The complex relationships between cystic fibrosis and congenital bilateral absence of the vas deferens: clinical, electrophysiological and genetic data

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Congenital bilateral absence of the vas deferens (CBAVD) is found in 1–2% of infertile males and in most male cystic fibrosis (CF) patients. CF and some of the CBAVD cases were found to share the same genetic background. In this study, 21 males with CBAVD had extensive physical and laboratory testing for symptoms of CF. Possible defective cellular chloride transport was measured by interstitial current measurement of rectal suction biopsies. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation analysis was performed for 10 common CFTR mutations. CF-related symptoms were found in six men. On laboratory testing slightly abnormal liver and pancreatic function was found in seven patients. The sweat test was found to be abnormal in four patients; interstitial current measurement showed defective chloride excretion in 11 patients. CFTR gene mutations were found in 66% of the patients: eight were compound heterozygotes; in six, only one common mutation could be detected. The 5T allele in one copy of intron 8 was found in four men. CBAVD appears to be a heterogeneous clinical and genetic condition. A CFTR gene mutation was found in both copies of the allele or interstitial current measurement showed defective chloride excretion in 14/21 cases. Genetic counselling is clearly indicated for couples seeking pregnancy through epididymal or testicular sperm aspiration and intracytoplasmic sperm injection.
Key words: CFTR gene analysis/clinical symptoms/congenital bilateral absence of the vas deferens/cystic fibrosis/intestinal current measurement

Introduction

Congenital absence of the vas deferens (CBAVD), due to bilateral regression of the mesonephric duct, occurs in 1–2% of infertile males and in 6% of azoospermic men (Oates and Amos, 1994). Clinical symptoms of CBAVD are bilateral non-palpable vas deferens, absence of the distal part of the epididymis and hypoplasia of the vesicula seminalis. Azoosper-mia with low semen plasma volume (<1.5 ml) and low pH (<7.5) is consistently found. Testis volume and serum gonadotrophins are usually normal. Testicular biopsy shows normal or slightly defective spermatogenesis. Viable spermato-zoa can be harvested from the epididymis by micropuncture (MESA) and pregnancy can be obtained by in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI; Silber et al., 1994).

The aetiology of CBAVD is unknown. Most male cystic fibrosis (CF) patients have CBAVD, and it was suggested that CBAVD represents an incomplete form of CF (Holsclaw et al., 1971). Since the identification of the cystic fibrosis transmembrane regulator (CFTR) gene (Riordan et al., 1989), mutations have been found in 62% of cases of CBAVD (Patricio et al., 1993; Mercier et al., 1995; De Braekeleer and Ferec, 1996).

CBAVD patients have been reported to carry two, one or no CFTR gene mutations, one of them being ΔF508, the most frequent CF mutation. Recently, the R117H mutation, a rare mutation in CF patients, was found to occur frequently in CBAVD patients (Gervais et al., 1993). Furthermore, the 5T variant of the polypyrimidine stretch in intron 8, which is thought to influence splicing, was shown to occur more frequently in CBAVD as compared to controls (Chu et al., 1993; Chillon et al., 1995). In cases where CBAVD is associated with urogenital malformation, CFTR gene mutations appear to be absent, suggesting a different aetiology (Dumur et al., 1996).

The aim of this study was to investigate whether patients with CBAVD have other CF related non-genital manifestations, and if so, how to improve genetic counselling in case of demand for MESA/ICSI treatment.

Materials and methods

Patients

This study was approved by the Hospital Medical Ethical committee and by patients by written informed consent. Twenty-one infertile patients with obstructive azoospermia due to bilateral absence of the scrotal vas deferens were enrolled in the study. The diagnosis was made by physical examination and confirmed by demonstration of azoospermia with low semen plasma volume and low pH.

A medical history was obtained focused on symptoms common in CF, such as rhino-sinusitis, nasal polyps, obstructive lung disease, recurrent pulmonary infections, gastro-intestinal malabsorption, fat intolerance, oily stools, cholelithiasis, liver dysfunction and intestinal obstruction. Family history was documented for CF and other genetic abnormalities.
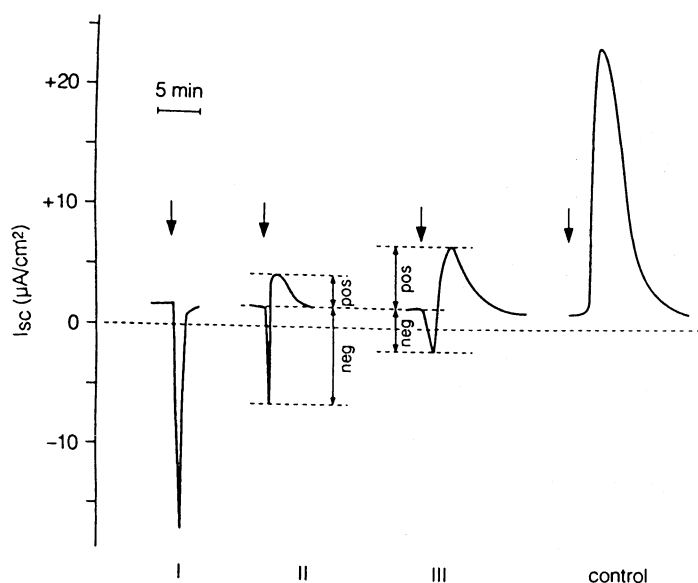
Figure 1. Interstitial current measurements of a rectal biopsy.
pos = positive, neg = negative. I = CF response, II = low
residual chloride secretion, III = high residual chloride secretion.

Physical examination

This included urogenital and pulmonary investigation and measurement of weight, height and nutritional state. Pulmonary function tests included forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1). A chest X-ray was performed. The Shwachman score was determined for all patients (Shwachman, 1990). Sonography of the kidneys and transrectal ultrasound was performed to detect urogenital malformations.

Laboratory testing

Laboratory testing included bacterial cultures in sputum, measurements of gonadotrophins [luteinizing hormone (LH) and follicle stimulating hormone (FSH)], liver function, serum glucose level and faecal chymotrypsin. Bilateral sweat tests were performed and, in the absence of normal values for adults, judged as abnormal if chloride >50 mmol/l.

Electrophysiological study

Interstitial current measurement on rectal tissue were performed (Veeze *et al.*, 1994). Rectal suction biopsies were mounted in an Ussing chamber with an exposed area of 1.13 mm^2. Sodium channels were blocked by adding amiloride (10^{-4} mol/l). Endogenous prostaglandin synthesis that is possibly linked to cAMP-mediated chloride secretion was inhibited by adding indomethacin (10^{-5} mol/l). Carbachol (10^{-4} mol/l) was added for cholinergic activation of chloride secretion.

In healthy controls, a carbachol-provoked change in interstitial current measurement values was found (control, Figure 1). The inward current in controls reflects transcellular chloride transport (serosa to mucosa) through the Na–K–Cl co-transporter in the basolateral membrane and the CFTR–chloride channel in the apical membrane. In the majority of CF patients, a carbachol-induced outward current response (type I negative, Figure 1) occurs. This reversed response probably results from apical potassium secretion that is unmasked in the case of absent or largely reduced chloride secretion.

In a subclass of CF patients, usually after a negative peak response, a separate and small positive peak response was observed, the result of low residual chloride secretion (type II, Figure 1). In another subset of cases, a high residual chloride secretion was seen (type III, Figure 1), due to chloride excretion by non-CFTR channels. The amount of residual chloride secretion appeared to be associated with preserved pancreatic function and delayed presentation of the disease, which was not exclusively determined by the CF genotype (Veeze *et al.*, 1994).

Interstitial current measurements are especially indicated in individuals with borderline or high normal sweat test results and an inconclusive CFTR mutation analysis, who cannot otherwise be distinguished from CF-carriers.

DNA analysis

DNA was isolated from peripheral leukocytes. CFTR mutation analysis was performed for 10 mutations: we analysed for the mutations R117H, A455E, ΔF508, 1717–1G→A, G542X, R553X, R1162X, S1251N, W1282X, and N1303K. The length of the T-stretch in intron 8 was determined (Kiesewetter *et al.*, 1993). Only the allele specific oligonucleotide for the identification of the 9 T-stretch was changed into: 5′-TGTGTG TTT TTT TTT AAC AG-3′, using a hybridization temperature of 37°C for all allele specific oligonucleotides.

Results

Table I summarizes the abnormal physical and laboratory findings. The history revealed nasal polyps/rhino-sinusitis (n = 3), obstructive lung disease (n = 1) and fatty stools (n = 2). Two patients had a positive family history for CF. Pulmonary function was abnormal in one case with a history of pertussis in childhood: on chest X-ray atelectasis and bronchiectasis were found. The Shwachman score was abnormal in one (80) and borderline (95) in another case.

High gamma-glutamyl transpeptidase, not related to alcohol consumption, was found in six cases. Faecal chymotrypsin was low in four cases, indicating exocrine pancreatic dysfunction. In four cases, the sweat test was borderline by using strict criteria.

Interstitial current measurement showed either a typical CF response (Figure 1, type I) (n = 4), a low residual chloride secretion (Figure 1, type II) (n = 1) or a high residual secretion (Figure 1, type III) (n = 6). The test was found inconclusive in one case and normal (Figure 1, control) in 10 patients.

CFTR gene analysis showed one or two mutations in 14/21 cases. In eight patients two different mutations (compound heterozygosity) were found; in six patients only one mutation could be identified. In seven cases, no common CFTR gene mutation could be detected: four out of seven of these were non-Caucasians. A 5T allele in one copy of the CFTR gene was found in four cases, three times in combination with a mutation in the other allele.

The ΔF508 mutation was found in eight patients, R117H in six, A445E in three and 1717–1G→A and R553X both in one. Three partners were found to have a single CFTR gene mutation (R117H, R117H, ΔF508).

Discussion

The observation that the vas deferens is absent in almost all male CF patients suggested that CBAVD is a primary genital form of CF (Holsclaw *et al.*, 1971). Following the identification of the CFTR gene, CBAVD and CF were also often found to share the same genetic background (Mercier *et al.*, 1993; Patricio *et al.*, 1993). CFTR, the product of the CFTR gene,

Table I. Summary of physical and laboratory findings in patients with CBAVD

Case no.	Age (years)	History tests	Laboratory Cl⁻/Na⁺	Sweat test	ICM	CFTR mutations	T-stretch intron 8	Remarks
1	36	NA	NA	38/46	CF response (I)	ΔF508/R117H	9/7	Non-Caucasian
2	27	Sinusitis/fat intol.	Chymotr.<	23/22	CF response (I)	ΔF508/R117H	9/7	CF in family
3	33	CARA/oily stools	Ggt>,Chymotr.<	23/36	CF response (I)	ΔF508/–	9/7	
4	31	Pelvic re kidney	NA	10/22	CF response (I)	–/–	7/7	
5	32	Sinusitis/nasal polyps	Chymotr.<	50/52	CF low residual (II)	A455E/–	9/5	Partner ΔdF508
6	38	NA	NA	40/43	CF high residual (III)	A445E/R117H	9/7	
7	27	NA	Ggt>,Chymotr.<	28/44	CF high residual (III)	R117H/R553X	7/7	Partner R117H
8	38	Nasal polyps	NA	34/51	CF high residual (III)	ΔF508/R117H	9/7	Pertussis
9	36	NA	NA	58/70	CF high residual (III)	ΔF508/–	9/5	
10	31	NA	Ggt>	54/70	CF high residual (III)	ΔF508/–	9/5	Partner R117H
11	32	Maldescended testis	Ggt>	16/34	CF high residual (III)	–/–	9/7	Single kidney in family
12	35	NA	NA	14/21	Inconclusive	ΔF508/–	9/7	
13	29	NA	NA	43/70	Normal response (IV)	A455E/R117H	9/7	
14	38	NA	NA	32/55	Normal response (IV)	R117H/1717–1→G→A	7/7	
15	29	NA	Ggt>	44/66	Normal response (IV)	ΔF508/R117H	9/7	
16	28	NA	NA	42/48	Normal response (IV)	R117H/–	7/7	
17	36	NA	NA	22/44	Normal response (IV)	–/–	7/5	Non-Caucasian
18	34	NA	NA	57/30	Normal response (IV)	–/–	7/7	Non-Caucasian
19	39	NA	NA	36/52	Normal response (IV)	–/–	7/7	Non-Caucasian
20	31	NA	NA	16/30	Normal response (IV)	–/–	7/7	Non-Caucasian
21	34	NA	NA	20/41	Normal response (IV)	–/–	7/7	

NA = no abnormalities, GgT = gamma glutamyl transpeptidase, Chymotr. = chymotrypsin, ICM = interstitial current measurement (see Figure 1), CFTR = cystic fibrosis transmembrane conductance regulator gene, –: none of 10 CF mutations, T-stretch = polypyrimidine (T) stretch intron 8.
Shwachman score was 95 in case 1 and 80 in case 8. Physical examination showed bronchiectasis in case 8 and hypogonadism in case 19.

a cell membrane protein of 1480 amino acids, regulates transmembrane chloride transport. Over 750 mutations of the CFTR gene have been reported, ΔF508 being the most frequent mutation in CF patients. CF is an autosomal recessive disease; a patient with CF receives two defective alleles. The carrier risk in Caucasians is 1:25.

In CF, conductive chloride transport is defective in epithelial tissues, resulting in viscous secretions associated with pulmonary infections, malabsorption and intestinal obstruction. The severity of the disease varies widely: homozygosity for the ΔF508 mutation was found to be associated with pancreatic insufficiency, early manifestations, poor lung function and high mortality (Kerem *et al.*, 1990). Other mutations, like R117H, are associated with a milder form of CF where conductive chloride transport is defective, but not absent (Gervais *et al.*, 1993).

The CFTR gene mutations occur frequently in CBAVD (Patricio *et al.*, 1993; Oates and Amos, 1994; de Braekeleer *et al.*, 1996), but the molecular basis of CBAVD is not completely understood. Mutations with a low frequency in classic CF, such as R117H, were found to occur regularly in CBAVD (Gervais *et al.*, 1993). Homozygosity for ΔF508 or compound heterozygosity for two severe mutations were not found in cases of CBAVD. It has been suggested that CBAVD patients are compound heterozygotes for a severe mutation on one allele in combination with a mild CFTR gene mutation on the other allele. In the majority of cases, however, only one CFTR gene mutation could be detected in CBAVD. Recently alterations in the non-coding regions of the gene, such as the polypyrimidine stretch in intron 8, in combination with a mutation in the other allele, were found to cause abnormal levels of CFTR protein (Chu *et al.*, 1993). Impaired CFTR protein function may cause defective, but not absent

chloride excretion, resulting in absence of the vas deferens, but not in pulmonary or pancreatic insufficiency (Anguiano *et al.*, 1992). The epididymis may be more susceptible to defective chloride transport, resulting in an early regression of the mesonephric duct. In contrast, only 6% of CFTR protein function is necessary for normal pancreatic function (Tizzano *et al.*, 1994). Also, the wide variability of symptoms related to various combinations of CFTR mutations suggests a possible role for unlinked genetic factors in the expression of these mutations.

In this study, 21 patients with CBAVD were investigated for non-genital manifestations of CF: in six patients mild CF symptoms were present. Slightly abnormal liver and pancreatic function were detected in seven, sweat tests showed high levels of chloride in four patients. Electrophysiology of rectal suction biopsies, not previously performed in CBAVD, showed defective chloride excretion in 11 patients. Three of these patients showed very low sweat test results, indicating different tissue expression of impaired CFTR function.

CBAVD appears to be a heterogeneous clinical and genetic condition: two CFTR gene mutations were detected in eight patients, five of them showing CF characteristics on interstitial current measurement. In these men the CBAVD might represent a mild form of CF. Of the patients carrying a single CFTR mutation, four also showed defective chloride excretion on interstitial current measurement, suggesting mutations going undetected with the current screening technology. So far, no convincing evidence has been brought forward that the presence of a single CFTR mutation (i.e. simple heterozygosity) has any phenotypic consequences (Meschede *et al.*, 1997). Therefore, in the case of CBAVD and defective chloride excretion further analysis of the CFTR gene is required to detect rare variant mutations.

In most cases of CBAVD, residual or normal chloride excretion was found in combination with either an abnormal sweat test or CFTR gene mutations. Only in five cases of CBAVD no abnormalities could be found, four of these men being non-Caucasians. These results suggest that there is a wide spectrum of phenotypic expression of cystic fibrosis, with pancreatic and pulmonary insufficiency at one end and CBAVD at the other.

Since the introduction of microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection (Silber *et al.*, 1994) infertility due to CBAVD has been treated successfully, resulting in ongoing pregnancy. Biological parenthood is now a realistic option for males with CBAVD, producing ongoing pregnancies in 20–30% of cases (Dohle *et al.*, 1998).

For couples with CBAVD-related infertility CFTR mutation analysis and genetic counselling of the patient and his partner is essential before MESA/ICSI procedures are performed (Pauer *et al.*, 1997). Although the *a priori* carrier risk for a CFTR gene mutations is only 3–4%, three partners of the male CBAVD group had a single CFTR gene mutation. In these cases the risk of offspring with a (mild or severe) form of CF could be 50%.

As there is no straightforward relationship between the genotype and the phenotype for most CFTR gene mutations, genetic counselling in these situations is complex, as no precise predictions on rare compound phenotypes of CF are possible. Considering all the medical and psychological burdens of MESA and ICSI procedures, followed by chorionic biopsy for early prenatal diagnosis of CF, reproduction becomes complicated for these couples. Pre-implantation screening of embryos would be an alternative technique for prenatal diagnosis, but does not solve all ethical problems. In case of a CFTR mutation in the partner and no detectable mutation in the CBAVD male, a positive interstitial current measurement test in the patient will indicate rare variant alleles of the CF gene. However, if no CFTR gene mutations are found in the female partner, the risk of offspring with CF is at the most 1:400. Prenatal or preimplantation screening for CF is not possible in these cases.

In conclusion, CBAVD appears to be a heterogeneous condition with respect to CF symptoms, tissue expression of defective chloride excretion and CFTR gene mutation analysis. Only in a small subset of men with CBAVD could no abnormalities be detected.

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