Hum Genet (1995) 95:205–211

ORIGINAL INVESTIGATION

T. Casals · L. Bassas · J. Ruiz-Romero · M. Chillón
J. Giménez · M. D. Ramos · G. Tapia · H. Narváez
V. Nunes · X. Estivill

Extensive analysis of 40 infertile patients with congenital absence of the vas deferens: in 50% of cases only one CFTR allele could be detected

Received: 28 June 1994 / Revised: 29 August 1994

Abstract Mutations in the cystic fibrosis (CF) conductance transmembrane regulator (CFTR) gene have been detected in patients with CF and in males with infertility attributable to congenital bilateral absence of the vas deferens (CBAVD). Thirty individuals with CBAVD and 10 with congenital unilateral absence of the vas deferens (CUAVD) were analyzed by single-strand conformation analysis and denaturing gradient gel electrophoresis for mutations in most of the CFTR gene. All 40 individuals were pancreatic sufficient, but twenty patients had recurrent or sporadic respiratory infections, asthma/asthmatic bronchitis, and/or rhino-sinusitis. Agenesia or displasia of one or both seminal vesicles was detected in 30 men and other urogenital malformations were present in six subjects. Among the 40 samples, we identified 13 different CFTR mutations, two of which were previously unknown. One new mutation in exon 4 was the deletion of glutamic acid at codon 115 (ΔE115). A second new mutation was found in exon 17b, viz., an A→C substitution at position 3311, changing lysine to threonine at codon 1060 (K1060T). CFTR mutations were detected in 22 out of 30 (73.3%) CBAVD patients and in one out of 10 (10%) CUAVD individuals, showing a significantly lower incidence of CFTR mutations in CBAVD/CUAVD patients ($P \ll 0.0001$), compared with that found in the CF patient population. Only three CBAVD patients were found with more than one CFTR mutation (ΔF508/L206W, ΔF508/R74W + D1270N, R117H/712–1G→T), highlighting L206W, R74W/D1270N, and R117H as benign CF mutations. Sweat electrolyte values were increased in 76.6% of CBAVD patients, but three individuals without CFTR mutations had normal sweat electrolyte levels (10% of the total CBAVD patients), suggesting that factors other than CFTR mutations are involved in CBAVD. The failure to identify a second mutation in exons and their flanking regions of the CFTR gene suggests that these mutations could be located in introns or in the promoter region of CFTR. Such mutations could result in CFTR levels below the minimum 6%–10% necessary for normal protein function.

T. Casals · M. Chillón · J. Giménez · M. D. Ramos · V. Nunes
X. Estivill (✉)
Molecular Genetics Department, Cancer Research Institute, Hospital Duran i Reynals, Autovía de Castelldefels Km. 2.7, L'Hospitalet de Llobregat, E-08907 Barcelona, Catalonia, Spain

L. Bassas · J. Ruiz-Romero · H. Narváez
Andrology Department, I.U.N.A., Fundació Puigvert, Barcelona, Catalonia, Spain

G. Tapia
Pediatrics Department, Hospital de Sant Pau, Barcelona, Catalonia, Spain

Introduction

Congenital bilateral absence of the vas deferens (CBAVD) (McKusick 277180) is present in 1%–2% of the infertile healthy male population and accounts for at least 6% of cases of obstructive azoospermia (Holsclaw et al. 1971; Jequier et al. 1985). CBAVD has also frequently been observed in males with cystic fibrosis (CF) (about 95%), the most common (1 in 2500 individuals) severe autosomal recessive disease in the Caucasoid population (Kaplan et al. 1968; Boat et al. 1989; Heaton and Pryor 1990). CF is characterized by chronic pulmonary infections, pancreatic enzyme insufficiency, elevated electrolyte levels in sweat, and CBAVD in males (Boat et al. 1989). The molecular link between CF and CBAVD has been provided by the analysis of the CF conductance transmembrane regulator (CFTR) gene and the identification of mutations in CF patients (Kerem et al. 1989; Tsui 1992) and in CBAVD individuals (Dumur et al. 1990; Rigot et al. 1991; Anguiano et al. 1992; Gervais et al. 1993; Osborne et al. 1993; Culard et al. 1994). CF mutations have been found in 65% of individuals with CBAVD, suggesting that CBAVD patients with CFTR gene mutations have a primarily genital form of CF (Anguiano et al. 1992).

More than 400 mutations have been described in the CFTR gene (CF Genetic Analysis Consortium, CFGAC, personal communications). Phenotype/genotype correlation studies for common CF mutations have shown that

homozygosity or compound heterozygosity for these common mutations is associated with severe CF clinical presentation (The CF Genotype-Phenotype Consortium 1993). On the other hand, individual reports of uncommon mutations have indicated that some missense mutations are associated with a mild phenotype (Dean et al. 1990; Strong et al. 1991; Chillón et al. 1993; Nunes et al. 1993). The involvement of the CFTR gene in CBAVD is still not well understood. At the clinical level, most CBAVD patients have elevated sweat electrolytes, but present normal gastrointestinal and respiratory function. Obligate male CF carriers do not have clinical features of CF and their fertility is not compromised; rather it may even be increased (Pritchard 1991). It is believed that CBAVD is not attributable to only one mutated CFTR allele, but that two mutations in the patient contribute to the CBAVD phenotype. If CBAVD is a primarily genital form of CF, most CBAVD males should be compound heterozygotes for two mild mutations or for one mild and one severe CFTR mutation. Alternatively, mutations in the promoter region or in non-coding regions of CFTR, in association with a severe mutation, could contribute to CBAVD. The complete characterization of CFTR in CBAVD patients should reveal the spectrum of mutations involved in this type of infertility and possibly also in moderate forms of CF.

We report here the clinical features and mutational data obtained in the genetic screening for CFTR mutations in 40 unrelated men, 30 with CBAVD and 10 with unilateral congenital absence of the vas deferens (CUAVD). We have identified 13 different mutations in the CFTR gene (in 73.3% of CBAVD and 10% of CUAVD individuals). Two of the mutations were previously unknown and only three individuals had two CFTR mutations.

Patients and methods

Patients

Seventy six unrelated Spanish men, who had their fertility assessed from 1984 to 1993 and who were diagnosed as having CBAVD or CUAVD, were asked to participate in the study. This represents 1.65% of the total infertile male population studied during this period in the Andrology Department of the I.U.N.A., Barcelona. Forty individuals, aged between 15 and 49 years, completed the study. Patients underwent scrotal exploration, transrectal and abdominal ultrasonography, and semen analysis (volume, pH, sperm count, fructose and citrate concentrations) (Table 1). CBAVD was confirmed in 30 patients, and 10 subjects had CUAVD. None of the patients showed pulmonary or gastrointestinal manifestations typical of CF. However, several patients had symptoms of respiratory disease, sinusitis, or nasal polyps. Sweat chloride analysis was performed in all CBAVD and seven CUAVD individuals (Gibson and Cooke 1959) (Table 2).

DNA analysis

DNA was obtained from peripheral blood lymphocytes according to standard protocols. Genomic DNA from CBAVD and CUAVD subjects was first analyzed for the two most common mutations in the Spanish population, viz., ΔF508 and G542X (Casals et al. 1993). After amplification by the polymerase chain reaction, CFTR gene exons and their flanking sequences were screened by denaturing gradient gel electrophoresis (DGGE) multiplex analysis (exons 3, 5, 6a, 8, 9, 11, 12, 14a, 14b, 15, 17b, 18, 20, 21, and 23), or by single strand conformation analysis (SSCA) (exons 4, 6b, 7, 17a, 19, and 22), as described (Fanen et al. 1992; Audrézet et al. 1993; Chillón et al. 1994a). Microsatellite analysis for three dinucleotide repeats within CFTR (IVS8CA, IVS17BTA and IVS17BCA) was performed as described previously (Morral and Estivill 1992). Haplotypes associated with specific CF mutations were as described (Morral et al. 1993).

Results

The clinical and genetic data of CBAVD and CUAVD patients are shown in Tables 1–3. All 40 individuals were pancreatic sufficient, but one presented with pancreatitis as a consequence of alcohol consumption. Recurrent respiratory infection was observed in six patients (15%), and sporadic episodes of infection were detected in two cases. Other respiratory problems included asthma and asthmatic bronchitis in three cases, and rhino-sinusitis in nine patients (22.5%). Sweat electrolytes, measured in 37 patients, gave Cl^- values over 70 mEq/l in 26 cases (65%) (mean 85.8 mEq/l; range 15–170 mEq/l).

Azoospermia was found in all CBAVD patients in whom semen analysis was performed (Table 1). In men with CUAVD, sperm concentrations ranged from azoospermia to normal values. Semen volume and the concentration of biochemical markers reflected a wide spectrum of seminal vesicle involvement. Other malformations of the genitourinary system (cryptorchidism, renal agenesia) and hernias of the abdominal wall were fairly frequent (Table 3).

We identified 13 different CFTR mutations in the 40 samples analyzed (80 chromosomes). The overall frequency of CF mutations detected in this population was 32.5% (26/80), significantly higher than that expected in the general population ($P \ll 0.0001$), but also significantly lower than in our CF patient population ($P \ll 0.0001$). The two most frequent CF mutations in the Spanish population (ΔF508 and G542X) were found on 14 chromosomes (10 ΔF508 and 4 G542X). When exon 10 was analyzed for ΔF508, one sample showed a different heteroduplex pattern that corresponded to mutation 1677delTA.

SSCA of exon 4 allowed the identification of two different changes (Fig. 1). Direct sequencing of these two abnormal fragments identified mutation R117H, a known

Table 1 Semen analysis of patients with CAVD, given as the mean (range)

	CBAVD (n = 27)	CUAVD (n = 10)
Sperm ($\times 10^6$/ml)	0	10.6 (0–90)
Seminal volume (ml)	0.9 (0.2–3.1)	2.5 (0.4–5.4)
pH	6.7 (6.0–8.0)	7.3 (6.4–7.7)
[a] Fructose (mmol/l)	2.6 (0–9)	10.3 (3–18)
[a] Citrate (mmol/l)	77.5 (11–188)	48.6 (36–88)

[a] Reference values: fructose, 8–28 mmol/l; citrate, 10–35 mmol/l

Table 2 CFTR mutation analysis in 30 CBAVD and 10 CUAVD patients (*CBAVD* congenital bilateral absence of the vas deferens, *CUAVD* congenital unilateral absence of the vas deferens, *ND* not determined, - absence of mutations, *RRI* recurrent respiratory infection, *R* rhinitis, *RS* rhinosinusitis, *BR.ASTH* bronchitis asthmatic)

Patient	Age (years)	Phenotype	Sweat test (mEq/l)	Mutation	Other clinical features
1	37	CBAVD	108	1677delTA	
2	28	CBAVD	50	G542X	
3	28	CBAVD	118	–	
4	33	CBAVD	90	ΔF508/L206W	RRI, R
5	26	CBAVD	118	R117H/712-1G→T	
6	42	CBAVD	66	–	RS
7	31	CBAVD	170	ΔF508	R
8	27	CBAVD	100	ΔF508/R74W + D1270N	RRI, R
9	32	CBAVD	74	ΔE115	RS
10	35	CBAVD	90	–	Nasal polyps
11	33	CBAVD	78	K1060T	RI, family history
12	45	CBAVD	150	R334W	RS
13	42	CBAVD	60	–	
14	40	CBAVD	110	R1070W	RS
15	29	CBAVD	110	G542X	
16	37	CBAVD	80	R117H	RI, RS, BR.ASTH
17	37	CBAVD	85	–	Asthma
18	46	CBAVD	15	R1162X	
19	37	CBAVD	110	ΔF508	RS, diarrhoea
20	42	CBAVD	45	2789+5G→A	RI
21	49	CBAVD	95	ΔF508	
22	36	CBAVD	70	ΔF508	RRI, RS
23	42	CBAVD	90	–	
24	15	CBAVD	150	ΔF508	
25	26	CBAVD	60	–	
26	39	CBAVD	100	ΔF508	RRI, RS
27	33	CBAVD	57	ΔF508	RRI
28	33	CBAVD	80	G542X	
29	34	CBAVD	78	–	
30	32	CBAVD	113	G542X	
31	33	CUAVD	ND	ΔF508	RS, pancreatitis
32	37	CUAVD	ND	–	
33	31	CUAVD	77	–	BR.ASTH
34	39	CUAVD	ND	–	
35	40	CUAVD	40	–	
36	33	CUAVD	59	–	
37	40	CUAVD	90	–	
38	47	CUAVD	40	–	RRI
39	39	CUAVD	50	–	
40	35	CUAVD	100	–	

Table 3 Congenital malformations associated with CAVD in 40 patients

	CBAVD			CUAVD			Total
No. mutations/individual	2	1	0	2	1	0	
Agenesia or displasia of seminal vesicles	3	12	7	–	1	7	30 (75%)
Renal agenesia	–	–	2	–	–	4	6 (15%)
Cryptorchidism	–	1	–	–	–	1	2 (5%)
Hernia	–	4	1	–	–	1	6 (15%)

mild phenotype mutation found in CF and CBAVD patients (Dean et al. 1990; Gervais et al. 1993). This mutation had not previously been detected in the Spanish CF population (Chillón et al. 1994a). A second R117H mutation was detected in another CBAVD patient. The sequence of another abnormal exon 4 fragment showed the deletion of codon GAG at position 475–477, which corresponds to a glutamic acid at position 115 (ΔE115), a previously unknown mutation in the CFTR gene (Fig. 2). The patient with ΔE115 presented CBAVD, rhino-sinusitis, and a borderline sweat test (74 mEq/l). An abnormal SSCA pattern was detected in exon 19 of patient 18; this

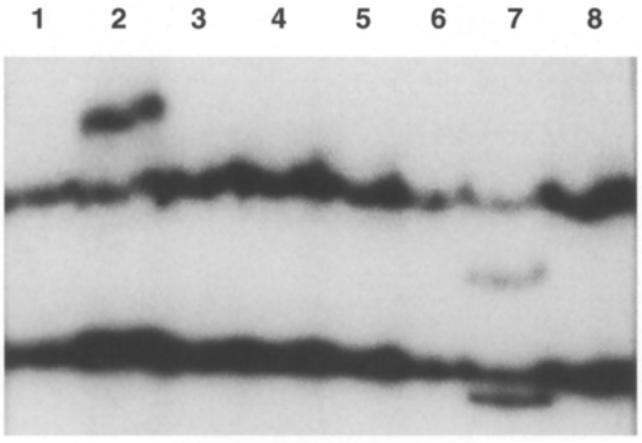

Fig. 1 SSCA of exon 4 of the CFTR gene using primers I4D1 and I4R1 (Chillón et al. 1994a). *Lane 2* corresponds to mutation R117H and *lane 7* to mutation ΔE115

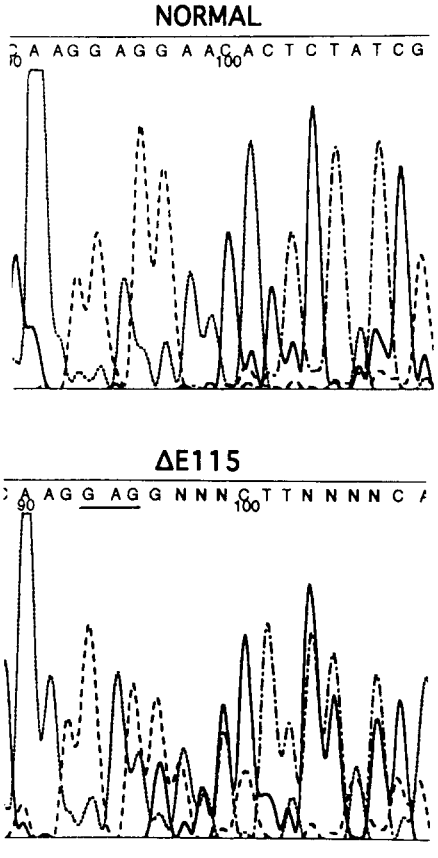

Fig. 2 Direct sequencing of exon 4 of the CFTR gene in a normal control and in a patient heterozygous for mutation ΔE115. At the GAG deleted trinucleotide (*underlined*), the intensity of the G and A is increased and the second G is decreased, in agreement with the GAG deletion

corresponded to mutation R1162X, which accounts for about 1.8% of Spanish CF chromosomes.

Fifteen exons were analyzed by multiplex DGGE. For the observed changes, we considered the strong association between mutations and CFTR microsatellite haplo-

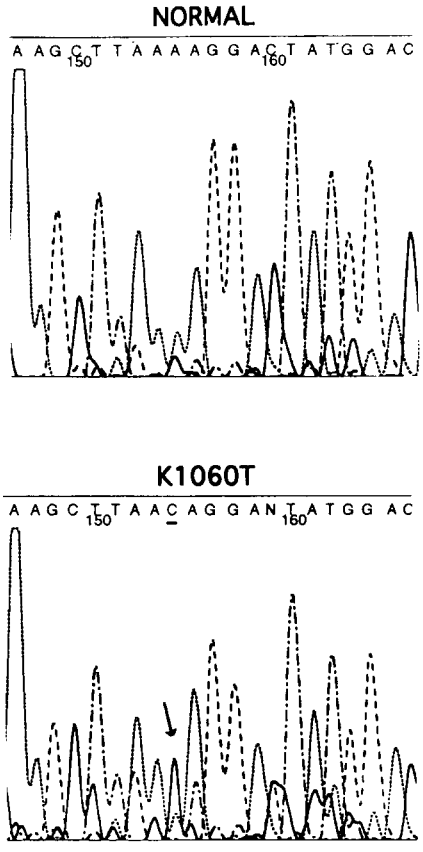

Fig. 3 Direct sequencing of exon 17b of the CFTR gene in a normal control and in a patient heterozygous for mutation K1060T. Sequencing involved the use of primer 17bi5' (Zielenski et al. 1991). The mutated nucleotide is indicated with an *arrow* on the trace and is *underlined* on the sequence

types (IVS8CA, IVS17BTA and IVS17BCA) (Morral et al. 1993). The analysis of this specific mutation/haplotype association allowed us to identify mutations L206W (16-7-17), 712-1G→T (23-31-13), R334W (17-46-13), and 2789 + 5G→A (17-7-17), on one mutated chromosome each. All mutations were confirmed by sequencing or by restriction enzyme analysis.

Abnormal DGGE fragments with no microsatellite haplotype associations were further analyzed by sequencing. One abnormal pattern in exon 17b was caused by an A→C substitution at position 3311, changing lysine to threonine at codon 1060 (K1060T); this is a new missense mutation in the CFTR gene (Fig. 3). The patient with mutation K1060T also belonged to the group of CBAVD, had an increased sweat Cl^- level (78 mEq/l), and a history of recurrent respiratory infections. A second change in exon 17b corresponded to R1070W (M. Macek Jr., personal communication to CFGAC), which has not previously been detected in Spanish CF chromosomes (Chillón et al. 1994b). Another abnormal DGGE fragment detected in exon 3 was the result of mutation R74W (Claustres et al. 1993); this mutation was found to be associated with mutation D1270N in exon 20 (Anguiano et al. 1992; C. Férec and M. Claustres, personal communication).

Discussion

The relationship between CBAVD and CFTR gene mutations has previously been demonstrated (Dumur et al. 1990; Anguiano et al. 1992; Gervais et al. 1993; Osborne et al. 1993; Culard et al. 1994). In the majority of cases, the mutations have only been found in the heterozygous state, i.e., only one mutation per CBAVD individual, even after extensive analysis of the CFTR gene (Osborne et al. 1993; Culard et al. 1994). We have also analyzed most of the CFTR gene in a sample of 30 CBAVD and 10 CUAVD individuals. CFTR mutations were detected in 22 out of 30 (73.3%) CBAVD patients and in one out of 10 (10%) CUAVD individuals. These results show a significantly lower incidence of CFTR mutations in CBAVD/CUAVD patients, compared with that expected in the CF patient population ($P \ll 0.0001$).

Only three CBAVD patients were found with more than one CFTR mutation. One patient was heterozygous ΔF508/L206W and had recurrent pulmonary infection and rhinitis episodes from the age of 24. Our data on Spanish CF patients with the mutation L206W (Claustres et al. 1993) suggest that L206W is a mild mutation associated with a benign CF phenotype (T. Casals, unpublished). The second patient was heterozygous for mutations R117H/712-1G→T, and had only a CBAVD phenotype, probably because of the benign mutation R117H (Dean et al. 1990). The third patient had ΔF508/R74W + D1270N, with clinical features of rhinitis and recurrent respiratory infections. We do not know which of the two mutations (R74W or D1270N, or both) is involved in the CBAVD phenotype. The proportion of these patients with more than one mutation in their CFTR gene (10%) is similar to that reported by others in previous extensive analyses of the CFTR gene (Osborne et al. 1993; Culard et al. 1994).

Nineteen patients (63.3%) had only one identified CFTR mutation. In 13 cases, the mutations are known to be associated with severe CF (ΔF508, G542X, R1162X and 1677delTA), whereas in five cases, the phenotypic effect of the mutation is still unknown (ΔE115, K1060T, R334W, R1070W, and 2789 + 5G→A); in one case (R117H), the mutation is known to result in mild CF. Of these mutations, R334W seems to cause pancreatic insufficiency with a variable age of onset (X. Estivill, in press), whereas mutation 2789 + 5G→A (W. E. Jr. Highsmith, personal communication to the CFGAC) has frequently been found in adult CF patients and is probably involved in a mild phenotype (T. Casals et al. unpublished). Genotype/phenotype information for the other CF mutations has still not been obtained.

It is unknown why most CBAVD patients have only one CFTR mutation. This is particularly important since the sequences have been determined for all the CFTR exons and their neighboring intronic regions in several samples from CBAVD individuals; only one CFTR mutation has been detected in most cases (Culard et al. 1994; C. Férec, personal communication). Studies on CFTR protein function show that only 6%–10% of CFTR is sufficient for normal ion transport (Chu et al. 1992; Johnson et al. 1992). The failure to find a second mutation in the CFTR gene exons and their flanking regions suggests that mutations could be located in intronic or promoter regions. These mutations could cause a high proportion of an abnormal, alternatively spliced CFTR mRNA, with little normal CFTR, and thus could be responsible for the CBAVD phenotype. Further studies should be performed to elucidate molecular changes, in addition to mutations in the CFTR coding region that cause CBAVD.

About 27% of CBAVD patients, 37.5% of whom have normal sweat electrolytes, were negative for the mutational analysis of most of their CFTR gene. For patients with one mutation previously identified, a second molecular defect in CFTR would be expected. However, those cases with negative results for CFTR gene analysis and with no CF-associated phenotypic abnormalities (which have also been found in other analyses: Culard et al. 1994; Oates and Amos 1994) suggest that there might be additional factors and genes involved in the CBAVD male infertile phenotype.

CFTR mutations were not detected in patients in which CBAVD or CUAVD was associated with unilateral renal agenesia (Table 3). These data further support the hypothesis of an alternative etiologic mechanism for CBAVD and CUAVD in several patients.

The two new mutations detected here (ΔE115 and K1060T) are located in transmembranic domains of CFTR, where it is well known that changes affect ion-channel transport (Cheng et al. 1990). Mutation ΔE115 occurs in a codon of the first transmembrane domain, which is not conserved in several species (Tucker et al. 1992). However, the loss of a charged residue in the transmembrane domain would probably modify the conformation of the protein and alter the ion permeability of the cell. For mutation K1060T, the lysine residue is conserved in the mouse, bovine, and *Xenopus* (Tucker et al. 1992), suggesting that this position in the second transmembrane domain might be more crucial. On the other hand, some missense mutations involving charged residues in transmembrane domains are associated with milder CF phenotypes (Chillón et al. 1993; Nunes et al. 1993). Although there is no doubt about the involvement of ΔE115 and K1060T in CBAVD, the genuine clinical significance of these two new mutations cannot be established, since only one mutation was detected in each individual. Further examples of mutations ΔE115 and K1060T should provide appropriate phenotype/genotype correlations.

The identification of CFTR mutations was not limited to CBAVD patients, as mutations were also found in CUAVD individuals. Although the detection rate in this group was lower (10%) than in CBAVD, a complete analysis of CFTR in CUAVD patients has not been performed. On the other hand, 42.8% of the CUAVD patients tested have elevated sweat test values, which suggests the involvement of CFTR in some CUAVD cases. Our data are in agreement with that of Culard et al. (1994), who also detected a CFTR mutation in a CUAVD man. CFTR seems to play a critical role in spermatogenesis and in the

differentiation of the male genital tract (Trezise et al. 1993; Tizzano et al. 1993). It is unknown whether the morphogenic alterations occur during development or whether they are the result of obstructions produced by abnormal secretions caused by CFTR abnormalities. The unilateral cases of vas deferens absence associated with CFTR mutations would suggest a later involvement of the CFTR gene or a non-uniform tissue response to CFTR dysfunction.

The identification of CFTR mutations in CBAVD and CUAVD patients has important implications for genetic counseling in these individuals and constitutes an indication for the diagnostic screening of them and their female partners. This is particularly true for those cases considering in vitro fertilization after microsurgical aspiration of sperm (Silber et al. 1990), especially if the partner is also a CF carrier. Although this situation would not be very common, the severity of each CFTR allele must be known to offer appropriate genetic counseling in each case.

In conclusion, we report here the characterization of 30 CBAVD and 10 CUAVD patients for most of the CFTR gene, with the identification of mutations in 73% of CBAVD and 10% of CUAVD individuals. Most CBAVD patients (63%) are heterozygous for a CFTR mutation, but only three patients (10%) have two mutations, being probably compound heterozygous for a severe and a mild mutation. Some 27% of patients are negative for CFTR mutations, 37.5% of them with negative values for the sweat test. We also present the identification of two new CFTR mutations (K1060T and ΔE115) involved in CBAVD.

Acknowledgements We thank M. Lynch for help with the manuscript, and M. Goossens for primers for multiplex CFTR DGGE analysis. This work was supported by the Fondo de Investigaciones Sanitarias de la Seguridad Social (93/0202), the Institut Català de la Salut, and the BIOMED1 programme of the European Union. M.D.R. received a fellowship from the Fundació Pi i Sunyer.

References

Anguiano A, Oates RD, Amos JA, Dean M, Gerrard B, Stewart C, Maher TA, White MB, Milunsky A (1992) Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. JAMA 267: 1794–1797

Audrézet MP, Mercier B, Guillermit H, Quéré I, Verlingue C, Rault G, Férec C (1993) Identification of 12 novel mutations in the CFTR gene. Hum Mol Genet 2: 51–54

Boat TF, Welsh MJ, Beaudet AL (1989) Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th edn. McGraw-Hill, New York, pp 2649–2680

Casals T, Nunes V, Palacio A, Giménez J, Gaona A, Ibáñez N, Morral N, Estivill X (1993) Cystic fibrosis in Spain: high frequency of mutation G542X in the Mediterranean coastal area. Hum Genet 91: 66–70

Cheng S, Gregory R, Marshall J, Paul S, Souza D, White G, O'Riordan C, et al (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63: 827–837

Chillón M, Casals T, Nunes V, Giménez J, Pérez Ruiz E, Estivill X (1993) Identification of a new missense mutation (P205S) in the first transmembrane domain of the CFTR gene associated with a mild cystic fibrosis phenotype. Hum Mol Genet 10: 1741–1742

Chillón M, Casals T, Giménez J, Nunes V, Estivill X (1994a) Analysis of the CFTR gene in the Spanish population: SSCP-screening for 60 known mutations and identification of four new mutations (Q30X, A120T, 1812–1G->A and 3667del4). Hum Mutat 3: 223–230

Chillón M, Casals T, Giménez J, Ramos MD, Palacio A, Morral N, Estivill X, Nunes V (1994b) Analysis of the CFTR gene confirms the high genetic heterogeneity of the Spanish population: 43 mutations account for only 78% of CF chromosomes. Hum Genet 93: 447–451

Chu C-S, Trapnell BC, Curristin SM, Cutting GR, Crystal RG (1992) Extensive post-transcriptional deletion of the coding sequences for part of nucleotide-binding fold 1 in respiratory epithelial mRNA transcripts of the CFTR gene is not associated with the clinical manifestations of cystic fibrosis. J Clin Invest 90: 785–790

Claustres M, Laussel M, Desgeorges M, Giansily M, Culard JF, Razakatsara G, Demaille J (1993) Analysis of the 27 exons and flanking regions of the cystic fibrosis gene: 40 different mutations account for 91.2% of the mutant alleles in Southern France. Hum Mol Genet 8: 1209–1213

Culard JF, Desgeorges M, Costa P, Laussel M, Razakatzara G, Navratil H, Demaille J, Claustres M (1994) Analysis of the whole CFTR coding regions and splice junctions in azoospermic men with congenital bilateral aplasia of epididymis or vas deferens. Hum Genet 93: 467–470

Dean M, White M, Amos J, Gerrard B, Stewart C, Khaw KT, Leppert M (1990) Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. Cell 61: 863–870

Dumur V, Gervais R, Rigot JM, Lafitte JJ, Manouvrier S, Biserte J, Mazeman E, Roussel P (1990) Abnormal distribution of CF ΔF508 allele in azoospermic men with congenital aplasia of the epididymis and vas deferens. Lancet 336: 512

Estivill X, Ortigosa L, Pérez-Frias J, Dapena J, Ferrer J, Peña J, Peña L, Llevadot R, Nunes V, Cobos N, Vázquez C, Casals T (1995) Clinical characteristics of 16 cystic fibrosis patients with the missense mutation R334W, a pancreatic insufficiency mutation with variable age of onset and interfamilial clinical differences. Hum Genet (in press)

Fanen P, Ghanem N, Vidaud M, Besmond C, Martin J, Costes B, Plassa F, Goossens M (1992) Molecular characterization of cystic fibrosis: 16 novel mutations identified by analysis of the whole cystic fibrosis transmembrane conductance regulator (CFTR) coding regions and splice site junctions. Genomics 13: 770–776

Gervais R, Dumur V, Rigot JM, Lafitte JJ, Roussel P, Claustres M, Demaille J (1993) High frequency of the R117H cystic fibrosis mutation in patients with congenital absence of the vas deferens. N Engl J Med 328: 446–447

Gibson LE, Cooke RE (1959) A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. Pediatrics 23: 545

Heaton ND, Pryor JP (1990) Vasa aplasia and cystic fibrosis. Br J Urol 66: 538–540

Holsclaw DS, Perlmutter AD, Jockin H, Shwachman H (1971) Genital abnormalities in male patients with cystic fibrosis. J Urol 106: 568–574

Jequier AM, Ansell ID, Bullimore NJ (1985) Congenital absence of the vasa deferentia presenting with infertility. J Androl 6: 15–19

Johnson LG, Olsen JC, Sarkadi B, Moore KL, Swanstrom R, Boucher RC (1992) Efficiency of gene transfer for restoration of normal airway epithelial function in cystic fibrosis. Nature Genet 2: 21–25

Kaplan E, Shwachman H, Perlmutter AD, Rule A, Khaw K-T, Holsclaw DS (1968) Reproductive failure in males with cystic fibrosis. N Engl J Med 279: 65–69

Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui L-C (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245: 1073–1080

Morral N, Estivill X (1992) Multiplex PCR amplification of three microsatellites within the CFTR gene. Genomics 13: 1362–1364

Morral N, Nunes V, Casals T, Chillón M, Giménez J, Bertranpetit J, Estivill X (1993) Microsatellite haplotypes for cystic fibrosis: mutation frameworks and evolutionary tracers. Hum Mol Genet 7: 1015–1022

Nunes V, Chillon M, Dörk T, Tümmler B, Casals T, Estivill X (1993) A new missense mutation (E92K) in the first transmembrane domain of the CFTR gene causes a benign cystic fibrosis phenotype. Hum Mol Genet 1: 79–80

Oates RD, Amos JA (1994) The genetic basis of congenital bilateral absence of the vas deferens and cystic fibrosis. J Androl 15: 1–8

Osborne LR, Lynch M, Middleton PG, Alton EWFW, Geddes DM, Pryor JP, Hodson ME, Santis GK (1993) Nasal epithelial ion transport and genetic analysis of infertile men with congenital bilateral absence of the vas deferens. Hum Mol Genet 2: 1605–1609

Pritchard DJ (1991) Cystic fibrosis allele frequency, sex ratio anomalies and fertility: a new theory for the dissemination of mutant alleles. Hum Genet 87: 671–676

Rigot JM, Lafitte JJ, Dumur V, Gervais R, Manouvrier S, Biserte J, Mazeman E, Roussel P (1991) Cystic fibrosis and congenital absence of the vas deferens. N Engl J Med 325: 64–65

Silber SJ, Patrizio P, Asch RH (1990) Quantitative evaluation of testicular histology in men with congenital absence of the vas deferens undergoing epididymal sperm aspiration. Hum Reprod 5: 89–93

Strong TV, Smit LS, Turpin SV, Cole JL, Tom Hon C, Petty TL, Craig MW, Rosenaw EC, Tsui L-C, Iannuzzi MC, Knowles MR, Collins FS (1991) Cystic fibrosis mutation in two sisters with mild disease and normal sweat electrolyte levels. N Engl J Med 325: 1630–1634

The Cystic Fibrosis Genotype/Phenotype Consortium (1993) Correlation between genotype and phenotype in patients with cystic fibrosis. N Engl J Med 28: 1308–1313

Tizzano EF, Chitayat D, Buchwald M (1993) Cell-specific localization of CFTR mRNA shows developmentally regulated expression in human fetal tissues. Hum Mol Genet 2: 219–224

Trezise AEO, Chambers JA, Wardle CJ, Gould S, Harris A (1993) Expression of the cystic fibrosis gene in human foetal tissues. Hum Mol Genet 2: 213–218

Tsui L-C (1992) Mutations and sequence variations detected in the cystic fibrosis transmembrane conductance regulator (CFTR) gene: a report from the Cystic Fibrosis Genetic Analysis Consortium. Hum Mutat 1: 197–203

Tucker SJ, Tannahill D, Higgins CF (1992) Identification and developmental expression of the *Xenopus laevis* cystic fibrosis transmembrane conductance regulator gene. Hum Mol Genet 1: 77–82

Zielenski J, Rozmahel R, Bozon D, Kerem B, Grzelczak Z, Riordan J, Rommens JM, Tsui L-C (1991) Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics 10: 214–228