



Analysis of mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene in patients with obstructive azoospermia

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Abstract

Congenital bilateral absence of the vas deferens (CBAVD) accounts for 1%-2% of sterility in men. A high incidence of mutations, as well as the involvement of the 5T variant of the T tract length in intron 8 of the cystic fibrosis conductance regulator (*CFTR*) gene, have been previously described in males with CBAVD. Herein we report the screening for mutations and for the 5T variant of the *CFTR* gene in 17 patients with CBAVD and three others with non-CBAVD obstructive azoospermia. In the CBAVD group, three patients (15%) were compound heterozygotes for mutations, and five patients (25%) had a mutation in one allele and the 5T variant in the other; the 5T variant was also present in two other patients, one of them being homozygous. The most frequent mutation was $\Delta F508$, present on five chromosomes (12.5%). A novel missense mutation (A399D) was detected in a Japanese CBAVD patient. Our results yield further evidence for a strong association between male obstructive azoospermia caused by CBAVD and mutation/5T variant in the *CFTR* gene. The search for *CFTR* mutations in such patients is thus recommended for genetic counseling of couples who undergo assisted fertilization due to CBAVD.

Key words: *CFTR* mutations, obstructive azoospermia, male infertility.

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Introduction

Congenital bilateral absence of vas deferens (CBAVD) accounts for approximately 6% of the cases of obstructive azoospermia. It is responsible for 1%-2% of cases of infertility in men and is also present in about 95% of male patients with cystic fibrosis (CF) (Jequier *et al.*, 1985). CF is a common severe autosomal recessive disorder characterized by chronic obstructive pulmonary disease, pancreatic exocrine insufficiency, and elevated concentrations of electrolytes in sweat (Boat *et al.*, 1989). Clinical manifestation varies greatly among patients, probably due, in part, to the multitude of disease-causing mutations in the *CFTR* gene. More than 980 mutations in the *CFTR* gene have been reported (Bernardino *et al.*, 2000). In CF patients, the vas deferens, the tail and body of the epididymis, and the seminal vesicle are atrophic, fibrotic, or completely absent. The pathogenesis of these structural changes probably relates to early, often intrauterine, obstruction of the genital tract by inspissant secretions (Kaplan *et al.*, 1968).

CBAVD is a genetically heterogeneous and complex disorder, involving mutations and variants of the *CFTR* gene in over 80% of cases (Dörk *et al.*, 1997). Approximately 10% of CBAVD patients carry CF mutations on both chromosomes, but in most cases the mutations have been detected in just one of the *CFTR* alleles.

Genotype-phenotype correlation studies have associated CF mutations with male infertility caused by CBAVD, suggesting that this condition is primarily a genital form of CF (Anguiano *et al.*, 1992; Chillón *et al.*, 1995; Pignatti *et al.*, 1996; Cohn *et al.*, 1998; Sharer *et al.*, 1998). Other studies have also shown that there is a strong association of the IVS8-T5 variant with CBAVD (Chu *et al.*, 1993). In patients with CBAVD, the frequency of the 5T variant is higher than in the general population. In some patients, homozygosity for this variant suffices to cause CBAVD and mild CF symptoms (Dörk *et al.*, 1997). These data support the suggestion that the 5T variant is a "very mild" *CFTR* mutation, specifically causing CBAVD (Zielenski *et al.*, 1995).

In the present study we performed *CFTR* gene analysis on 20 patients who were referred to our center due to obstructive azoospermia, 17 of which had CBAVD.

Patients and Methods

Patients

The present investigation was performed on a group of 20 unrelated men (ages 26 to 49 years) with obstructive azoospermia. Seventeen of them were found to have CBAVD. The diagnosis of CBAVD or obstructive azoospermia was based on scrotal examination, ultrasound and semen analysis. None had been diagnosed with CF or had any pulmonary problems, except for one patient who reported bronchitis in childhood. Nineteen patients were pancreatic-sufficient, and one had pancreatitis.

DNA Analysis

Mutation detection studies were carried out on all 27 exons and exon/intron boundaries of the *CFTR* gene by means of single-strand conformation polymorphism (SSCP) and heteroduplex analysis. A 100 ng sample of genomic DNA was amplified under the following conditions: 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each primer, and 2 units of Taq polymerase (Gibco BRL) for 35 cycles. All PCR reactions were performed in a total volume of 10 μ L. SSCP/heteroduplex analysis was performed according to the method described by Ravnik-Glavac *et al.* (1994). Abnormally migrating fragments were subsequently sequenced to confirm the presence of mutations. *CFTR* gene variants of the T tract length of intron 8 were investigated. Microsatellite IVS8 (T_n) analysis was made by PCR, and the amplified fragments were subjected to electrophoresis at 1500 V for 4 h in a 6.5% denaturing polyacrylamide gel.

Results

Of the 17 patients with CBAVD, ten were found to carry mutations/variants: three (17.7%) carried two *CFTR* mutations (Δ F508/R117H; Δ F508/P205S; Δ F508/L206W), five (29.4%) had one *CFTR* mutation and the 5T variant in the second allele (A399D/5T; W1282X/5T; two Δ F508/5T; L206W/5T), one patient was homozygous, and another one was heterozygous for the 5T variant. In seven CBAVD patients and in the three patients with non-CBAVD obstructive azoospermia no mutation/variant was detected.

Overall, seven different mutations, including the 5T variant, were detected by screening the 27 *CFTR* exons and exon/intron boundaries. The 5T variant was the most common mutated allele (7/17), and Δ F508 was the second most frequent (5/17). One novel mutation, A399D, was found: this C \rightarrow A transition at position 399 in exon 8 leads to the substitution of alanine for aspartic acid. This mutation was detected in a 39-year-old Japanese CBAVD patient, who also carried the 5T variant at the other allele.

The patient who reported childhood bronchitis was found to carry two mutations (Δ F508/P205S). This was

also the case of the patient with pancreatitis (Δ F508/L206W).

Discussion

The aim of this study was to determine the frequency of mutations/variants of the *CFTR* gene in Brazilian patients with CBAVD or with non-CBAVD obstructive azoospermia. Among the 17 patients with CBAVD, eight were compound heterozygotes for mutations, or for a mutation and the 5T variant, and two patients carried only the 5T variant, one of them being homozygous for it. No alterations were found in seven patients with CBAVD and in the three azoospermic patients without CBAVD. A high number of different *CFTR* mutations have been reported in patients with CBAVD (Chillón *et al.*, 1995; Dörk *et al.*, 1997; Mak *et al.*, 1999; Casals *et al.*, 2000). Most of these mutations correspond to amino acid changes or splice site mutations, and they are likely to cause a mild CF phenotype (Casals *et al.*, 2000). However, less than 60% of these mutations are of the same types as those found in the patients with CF (Casals *et al.*, 1997). Thus, in the majority of CBAVD patients, a complete screening of the *CFTR* gene is needed to identify mutations in one or both alleles. In the 17 CBAVD patients of our sample, we detected seven different mutations, including the 5T variant. This variant was found at a frequency of 23.5%, which is in accordance with previous reports (Chillón *et al.*, 1995, Casals *et al.*, 2000), thus confirming its association with CBAVD. The Δ F508 deletion was found on five chromosomes and was the second most frequent mutation. Other five mutations were identified (R117H, P205S, L206W, A399D, and W1282X). The L206W mutation, found in two out of the 17 CBAVD patients, had previously only been detected in two Brazilian CF patients of a *CFTR* gene screening study with 160 patients (Bernardino *et al.*, 2000). This preliminary observation suggests that the Δ F508 and L206W mutations, together with the 5T variant, should be the first ones to be analyzed in Brazilian CBAVD patients, but the frequency of the L206W mutation should be confirmed in a larger sample. The A399D mutation identified in a Japanese patient was unexpected, since mutations in the *CFTR* gene are very rare in this ethnic group (Beaudet *et al.*, 1989).

The 5T variant in intron 8 of the *CFTR* gene leads to a higher proportion of mRNA transcripts lacking exon 9 than the other variants, 7T and 9T. Consequently, the 5T variant produces abnormally low levels of the CFTR protein (Chu *et al.*, 1993; Chillón *et al.*, 1995). On the other hand, the observation that in the majority of the CBAVD cases the mutations have been found in only one of the *CFTR* alleles is still not well understood. Studies on CFTR protein function show that 6%-10% of the normal levels of this protein are sufficient for normal ion transport (Chu *et al.*, 1993); therefore, carrying one mutation only would not lead to the CF phenotype. A possible explanation is that the second alter-

ation could be located at non-screened intronic, promoter, or regulatory sites, resulting in a CFTR protein with normal structure but low levels of expression, thus altering the development of susceptible tissues, such as the vas deferens and epididymis (Culard *et al.*, 1999; Casals *et al.*, 1995; Anguiano *et al.*, 1992). Interestingly, we recently examined a male patient presenting with alcoholic pancreatitis, who carried two CF mutations and had fathered two children (unpublished observation). This indicates that some male patients may still be fertile, even carrying mutations at both CF alleles.

In summary, our data provide further evidence of a strong association between male obstructive azoospermia caused by CBAVD and mutations/5T variant in the *CFTR* gene in Brazilian patients. In addition to diagnosis confirmation, the identification of mutations in these patients provides important information for the genetic counseling of couples, prior to assisted fertilization.

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