Molecular analysis of the cystic fibrosis gene reveals a high frequency of the intron 8 splice variant 5T in Egyptian males with congenital bilateral absence of the vas deferens

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It has previously been shown that defects in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are largely responsible for the condition of congenital bilateral absence of the vas deferens (CBAVD), without associated renal abnormalities, in Caucasian populations. To assess the involvement of the CFTR in CBAVD in a population with presumed low cystic fibrosis (CF) frequency, we have analysed 20 CBAVD males from Egypt for the presence of 12 common Caucasian CFTR mutations and the intron 8 5T splice variant, IVS-5T, known to be a major cause of CBAVD in Caucasian patients. In 16 of the males without associated renal abnormalities only one ΔF508 carrier was identified, but an exceptionally high frequency of the IVS-5T variant was found (14 of 32 alleles or 43.7%), confirming that this variant is involved in many cases of CBAVD, even in populations where CF is rare. CFTR mutations or the IVS-5T variant were found neither in the remaining four patients with associated renal abnormalities nor in the spouses of the 20 CBAVD patients. However, one patient was homozygous for a leucine to proline substitution at amino acid position 541 (L541P) of the CFTR. It is as yet not clear whether this change is involved in CBAVD in this male.

Key words: congenital bilateral absence of the vas deferens/cystic fibrosis/Egypt/male infertility/obstructive azoospermia

Introduction

Congenital bilateral absence of the vas deferens (CBAVD) is responsible for up to 2% of male infertility and 6% of all obstructive azoospermia cases (Dubin and Amelar, 1971). Obstructive azoospermia due to CBAVD is also a common feature in males with cystic fibrosis (CF), an autosomal recessive disease primarily characterized by respiratory tract disease and exocrine pancreatic insufficiency (Welsh et al., 1995; Zielenksi and Tsui, 1995). CF is a heterogeneous condition (Dean and Santis, 1994; Pignatti, 1994; Estivill, 1996; Ferrari and Cremonesi, 1996; Kerem and Kerem, 1996a,b; Kerem et al., 1997). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989) and more than 700 mutations have already been reported to the Cystic Fibrosis Genetic Analysis Consortium. In recent years, a genetic link between infertility in CF and in isolated CBAVD has been established by the frequent detection of mutations in the CFTR genes of CBAVD patients (for reviews see De Braekeleer and Férec, 1996; Lissens et al., 1996; Lissens and Liebaers, 1997). In the majority of cases, CBAVD can now be considered as a genital form of CF, presenting without the other clinical features of CF. Nevertheless, in ~20% of patients, CBAVD is associated with urinary tract malformations and in these cases the aetiology of CBAVD is not related to defects in the CFTR gene (Augarten et al., 1994; Dumur et al., 1995; Casals et al., 1995; Mercier et al., 1995; Rave-Harel et al., 1995).

The mutations found in the CFTR genes of CBAVD males have recently been reviewed (De Braekeleer and Férec, 1996). Although a substantial fraction of patients are carriers of a mutation also seen in CF (such as the ΔF508 mutation, the commonest mutation in CF worldwide), some patients carry a second mutation that seems to be specific to the condition of isolated CBAVD. In other patients, a second mutation has not been identified. An intron 8 splice variant, called 5T (IVS8–5T), is also frequently observed in CBAVD and seems to be specific for this condition, and probably also for other CF-related disease presentations without the major features of CF (Chillon et al., 1995; Costes et al., 1995; Jarvi et al., 1995; Zielenski et al., 1995; Dumur et al., 1996; Kerem et al., 1997). The splice variant is localized at the branch acceptor site of exon 9 and alternative variants consist of 7T or 9T nucleotides. The 5T allele is associated with high levels of exon 9 skipping which results in the production of non-functional CFTR protein (Delaney et al., 1993; Strong et al., 1993). The splicing efficiency of the 5T allele shows inter-individual and inter-organ variability, and this variability could explain why the presence of this variant in males will not always lead to CBAVD, i.e. that the 5T allele is a mutation with variable penetrance (Rave-Harel et al., 1997; Teng et al., 1997).

We have studied 20 males with CBAVD from Egypt with the aim to determine the incidence of cystic fibrosis mutations...
and the 5T variant in these patients. Since no data are available on either the frequency of CF or on the mutations leading to CF in Egypt, we have included the most common mutations seen in CF worldwide (The Cystic Fibrosis Genetic Analysis Consortium, 1994; Estivill et al., 1997). The DNA samples of the spouses of the 20 males were also studied for two reasons. First, they could serve as controls for the presence of CF mutations and the 5T variant. Second, since the patients were coming for fertility treatment by testicular sperm extraction (TESE) or microsurgical epidydymal sperm aspiration (MESA) and intracytoplasmic sperm injection (ICSI) of oocytes, these couples could be at risk for children with CF if the females are also carriers of a CF mutation.

Materials and methods

Patients

Blood samples were collected from 20 males with CBAVD visiting the Kamal Shaer Hospital Center of Fertility and Andrology Care in Orman-Dokki, Egypt. The diagnosis of CBAVD was based on the following examinations: (i) semen analysis proving low volume (< 0.5 ml), non-coagulability and azoospermia; (ii) absence or low levels of fructose and α-glucosidase in seminal plasma; (iii) absence of palpable vasa deferentia on both sides of the scrotal compartment; (iv) absence of the pelvic part of the vas deferens and the seminal vesicles by transrectal ultrasonography (TRUS); (v) routine plain and intravenous urography to screen for urinary abnormalities; (vi) family history. Blood samples were also drawn from the spouses of these males. Three couples were consanguineous (first cousins). Four CBAVD patients also had renal anomalies (see Results section). In none of the patients could a sweat test be performed.

CFTR mutation analysis

DNA was prepared from EDTA blood according to standard procedures. Initially, eight of the males’ samples were analysed by a commercial kit allowing the detection of eight mutations in the CFTR gene: ΔF508, ΔI507, G542X, G551D, R553X, 1717–1G→A, W1282X and N1303X (INNO-LiPA CF, Innogenetics, Zwijnaarde, Belgium). Later on, all samples, including the previous ones, were studied by using the CF(12)m polymerase chain reaction (PCR) kit (Zeneca Diagnostics, Abingdon, UK) allowing the detection of the mutations present in the INNO-LiPA CF kit (with the exception of the ΔI507 mutation) and the R117H, R1162X, R334W, 621+1G→T and 3849+10kbC→T mutations.

Sequencing of exon 11 of the CFTR gene was performed directly on a PCR-amplified fragment using the amplification primers 11i5 and 11i3 for sequencing (Sequenase Version 2.0, Amershams, UK) (Zielenski et al., 1991). Screening for the presence of the L541P change was done by restriction enzyme digestion of the PCR amplified exon 11 (with primers 11i5 and 11i3) with ScrFI. The normal 425 bp PCR product contains one ScrFI site (resulting in fragments of 323 and 102 bp); the T to C substitution introduces an additional site resulting in fragments of 153, 170 and 102 bp. The difference in patterns could easily be detected by 2% agarose gel electrophoresis and ethidium bromide staining.

Amplification and sequencing of the polypyrrolimidine tract in front of exon 9 was performed using primers CF13 and CF16 (Chu et al., 1991). For some patients, the poly-T tract was identified by using the radioactively labelled CF13/CF16 PCR fragment in single-strand conformation polymorphism (SSCP) analysis.

Results

Four of the 20 CBAVD patients had renal anomalies: (i) absent right kidney and an ectopic left kidney; (ii) ectopic pelvic kidney; (iii) absent right kidney and (iv) ectopic malrotated right kidney.

Mutation analysis of the 20 CBAVD males and their spouses revealed one carrier male for the ΔF508 mutation. This patient has no renal anomalies. None of the other individuals showed a mutation except the male with the ectopic pelvic kidney (patient ii in the previous paragraph) who had neither a normal nor a mutated band at the G542X site when studied by the INNO-LiPA CF kit. In this patient, normal bands were visible at the sites of the other mutations localized in exon 11 (1717–1G→A, G551D, R553X) thereby excluding a deletion of exon 11. PCR amplification and sequencing of the whole coding region of exon 11 showed that he was homozygous for a T to C substitution at cDNA position 1754 of the CFTR (1754T→C), predicting an amino acid change of leucine to proline at amino acid position 541 (L541P). This base substitution probably inhibits the binding of the exon 11 PCR fragment to the normal (and mutant) sequence at the G542X locations in the INNO-LiPA CF kit. Since this substitution would probably not be detected in heterozygotes with a normal and an L541P allele, another approach, based on the creation of an additional ScrFI restriction site in the PCR fragment defined by primers 11i5 and 11i3, was used to screen for it in the other individuals (Figure 1). The 1754T→C change was not found in any of the other individuals except in his consanguineous spouse who was a carrier.

Study of the polypyrrolimidine tract in front of exon 9 revealed a high frequency of the 5T allele in the CBAVD males without renal anomalies (Table I). Five males were homozygous for 5T while another four males were heterozygotes with a 7T or a 9T on the other allele. In contrast, none of the females and none of the males with renal anomalies were found to carry a 5T allele. The allele distribution was 43.7, 0 and 0% for 5T, 46.9, 100 and 85% for 7T and 9.4, 0 and 15% for 9T for the

![Figure 1](https://academic.oup.com/molehr/article-abstract/5/1/10/1162719/12-February-2019)
The preliminary conclusion of Dörk et al., 1995; Jarvi et al., 1995; Zielenski et al., 1997). In the Egyptian patients the ΔF508 mutation is almost exclusively found on a 9T background and this is probably also the case for the carrier male detected in this study since he has 7T and 9T alleles.

CBAVD males without and with renal anomalies, and their spouses, respectively. The L541P change was found on a 7T background. The ΔF508 change is a minor fraction of CBAVD patients, the condition is associated with a proposed different, but as yet unidentified, aetiology of the condition of CBAVD in these patients. However, one patient was homozygous for an L541P substitution in the CFTR gene, a change that so far has not been described either as a polymorphism or as a mutation. The L541P substitution is in the first nucleotide binding fold of the CFTR and would be localized, according to the model of Hyde et al. (1990), in the third β-sheet of the ATP-binding cassette (ABC) of the protein. It is positioned in a stretch of three amino acids that is highly conserved among ABC proteins. Leucine and proline both have non-polar side groups but in addition proline has a rigid ε-imino acid structure. Therefore, the replacement of a leucine by a proline could lead to the disturbance of the β-sheet and impair the function of the ATP-binding cassette. The patient did not show signs of CF, but unfortunately a sweat test could not be performed. The question remains whether the observation in this patient is either a pure coincidence of renal anomalies and a rare polymorphism or mild mutation in the CFTR gene or that both contribute to the aetiology of CBAVD in this male.

In contrast, in the four CBAVD males presenting with renal anomalies the IVS8–5T variant was absent and all patients were found to carry 7T variants. These results are in agreement with a proposed different, but as yet unidentified, aetiology of the condition of CBAVD in these patients. However, one patient was homozygous for an L541P substitution in the CFTR gene, a change that so far has not been described either as a polymorphism or as a mutation. The L541P substitution is in the first nucleotide binding fold of the CFTR and would be localized, according to the model of Hyde et al. (1990), in the third β-sheet of the ATP-binding cassette (ABC) of the protein. It is positioned in a stretch of three amino acids that is highly conserved among ABC proteins. Leucine and proline both have non-polar side groups but in addition proline has a rigid ε-imino acid structure. Therefore, the replacement of a leucine by a proline could lead to the disturbance of the β-sheet and impair the function of the ATP-binding cassette. The patient did not show signs of CF, but unfortunately a sweat test could not be performed. The question remains whether the observation in this patient is either a pure coincidence of renal anomalies and a rare polymorphism or mild mutation in the CFTR gene or that both contribute to the aetiology of CBAVD in this male.

In all studies performed so far, a high frequency of CFTR mutations or the IVS8–5T variant has been found in patients without urinary tract dysfunction. However, a substantial fraction of patients have only one mutation or one IVS8–5T variant or no identifiable abnormalities in the CFTR gene. Some authors suspect that the inability to detect CFTR gene abnormalities in these patients is probably not a technical problem. It is likely that renal anomalies are not easily recognized in these patients, perhaps due to only very subtle changes. Another explanation could be that a third group of patients exists in which the aetiology of CBAVD is neither related to CFTR dysfunction nor to the presence of renal abnormalities.

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