Detection of Azoospermic Factor Genes in Chinese Men with Azoospermia or Severe Oligozoospermia

SHIUH YOUNG CHANG[1,2,3] and MENG-YIN TSAI[1]

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Purpose: *We investigated the prevalence of deletions in the azoospermic factor (AZF) region of chromosome Yq11 in Chinese men with infertility due to idiopathic azoospermia or severe oligozoospermia. The DAZ gene cluster was also examined for mutations.*

Methods: *Sixty-eight men with azoospermia or severe oligozoospermia taking part in an intracytoplasmic sperm injection program were recruited. Four loci specific for AZFa, AZFb, and AZFc were amplified from genomic DNA via polymerase chain reaction to determine whether deletions were present in the AZF region. Direct DNA sequencing of amplified products was also performed to look for mutations or polymorphism from exon 2 to exon 6 of the DAZ gene cluster.*

Results: *Six (9%) of the 68 patients had AZF deletions. None had mutations in exons 2 to 6 of DAZ.*

Conclusions: *The prevalence of AZF deletions in our study was similar to those in Western reports, as was the lack of DAZ mutations.*

KEY WORDS: AZF; azoospermia; DAZ; oligozoospermia.

INTRODUCTION

Nonobstructive azoospermia or severe oligozoospermia is found in about 2% of infertile couples (1). It has been reported that chromosomal abnormalities, at either the cytogenetic or the molecular level, may be responsible for the defects in spermatogenesis (2). Current evidence strongly supports the presence of at least three spermatogenesis loci in Yq11, which have been designated azoospermic factor (AZF)a, AZFb, and AZFc (3). Each of these loci should contain at least one gene that is involved in spermatogenesis, and mutations in these loci should induce male infertility or sterility. Though these genes have not yet been found, some candidate genes have been proposed, including RBM (RNA binding motif) for AZFb and the DAZ (deleted in azoospermia) gene cluster and SPGY (spermatogenesis gene on the Y) for AZFc. DAZ and SPGY are members of the same gene family. The DAZ gene cluster has been reported to be deleted in 5–13% of men with azoospermia or severe oligozoospermia (4,5).

The prevalence of AZF deletions in Chinese men is not known. Intracytoplasmic sperm injection (ICSI) has allowed men with azoospermia or severe oligozoospermia, who in the past had little hope of fathering offspring, to have children. However, with this ability comes the very real risk of the transmission of infertility or sterility from father to son. In light of this, understanding the genetic mechanisms of azoospermia and oligozoospermia is highly relevant.

This study was performed to investigate the prevalence of AZF deletions in Chinese men with infertility due to azoospermia or severe oligozoospermia who were taking part in an ICSI program. In addition, direct DNA sequencing was performed for each subject to examine sequence polymorphisms or mutations of any kind from exon 2 to exon 6 of the DAZ gene cluster.

MATERIALS AND METHODS

Patients

The patients involved were participants in the ICSI program at Chang Gung Memorial hospital between August 1997 and April 1998. A total of 68 patients with either idiopathic azoospermia or severe oligo-

[1] Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan.
[2] Medical School, Chang Gung University, Taoyuan, Taiwan.
[3] To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, 123 Ta-Pei Road, Niao-Sung Hsiang, Kaohsiung County, Taiwan, Republic of China.

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zoospermia was recruited. Patients with chromosomal anomalies or congenital absence of the vas deferens were excluded from the study. Severe oligozoospermia was defined as a sperm concentration of less than 5×10^6/ml. Live spermatozoa or, in the case of no spermatozoa being present, elongated spermatids recovered either from testes or ejaculates were selected for ICSI. Y chromosome-specific sequence tagged sites (STSs) within the AZF regions were used to screen for AZF deletions.

Screening for AZF Deletions

Genomic DNA was obtained from peripheral leukocytes using the guanethidine isothiocyanate method (DNAzol; GIBCO BRL, Gaithersburg, MD). Four pairs of oligonucleotide primers for the loci of sY84, sY143, sY254, and sY255, specific for AZFa, AZFb, AZFc, and AZFc, respectively, were used for polymerase chain reaction (PCR) amplification (6). Amplification with primers for sY84, sY143, sY254, and sY255 should yield products of 326, 143, 330, and 126 bp, respectively. The PCR reaction profiles were as follows: sY84, sY143, and sY254 were amplified with 35 cycles of denaturation at 94°C for 1 min, primer annealing at 58°C for 1 min 30 sec, and primer extension at 72°C for 1 min. sY255 were amplified with 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min 30 sec, and elongation at 72°C for 1 min. A pair of sex-determining region Y (SRY-)-specific primers which yields a 472-bp product was coamplified in all reactions to prove the presence of Y-specific DNA and to exclude failure of amplification (7). The conditions for amplification with the SRY-specific primers were 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and elongation at 72°C for 1 min. If any of the loci were not amplified, PCR was repeated. If a locus was not amplified on the second attempt, it was considered to be deleted.

Direct Sequencing of Exons 2 to 6

To screen for the possible point mutations, direct sequencing was performed in patients without AZF deletions. Exons 2 through 6 of the DAZ gene cluster were sequenced. Five pairs of the intron primers located close to the intron/exon junctions were designed (8). The sequences of the designed primers were as follows (from 5′ to 3′ end).

Exon 2:
Forward, GCCTGCATACGTAATTATGA
Reverse, GTACGAATACAATACCCTAG

Exon 3:
Forward, CGTTCAGTTCTTTTCCATAG
Reverse, AGAATAGGTGACTGGAGTTC

Exon 4:
Forward, TTTTATGCTTCATTTGTCTGCC
Reverse, CACTAAACGTTGTAGCAATAAG

Exon 5:
Forward, CTAGTTTTGTTAACGGTTC
Reverse, GAACCATTTCTTTTCCTAC

Exon 6:
Forward, CTGACCCGTGTAGCTTTTCA
Reverse, GAGAACAGGAACATAAGCAG

DNA used for the sequencing was obtained by amplification of genomic DNA using these five sets of primers. The conditions for PCR were as follows:

Exons 2 and 3: 30 cycles of denaturation at 94°C for 1 min, primer annealing at 52°C for 1 min, and elongation at 72°C for 1 min.

Exon 4: 30 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and elongation at 72°C for 1 min.

Exon 5: 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 1 min.

Exon 6: 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min 30 sec, and elongation at 72°C for 1 min.

Two microliters of the amplified products was used for direct sequencing. A commercial sequencing kit that employs the Sanger sequencing method (Sequenase II, Amersham Life Science, Buchinghamshire, England) was used, according to the manufacturer's instructions.

RESULTS

Deletion of AZF Regions

Six (9%) of the 68 patients had a deletion in either the sY84, the sY143, the sY254, or the sY255 locus. Three (4%) had a deletion in the sY84 locus (Fig. 1), which means that they could have a defect in the AZFa region. The other three (4%) had deletions in both the sY254 and the sY255 loci (Fig. 1). Both loci are within the DAZ gene cluster, indicating that this gene cluster was deleted in the three patients. None of the patients had deletions in the sY143 locus.

Results of Direct Sequencing

In the remaining 62 patients, results of the direct sequencing were compared with the published gene

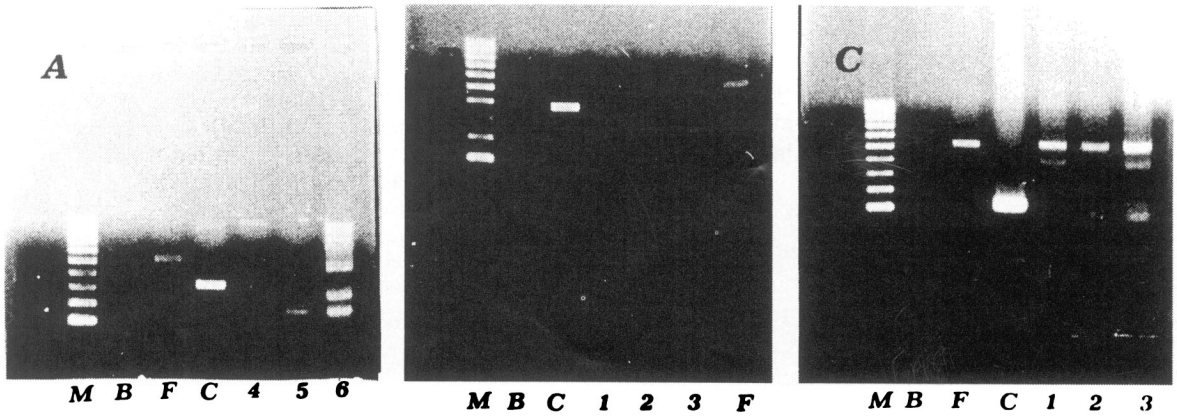

Fig. 1. Three patients had a deletion in the sY84 locus (*A*). The other three had deletions in both the sY254 (*B*) and the sY255 loci (*C*). Primer pairs for the loci of sY84, sY254, and sY255, specific for AZFa, AZFc, and AZFc, respectively, were used for polymerase chain reaction amplification. The sizes were 326, 330, and 126 bp, respectively. Electrophoresis was carried out in 6% polyacrylamide gels containing 10% glycerol at room temperature. M, marker; B, blank; C, positive control; F, female control; 1–6, patients.

sequence (8). No sequence polymorphisms or mutations of any kind were found in any of the five exons examined.

Spermatogenic Defects

Wet preparation in the ICSI laboratory revealed the presence of spermatozoa in two of three patients with the AZFa (sY84) deletion. The other one had maturation arrest. The testicular size and serum follicle-stimulating hormone (FSH) level were all within normal limits in the three patients with the AZFa deletion. Among the three patients with AZFc (sY254 and sY255) deletions, one had scanty spermatozoa in the testes, while another two were Sertoli cell-only syndrome. All the three patients with the AZFc deletion had small testes and elevated FSH levels.

DISCUSSION

Tiepolo and Zuffardi first proposed the existence of a spermatogenesis gene, or AZF, on the long arm of the Y chromosome, Yq11 (9). It is estimated that about 1.9 to 20% of males with nonobstructive azoospermia or severe oligozoospermia may have a disruption of AZF (10,11). The wide range of the reported frequencies of AZF deletion may be due to the selection criteria of the patients or ethnic differences (6,8,10–12).

In this study, we report on the screening of the AZF deletion in 68 Chinese men with idiopathic azoospermia or severe oligozoospermia who were enrolled in an ICSI program. We used 4 oligonucleotide primer pairs because these 4 pairs have been reported to yield results similar to those using 28 primer pairs (6). Among our 68 patients, 6 had an AZF deletion. This prevalence, 9% (6/68), fell within the range reported previously. In performing direct DNA sequencing, we found no mutations or sequence polymorphisms in exons 2 to 6 of the DAZ gene cluster. This finding is also in agreement with the results of previous studies (5,12).

AZF has been mapped to at least three distinct loci located on the distal portion of Yq11, designated AZFa, AZFb, and AZFc (3,11). AZF deletions are associated with diverse histologic pictures (8). Evidence indicates that expression of genes within AZFa and AZFb is needed prior to proliferation and at meiosis, respectively (8). In addition, DAZ genes, the candidate genes for AZFc, are active in late spermatids and spermatozoa (13). Deletion of DAZ genes is supposed not to interfere with human sperm maturation, but to result in a gradual reduction in the number of mature spermatozoa (13). We did not perform fixative histologic examination in every patient. However, wet preparation in the ICSI laboratory in this study revealed the presence of spermatozoa in two of three patients with the AZFa (sY84) deletion. The other one had maturation arrest. Among the three patients with AZFc (sY 254 and sY 255) deletions, one had scanty spermatozoa in the testis, while another two had Sertoli cell-only syndrome. It is of interest to see normal testicular sizes and serum FSH levels among the three patients with the AZFa deletion. In contrast, all the three patients

with the AZFc deletion had small testes and elevated serum FSH levels. Simoni *et al.* reported elevated serum FSH levels in four of five patients with DAZ deletions. All five cases in their series had small testes (6). The variability in spermatogenic defects suggests that other factors may exist.

Vertical transmission of the AZF deletion from father to son may result rarely from natural conception; presumably, the fathers in such cases are oligozoospermic. With the advent of ICSI, vertical transmission of deleted AZF, and hence inheritance of sterility/infertility from father to son, will be expected to increase substantially (4). It is therefore imperative that infertile couples are well informed about the possible consequences. Effort should be exerted to elucidate AZF in the future. Only with more data can the responsible genes, the extent of the problem, and the relevance of screening be established. It is hoped that, with further elucidation of AZF, gene therapy at the embryo stage may become a reality for avoiding AZF disruption-induced male infertility.

Evidence from recent animal studies suggests that many other autosomal genes may be involved in spermatogenesis (5,14–16). It is likely that similar autosomal genes might be needed for fertility in humans. However, it is unlikely that a single gene contributes to infertility in a large fraction of patients, making general screening for mutations in these autosomal genes cost-ineffective at present.

In conclusion, the prevalence of AZF deletions in Chinese men with idiopathic azoospermia or severe oligozoospermia enrolled in an ICSI treatment appears to be similar to that in Western reports. As in Western reports, we found no other mutations or sequence polymorphisms in this study.

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