Increased frequency of cystic fibrosis transmembrane conductance regulator gene mutations in infertile males

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Objective: To investigate the frequency of mutations of the cystic fibrosis transmembrane regulator (CFTR) gene in males with reduced sperm quality before intracytoplasmic sperm injection (ICSI).

Design: The nine most frequent cystic-fibrosis-causing mutations in the German population and IVS8T alleles were analyzed.

Setting: University-based centers for reproductive medicine and clinical genetics.

Patient(s): An unselected group of 597 males with oligo-, astheno-, terato-, crypto-, oligoasthenoteratozoospermia, or azoospermia, which underwent pre-ICSI genetic counseling over a 5-year period.

Intervention(s): Blood samples were collected from the patients during genetic counseling.

Main Outcome Measure(s): Frequency of mutations of CFTR gene in infertile males.

Result(s): A heterozygous CFTR mutation was observed in 34 of 597 patients (5.70%). None of the patients had two CFTR mutations. Given that our mutation panel recognizes about 82% of heterozygotes, it can be assumed that the frequency of CFTR heterozygotes in our cohort is about 6.94%. The frequency of CFTR mutations in our cohort did not correlate with a reduced sperm count.

Conclusion(s): The frequency of cystic fibrosis in the German population is 1:3300. Thus, a CFTR heterozygosity of 3.42% can be estimated. This indicates that in our cohort of infertile males, the frequency of CFTR heterozygosity is twofold higher than in the general population (P<.0001). (Fertil Steril® 2006;85:135–8. ©2006 by American Society for Reproductive Medicine.)

Key Words: CFTR mutations, ICSI, infertility, oligozoospermia

It is estimated that about 30%-40% of couples seeking fertility treatments are diagnosed with male factor infertility. These males mainly present with reduced sperm quality including oligozoospermia (i.e., sperm count less than 20 million/mL), cryptozoospermia (i.e., sperm count less than 1 million/mL), asthenozoospermia (i.e., progressive sperm motility less than 50%), teratozoospermia (i.e., sperm with normal morphology less than 15%, strict criteria), and azoospermia (i.e., no sperm count).

Infertility caused by obstructive azoospermia has been reported in >95% of men with cystic fibrosis (CF). It is well established that 60%-70% of patients with congenital bilateral aplasia of the vas deferens (CBAVD) have mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, with no other clinical symptoms of CF (1). However, contradictory results have been reported concerning the frequency of CFTR mutations in infertile males without CBAVD. van der Ven et al. have reported a fivefold elevated CFTR mutation rate in a cohort of 80 healthy men with infertility due to reduced sperm quality (2). We have previously reported a twofold elevated mutation rate in the CFTR gene in a cohort of 197 males with idiopathic infertility (3). Cruger et al. found an elevated CFTR mutation rate only in males with cryptozoospermia (4). In contrast, Boucher et al., Tuerlings et al., and Ravnik-Glavac et al. did not detect an increased CFTR mutation frequency in males with nonobstructive azoospermia or oligoasthenoteratozoospermia (5–7).

Thus, it is uncertain whether or not screening for CFTR mutations should be recommended to infertile males without CBAVD during pre-intracytoplasmic sperm injection (ICSI) genetic counseling. In the present study, we significantly increased the size of our cohort and evaluated the frequency of mutations in the CFTR gene in 597 men with reduced sperm quality.

MATERIALS AND METHODS

Study Population

The study population consisted of 597 males with oligo-, oligoastheno-, astheno-, asthenoterato-, oligoasthenoterato-, crypto-, or azoospermia, who underwent pre-ICSI genetic counseling over a 5-year period. A full history was obtained from each subject. Physical examination of the patients was performed. Semen analysis was achieved according to the
World Health Organization (WHO) guidelines. Males with chromosomal aberrations or deletions of azoospermia factors in Yq were not excluded. The study population included 67 men with azoospermia. A testicular biopsy was performed in 62 of these cases. In six of these patients, a bilateral normospermatogenesis was observed suggesting an obstructive azoospermia. Informed consent was obtained from all patients before the study was initiated.

Genetic Analyses
Genetic analyses included conventional chromosome analysis and DNA analysis for microdeletions in the azoospermia factor (AZF) regions of the Y chromosome and for mutations in the CFTR gene on chromosome 7. A DNA analysis was performed on genomic DNA obtained from peripheral leucocytes.

Chromosome Analysis
Chromosome analysis was performed by standard methods using Q-banding.

CFTR Mutation Analysis.
For mutation analysis, DNA was isolated from leukocytes following standard procedures (8). Mutation F508del was tested by polymerase chain reaction (PCR) and acrylamide gel electrophoresis. Mutations R117H, R347P, G542X, G551D, R553X, 3849+10kbC>T, and N1303K were analyzed by PCR and restriction enzyme cleavage. Mutations CFTR2,3dele(21kb) and the presence of IVS(8)5T-allele were tested by allele-specific PCR. Among CFTR mutations detected in the German population, F508del, R117H, R347P, G542X, G551D, R553X, 3849+10kbC>T, N1303K, and CFTR2,3dele(21kb) occur with a frequency of 72%, 1%, 1.2%, 1.2%, 0.9%, 2%, 1%, 1.8%, and 1.2%, respectively (9–11). Thus, our mutation panel recognizes about 82% of heterozygotes in the German population.

Detection of Microdeletions in the Y Chromosome.
Y chromosome microdeletions were tested by multiplex-PCR with primers for following loci: DYS273, DYS148, DYS218, DYS224, and DAZ.

Statistical Analysis
The statistical analyses have been performed using the Software SAS for Windows (Statistical Analysis System, version 8.0.2; SAS Institute Inc., Cary, NC). The determination of the frequencies of heterozygotes in the observed patients, together with the corresponding exact 95% confidence interval (CI) and a two-sided binomial test to compare the observed frequency of heterozygotes with the published frequency for the normal population, were performed with the PROC FREQ function of the SAS program package. Furthermore, differences between subgroups of patients were tested using χ². We complemented the general Pearson χ² test by the special Mantel-Heanszel version, taking into account the ranking of the subgroups with reduced sperm concentration, both using the “exact” option in PROC FREQ. A P value of .05 was used in all tests.

RESULTS
The results of CFTR mutation testing are presented in Table 1. In the present study, we examined 597 infertile males for 9 different CFTR mutations before ICSI therapy. A heterozygous CFTR mutation was observed in 34 (5.70% [95% CI, range 4.01%–7.87%]) of 597 patients. Given that our mutation panel recognizes about 82% of heterozygotes (9–11), it can be assumed that the frequency of CFTR heterozygotes in our cohort is approximately 6.94%. The frequency of CF in the German population is 1:3300 (9). Thus, a CFTR heterozygosity of 3.48% can be estimated. This indicates that in our cohort of infertile males, the frequency of CFTR heterozygosity is about twofold higher than in the general population (P<.0001).

The only 3 mutations identified were F508del (26 cases), R117H (5 cases), and CFTRdele2,3 (3 cases). The 5T variant was identified 28 times accounting for an allele frequency of 4.7%, indicating that the 5T allele frequency was in the same range as in the normal population. None of the patients showed two “classical” CFTR mutations. We found three cases with a F508del heterozygous mutation and a 5T allele. In one of these males, a testicular sperm extraction was performed, and normal spermatogenesis was diagnosed, suggesting a case of obstructive azoospermia, which has not been detected by clinical parameters.

The CFTR mutations were observed in 1 (7.69%) of 13 patients with oligozoospermia, in 5 (12.82%) of 39 patients with asthenozoospermia, in 7 (5.07%) of 138 patients with oligoasthenozoospermia, in 1 (4.60%) of 282 patients with oligoasthenoteratozoospermia, in 1 (1.96%) of 51 patients with cryptozoospermia, and in 7 (10.44%) of 67 patients with azoospermia. Statistical analysis revealed that the frequency of CFTR mutations in our cohort did not correlate with sperm count, neither with (P=.6840) nor without (P=.1687) inclusion of the ranking of patient subgroups.

Because infertile males have a higher prevalence of chromosomal abnormalities, we offered them chromosome analysis. We found chromosome aberrations in 14 (2.34%) of 597 males. The following chromosome aberrations were detected: 45,XYder(13;14) in four cases; 47,XY+mar in three cases; and in one case each 46,XYt(7;19); 46,XYt(1;10); 46,XYt(1;18); 46,XYt(2;16); 47,XXY/46,XY; 45,X/46,XY. In one case of oligoasthenoteratozoospermia, we found a Robertsonian translocation 45,XY,der(14;22) and a heterozygous F508del mutation. Half of these cases presented with oligoasthenoteratozoospermia and none with azoospermia. Exclusion of cases with chromosomal abnormalities from statistical analysis did not affect the CFTR mutation rate in our cohort. In addition, five males showed microdeletions of the AZF region (0.83%). None of the five patients with AZF deletions had an additional CFTR mutation.

136 Schulz et al. CFTR mutation frequency in infertile males Vol. 85, No. 1, January 2006