bars* indicate regions without microdeletions. **a** case 1. **b** case 2. **c** case 3

After informed consent was obtained, umbilical cord blood (cases 1 and 2) or peripheral blood (case 3) was analyzed for Y chromosomal microdeletions. DNA was extracted from the blood samples and digested with *EcoR* I, which allowed the polymerase chain reaction (PCR) to proceed smoothly. PCR was performed using 16 sets of primers located in the interval D16–22 of the Y chromosome (sY138, sY233, sY240, sY245, sY277, sY254, sY283, sY255, sY236, sY267,

sY272, sY269, sY273, sY158, sY159, and sY160 (Vollrath et al. 1992), as described previously (Kato et al. 2001). E76 primer (ACACAGAGCAAGGCCA GAAT) and E77 primer (CTTCATGGGCCTGAACT GAT) (Ma et al. 1993) were used as positive controls. Genomic DNA was dissolved in PCR buffer (50 mmol/l KCl, 15 mmol/l $MgCl_2$, 0.1% gelatin, 10 mmol/l Tris HCl at pH 8.3) and mixed with 20 mmol/l deoxyribonucleoside triphosphate (deoxyadenosine triphosphate, deoxythymidine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate), 1 mmol/l primer, and 1.87 units of *Thermus aquaticus* DNA polymerase (Applied Biosystems Division, Foster City, CA, USA). After preheating at 95°C for 5 min, PCR was performed by denaturing at 95°C for 1 min, annealing at 57°C for 2 min and ending with a primer extension at 72°C for 3 min in 35 cycles. The reaction mixture was then incubated at 72°C for 5 min. The products were analyzed by gel electrophoresis in 2% NuSieve GTG agarose (FMC BioProducts, Rockland, ME, USA) and 1% Seakem GTG agarose (FMC BioProducts) in Tris-acetate-EDTA buffer (40 mmol/l Tris-acetate, 1 mmol/l ethylenediamine tetraacetic acid).

Results

In case 1, PCR analysis detected a microdeletion in the Y chromosome that was the same as that found in his father's DNA (Fig. 1a). Case 2 also showed the same deletion in the Y chromosome as that of his father (Fig. 1b). Case 3 had a larger deletion in the Y chromosome than that of his father (Fig. 1c).

Discussion

Recent studies of mechanisms of infertility have identified some genetic disorders associated with spermatogenic defects in infertile males (Thielemans et al. 1998). In particular, close relationships between microdeletions in the Y chromosome and spermatogenic failure have been reported (Pryor et al. 1997; Kostiner et al. 1998; Vogt 1998; McElreavey and Krausz 1999; Foresta et al. 2001; Kato et al. 2001; Kleiman et al. 2001; Simoni 2001). About 10% of infertile males with azoospermia and oligozoospermia have Y chromosomal microdeletions around the AZF region. For males who are infertile because of severe spermatogenic defects, such as azoospermia and severe oligozoospermia with Y chromosomal microdeletions, ART such as ICSI is often used successfully to achieve fertilization (Kostiner et al. 1998; Vogt 1998; Foresta et al. 2001). However, since ICSI is the direct introduction of a spermatozoon into an oocyte, bypassing the induction of capacitation and acrosome reaction by a motile spermatozoon for oocyte penetration, the possibility exists that ICSI may transmit genetic abnormalities from a father to a son (Kretser and Baker 1999; Foresta et al. 2001).

In this study, we showed that all three sons examined carried microdeletions in the Y chromosome, two of which were the same as those found in their fathers. The third microdeletion was similar to but longer than that of his father. These results support the view that Y chromomal microdeletions can be transmitted and expanded through ICSI.

Stuppia et al. (1996) have reported that widening of microdeletions transmitted via ICSI may account for the infertility of a son (Stuppia et al. 1996). If the severity of spermatogenic failure is related to the extent of microdeletions, ICSI may result in worsening of spermatogenic defects in the next generation. The mechanism of the widening of microdeletions is obscure. Lupski (1998) indicated that homologous locus-specific clusters of repetitive sequence elements caused loss of megabases of DNA (Lupski 1998).

Several researchers demonstrated that the retroviral sequence containing homologous repetitive sequences caused microdeletions in the AZFa region because the homologous repetitive sequences induced intrachromosomal recombination events easily (Kamp et al. 2000; Blanco et al. 2000). The Y chromosome itself is also polymorophic and has many repetitive sequences. Therefore, the occurrence of recombinations in the Y chromosome may be more frequent than is the case with the other chromosomes. The mechanism of widening the microdeletion via generation may be the same reason. On the other hand, Chang et al. (1999) have reported that a father of five children, including four sons, was found to be azoospermic with Y chromosomal microdeletions at the age of 63 years. All the sons were infertile with Y chromosomal microdeletions. These facts suggest that spermatogenic defects with Y chromosomal microdeletions may worsen with age.

Although the biological effect of the Y chromosomal microdeletions found in the three infants in this study will remain unknown until they reach adulthood, the possibility exists that the ICSI treatment may lead to an increase in the severity of spermatogenic defects and the number of infertile males in future generations. Therefore, before using ICSI in infertile couples with severe spermatogenic defects, the explanation and screening for microdeletions should be performed. Nap et al. (1999) demonstrated the role of genetic counseling in making the decision for treatment (Nap et al. 1999). According to their study, most infertile couples chose ICSI after taking genetic counseling. Nap et al. (1999) also indicated that the process of genetic counseling was important in making the final-decision. The rationale for ART is to provide infertile couples with an opportunity to have a healthy baby. It is therefore advisable for an infertile male contemplating ICSI because of severe spermatogenic defects to be examined for Y chromosomal microdeletions and, if any are found, to be provided with genetic counseling.

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