Frequency of cystic fibrosis transmembrane conductance regulator gene mutations in infertile men

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INTRODUCTION

It is well established that 60-70% of patients with congenital bilateral aplasia of the vas deferens (CBVAD) have mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Increased CFTR mutation frequency was also reported in males with, non-obstructive azoospermia and oligoasthenoteratozoospermia, emphasizing the importance of screening for CF mutations not only in men with CBVAD, but in all men with reduced sperm counts.

AIM OF THE STUDY

The aim of this study was to investigate the frequency of the most common CFTR mutations in infertile males from the Republic of Macedonia, by a multiplex SnaPshot analysis.

MATERIALS AND METHODS

A total of 169 infertile men (73 with azoospermia, 46 with severe oligozoospermia, 23 with mild oligozoospermia and 27 with normozoospermia but unexplained couple infertility) and 136 fertile controls were included in the study.

Five common CFTR mutations (DF508, G542X, N1303K, 621+1G->T and R117H) and IVS 8 polyT alleles were analyzed by a multiplex PCR. The PCR products were cleaned up with ExoSAP-IT and multiplex single nucleotide extension reactions were performed using SNaPshot multiplex kit (Applied Biosystems). To remove the unincorporated ddNTPs, the SNaPhot reaction mixtures were incubated with shrimp alkaline phosphatase.

The SNaPshot products were run on ABI 310 Genetic Analyzer (Applied Biosystems). Analysis of electrophoreograms was performed using the GeneScan 3.1 software (Applied Biosystems) (Figure 1).

RESULTS

Six delF508 and one G542X mutations were detected among infertile men, while two DF508 mutations were present among fertile controls (Table 1). Two infertile men with CFTR mutation (one with obstructive azoospermia and one with mild oligozoospermia) were also carriers of a IVS 8(5T) allele. The frequency of CFTR heterozygosity was slightly higher in all groups of infertile males in comparison to the fertile controls (Figure 2).

The frequency of IVS 8 polyT (5T/9T) genotype is higher in infertile patients (2.37%) than in controls (0.74%), but no difference is observed in IVS 8 polyT (5T/7T) genotype (5.92% in infertile, 5.88% in controls) (Figure 3).

The frequency of IVS 8 polyT (5T) allele was 4.1% in infertile men and 3.3% in controls (Figure 4).

CONCLUSIONS

The use of multiplex SnaPshot analysis represents a fast, simple and inexspensive approach for detection of the most common CFTR mutations in infertile men.

ACKNOWLEDGMENTS

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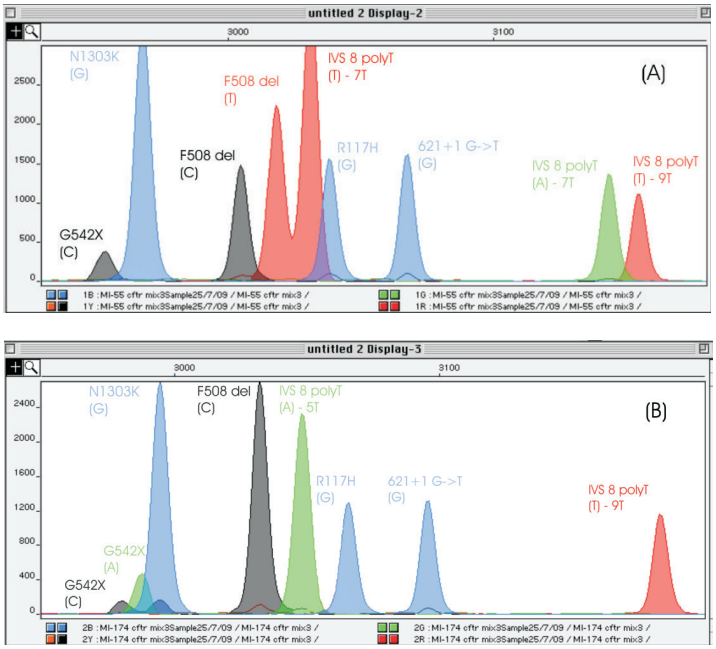

Figure 1. Electrophoreogram of the multiplex SNaPshot analysis for the detection of five common CFTR mutations and IVS 8 polyT alleles; (A)heterozygote for delF508 mutation and IVS 8 7T/9T genotype; (B)heterozygote for G542X mutation and and IVS 8 5T/9T genotype.

Table 1. IVS 8 polyT genotype and fertility status in men with CFTR mutations

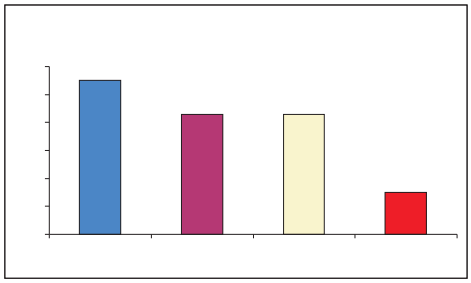

Figure 2. Frequencies of CFTR heterozygosity in infertile patients with different sperm counts (azoospermia, severe and mild oligozoospermia) and fertile controls.

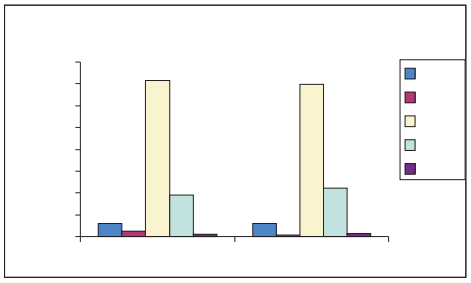

Figure 3. IVS 8 polyT genotype frequencies among infertile patients and fertile controls.

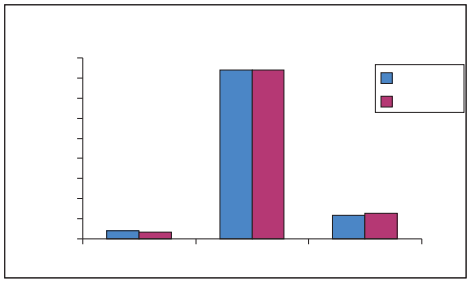

Figure 4. IVS 8 polyT allelle frequencies among infertile patients and fertile controls.