Mutations in the Cystic Fibrosis Transmembrane Regulator Gene and *In Vivo* Transepithelial Potentials

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Aim: **To examine the relationship between cystic fibrosis transmembrane regulator gene mutations (CFTR) and *in vivo* transepithelial potentials.**

Methods: **We prospectively evaluated 162 men including 31 healthy subjects, 21 obligate heterozygotes, 60 with congenital bilateral absence of the vas deferens (CBAVD) and 50 with CF by extensive CFTR genotyping, sweat chloride and nasal potential difference testing.**

Results: **Six (10%) men with CBAVD carried no CFTR mutations, 18 (30%) carried one mutation, including the 5T variant, and 36 (60%) carried mutations on both alleles, for a significantly higher rate carrying one or more mutations than healthy controls (90% versus 19%, $p < 0.001$). There was an overlapping spectrum of ion channel measurements among the men with CBAVD, ranging from values in the control and obligate heterozygote range at one extreme, to values in the CF range at the other. All pancreatic-sufficient patients with CF and 34 of 36 patients with CBAVD with mutations on both alleles carried at least one mild mutation. However, the distribution of mild mutations in the two groups differed greatly. Genotyping, sweat chloride and nasal potential difference (alone or in combination) excluded CF in all CBAVD men with no mutations. CF was confirmed in 56% and 67% of CBAVD men carrying 1 and 2 CFTR mutations, respectively.**

Conclusion: **Abnormalities of CFTR transepithelial function correlate with the number and severity of CFTR gene mutations.**

Keywords: CFTR mutations; congenital bilateral absence of the vas deferens; cystic fibrosis; nasal potential difference; sweat chloride

The heterogeneity of cystic fibrosis (CF) disease is partially explained by more than 1,300 different mutations in the CFTR gene (1). Most classically diagnosed patients with CF carry severe loss-of-function mutations on both alleles and have evidence of pancreatic insufficiency (PI) (2). Those with nonclassic CF carry a mild CFTR gene mutation on at least one allele, and usually retain sufficient residual pancreatic function to confer pancreatic sufficiency (PS) (3–6). Patients with PS are usually diagnosed in adolescence or adulthood with less severe symptoms and variable but generally milder pulmonary disease. Most men with CF are infertile due to obstructive azoospermia, which in its severest form presents as congenital bilateral absence of the vas deferens (CBAVD) (7–10). CBAVD also occurs in 1 to 2% of infertile males who are otherwise healthy, the majority of whom carry CFTR gene mutations on one or both alleles (11–20). Typically, the CFTR genotype in men with CBAVD includes at least one "mild" missense or splice variant. Similar combinations of CFTR gene mutations have been observed in patients with other CFTR-associated phenotypes, including idiopathic chronic pancreatitis (21–23) and chronic sinusitis (24), but the frequency of gene mutations in these patients is lower than in men with CBAVD.

The relation between the number and severity of CFTR gene mutations and the degree of CFTR-mediated dysfunction of transepithelial transport has not been comprehensively evaluated in men with CBAVD. We explored this association by prospectively evaluating men with CBAVD as well as healthy males, CF obligate heterozygotes, and men with CF disease. Some results of this study have been reported in the form of an abstract (25).

METHODS

Subject Selection and Study Design

The institutional human ethics committees approved the study. Informed, written consent was provided by all men who were prospectively ascertained over the same time period. The healthy men had no family history of CF, nor evidence of pulmonary or pancreatic disease. Men with CBAVD were ascertained after the diagnosis was confirmed by physical examination, transrectal ultrasound, and evidence of azoospermia on two separate occasions. Obligate heterozygotes were fathers or adult male siblings of patients with CF whose carrier status had been confirmed by genotyping. All men with CF had been previously diagnosed by characteristic clinical manifestations and confirmatory diagnostic tests. Exocrine pancreatic function status was defined according to previously described methods (2, 4).

Nasal Potential Difference and Sweat Chloride

Nasal transepithelial potential difference (PD) was performed as described by Knowles and colleagues (26), by a single operator (L.E.) who was masked to the test results of the men with CBAVD. Maximum PD (Max PD) was the highest basal measurement. The positive change in PD after superfusion with amiloride (ΔAmil) was the difference in PD from beginning the infusion. CFTR-mediated chloride diffusion potential was determined by first perfusing with amiloride and a chloride-free solution (ΔCl^--free) followed by isoproterenol (ΔCl^--free + Iso). Because the diagnostic reference limits for nasal PD have not been established, we used ΔCl^--free + Iso values below the lower 99% confidence limits for controls (−7.65 mV) as a conservative indicator of CF disease (27). Sweat chloride tests were performed by the Gibson and Cooke method (28) on the same day as the PD measurements. Sweat chloride concentrations were interpreted as normal (< 40 mmol/L), borderline (40–60 mmol/L), or abnormal (> 60 mmol/L).

Genotype Analysis

Extensive CFTR genotype analysis was performed using multiplex heteroduplex and sequencing analyses as previously described (29). The polythymidine tract (5T, 7T, or 9T) in the acceptor splice site of intron

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8 was also characterized (30). The number of adjacent TG repeats was evaluated in subjects who carried the 5T variant (31, 32). Throughout the article, the term "CF-causing mutations" refers to the 24 CF disease–causing mutations listed in the 1998 consensus statement on the diagnosis of CF (33), whereas the term "CFTR mutations" is used to refer to all mutations including 5T. The term "incidental heterozygotes" is used to define healthy control subjects who were found to carry a CFTR mutation on one allele. The term "severe" is used to describe loss-of-function mutations that are associated with "classic" CF; most patients with severe mutations on both alleles are pancreatic insufficient. In contrast, the term "mild" describes any mutation that retains, or is predicted to retain, some residual CFTR function. Most patients carrying at least one "mild" mutation are pancreatic sufficient and are considered to have "nonclassic CF."

Statistical Analysis

The relative similarities or differences in transepithelial transport characteristics of the different groups were demonstrated using box plots arranged along the horizontal axis according to the value of the median. Confidence intervals for medians were computed as a function of the quartiles and, as such, are robust to the presence of outliers. Independent proportions were compared using Fischer's exact test. Differences between two group medians were tested using the Mann-Whitney test. All p values are two-sided. Control subjects with identified CFTR mutations were excluded from all statistical comparisons and figures.

RESULTS

Subjects

One hundred and sixty-two men were prospectively ascertained, including 31 healthy control subjects, 21 obligate heterozygotes, 60 with CBAVD, and 50 with a conventional diagnosis of CF. The men with infertility were enrolled from 86 consecutive men diagnosed with CBAVD.

Genotype Analysis

Six of 31 (19%) healthy control subjects carried seven mutations in the CFTR gene (Table 1), which is similar to the findings of Bombieri and coworkers (34). One control subject carried mutations on both alleles, but had a normal sweat test and nasal PD. The clinical significance of one of his mutations (R75Q) remains uncertain. Ninety percent of men with CBAVD were found to carry at least one CFTR gene mutation and/or the 5T variant, which accounted for 90 mutant alleles in 120 (75%) chromosomes. The frequency of mutations among the men with CBAVD was significantly greater than that observed in the healthy control subjects ($p < 0.001$). Six (10%) men with CBAVD carried no CFTR mutation (CBAVD-0), 18 (30%) had one mutation (CBAVD-1), whereas 36 (60%) carried mutations on both alleles (CBAVD-2). Seven of 18 (39%) and 30 of 36 (83%) men in the CBAVD-1 and CBAVD-2 groups, respectively, carried at least one CF-causing mutation, but only two men in the CBAVD-2 group carried published CF-causing mutations on both alleles (33). The 5T variant was found in 22 of 60 (37%) men with CBAVD, which was significantly greater than the number (3%) observed in the healthy control subjects ($p < 0.001$). It is noteworthy that 9 of 53 (17%) patients with CBAVD of known ethnic background and who carried one or two CFTR gene mutations originated from the Far East (n = 4), Indian subcontinent (n = 2), and the Middle East (n = 3).

The nature and distribution of mutations varied considerably between the patient groups. Twenty of 26 (77%) CF-PI patients, but only 9 of 24 (38%) of the CF-PS patients carried published CF disease–causing mutations on both alleles (33). All CF-PS patients with identified mutations on both alleles, as well as 34 of 36 men in the CBAVD-2 group, carried at least one "mild" CFTR mutation. One exception, a man in the CBAVD-2 group, carried ΔF508 with a rare missense mutation (S549R), which has been observed in a CF-PI patient (35). The same genotype, ΔF508 in combination with 2789+5 G→A, was observed in both a patient in the CBAVD group and a patient in the CF-PI group. Most individuals in the CBAVD and CF-PS groups carried severe CF-causing mutations on one allele but the nature of the second mild mutation differed considerably between the two groups. Only two individuals in the CF-PS and CBAVD-2 groups carried a combination of the same mutations on both alleles (ΔF508/P67L). In keeping with a previous report (36), all 13

TABLE 1. CFTR GENE MUTATIONS IN THE PATIENT GROUPS

Control Subjects (n = 31)	Heterozygotes (n = 21)	CBAVD-1 (n = 18)	CBAVD-2 (n = 36)	CF-PS (n = 24)	CF-PI (n = 26)
G542X*/R75Q	ΔF508*/− (n = 16)	ΔF508* (n = 6)	ΔF508*/2789+5G→A*	ΔF508*/R117H [5T]* (n = 4)	ΔF508*/ΔF508* (n = 11)
ΔF508*	ΔF508*/5T	W1282X*/5T (n = 8)	R334W*/R334W*	ΔF508*/A455E* (n = 2)	ΔF508*/G542X* (n = 2)
G542X*	W1282X*/− (n = 2)	D1152H†	ΔF508*/R117H [7T] (n = 10)	ΔF508*/3849+10kbC→T* (n = 2)	ΔF508*/G551D* (n = 2)
R117H[7T]	G85E†/R75Q	L206W†	ΔF508*/R117C [7T]	G551D*/3849+10kbC→T*	ΔF508*/621+1G→T* (n = 2)
S431G	R75Q/−	A198P	G551D*/R117H [7T]	ΔF508*/3272–26A→G† (n = 2)	ΔF508*/2789+5 G→A*
5T			ΔF508*/5T (n = 8)	ΔF508*/P574H† (n = 2)	ΔF508*/W1282X*
			G542X*/5T	ΔF508*/I1234V†	ΔF508*/G85E*
			W1282X*/5T	ΔF508*/P67L†	ΔF508*/L1077P† (n = 2)
			ΔF508*/P67L†	ΔF508*/R347H†	G551D*/G480C†
			ΔF508*/L206W†	ΔF508*/5T	ΔF508*/− (n = 2)
			ΔF508*/M952T†	ΔF508*/875+1G→C†	−/−
			ΔF508*/S549R†	G551D*/R75Q	
			A455E*/L206W†	ΔF508*/− (n = 2)	
			621+G→T*/R117C [7T]	A455E*/−	
			R117H [7T]/5T	ΔI507*/−	
			R117L[7T]/5T	−/−	
			R117H/R117H [7T/7T]		
			D979A/5T		
			5T/-741T→G		
			4016insT†/D110H		

Definition of abbreviations: CBAVD = congenital bilateral absence of the vas deferens; CF-PI = pancreatic-insufficient cystic fibrosis; CF-PS = pancreatic-sufficient cystic fibrosis.

* Cystic fibrosis–causing mutations (30).

† Mutations not listed in the consensus document, which subsequently fulfilled the criteria as CF disease causing.

TABLE 2. THE NUMBER OF TG REPEATS IN SUBJECTS CARRYING THE 5T POLYTHYMIDINE VARIANT

		Number of TG Repeats			
Diagnosis	n*	TG11	TG12	TG13	TG14
Control	1	1	0	0	0
Obligate heterozygotes	1	1	0	0	0
CBAVD-1	5	1	2	2	0
CBAVD-2	11	1	6	3	1
CF-PS	1	0	1	0	0
Total	19	4	9	5	1

Definition of abbreviations: CBAVD = congenital bilateral absence of the vas deferens; CF-PS = pancreatic-sufficient cystic fibrosis.

* Results not available for six men (CBAVD-1 [n = 3], CBAVD-2 [n = 3]), because the DNA was denatured.

patients with CBAVD carrying the R117H allele cosegregated with the 7T variant, whereas the R117H allele was associated with the 5T variant in all four CF-PS patients. The frequency of TG repeats adjacent to the 5T variant for 19 subjects with available results is summarized in Table 2. The control subject and CF obligate heterozygote each carried TG11 on the same allele as 5T. In contrast, all but two patients with CBAVD (5 with CBAVD-1 and 11 with CBAVD-2) and the patient with pancreatic-sufficient CF disease carried a higher frequency of TG repeats (TG12, TG13 or TG14) on the same allele as the 5T variant.

Sweat Chloride and Nasal PD Measurements

Transepithelial transport measurements in each patient group are summarized in Table 3, with the healthy subjects with and without CFTR gene mutations shown separately. The reference range in Figures 1 and 2 are based on the 25 control subjects without identified CFTR gene mutations. These figures are arranged by group medians to permit clearer visualization of the similarities and differences among the groups. Groups with closely contiguous values are similar, whereas the degree of separation of values shows the level of disparity among groups. Sweat chloride concentrations in the CBAVD-0 group (Figure 1A) were contiguous with those seen in control subjects and similar to the CF obligate heterozygotes, but significantly lower than the values observed in the CBAVD-1 and CBAVD-2 groups ($p = 0.04$ and $p = 0.0008$, respectively). Values in the CBAVD-1 group were higher than those in the heterozygote group ($p = 0.01$) but not significantly different from those of the CBAVD-2 group. Similarly, the CF-PS patients showed intermediate sweat chloride values between the CF-PI group ($p < 0.0001$) at one extreme and the CBAVD-1 ($p < 0.0001$) and CBAVD-2 patients ($p < 0.0001$) at the other.

Max PD (Figure 1B) demonstrated contiguity of the control, heterozygote, CBAVD-0, and CBAVD-1 groups, whereas the CBAVD-2 group had intermediate values between the control subjects ($p < 0.0001$) and heterozygotes ($p < 0.0001$) at one extreme and the CF-PS and CF-PI patients ($p = 0.006$ and $p < 0.0001$, respectively) at the other. Similar results were obtained with basal PD (not shown).

Response to amiloride (Figure 1C) showed the CBAVD-0 group aligning closely with the control subjects and obligate heterozygotes, whereas the CBAVD-1 group showed a slightly greater response than the control subjects ($p = 0.01$). In contrast, the amiloride response in the CBAVD-2 group, which was similar to the CF-PS group ($p = 0.33$), showed a significantly greater change than that in the control subjects ($p < 0.0001$), CBAVD-0 patients ($p = 0.005$), and heterozygotes ($p = 0.0005$), and a significantly lesser change than that in the CF-PI patients ($p < 0.0001$).

Stimulated chloride diffusion potential (ΔCl^--free + Iso) in the CBAVD-0 group (Figure 1D) aligned with the heterozygotes ($p = 0.84$). The response in the CBAVD-1 group, while significantly different from control subjects and heterozygotes ($p < 0.0001$ and $p = 0.0003$, respectively), was only marginally different from the CBAVD-2 group ($p = 0.08$). The CBAVD-2 group showed an intermediate response between the obligate heterozygotes ($p < 0.0001$) and the patients with CF ($p < 0.0001$). Unlike the observations for sweat chloride and amiloride, ΔCl^--free + Iso in the CF-PS and CF-PI patients was not significantly different ($p = 0.08$). Similar results were observed with ΔCl^--free (not shown).

Figure 2 shows the combined results of amiloride and chloride diffusion potential. The median value for the CBAVD-0 group was not significantly different from the control or heterozygote groups, which were marginally different from each other ($p = 0.07$). All other groups were significantly different from each other (all p values ≤ 0.004).

Diagnosis of CF

Figure 3 shows individual sweat chloride measurements plotted against stimulated nasal chloride conductance (ΔCl^--free + Iso). The reference limits for both tests are shown to identify patients with the following: normal sweat chloride and nasal PD values (*bottom left quadrant*), abnormal sweat chloride and nasal PD (*top right quadrant*), normal sweat test but abnormal nasal PD (*bottom right quadrant*), and abnormal sweat test but normal nasal PD (*top left quadrant*). Sweat chloride and nasal PD values in the three CBAVD groups and the reference groups show

TABLE 3. SWEAT CHLORIDE AND NASAL POTENTIAL DIFFERENCE MEASUREMENTS IN PATIENT GROUPS

				Nasal Potential Difference (*mV*)				
Group	n	Age (*yr*)	Sweat Chloride (*mmol/L*)	Max PD	ΔAmil	ΔCl^--free	ΔCl^--free + Iso	ΔAmil + Cl^--free + Iso
Control	25	30 ± 6	20 ± 11	−24 ± 8	13 ± 4	−15 ± 9	−29 ± 10	−16 ± 12
Incidental Heterozygote	6	24 ± 2	34 ± 16	−26 ± 9	15 ± 6	−12 ± 4	−27 ± 3	−12 ± 5
CF heterozygote	21	37 ± 10	26 ± 13	−22 ± 6	14 ± 5	−11 ± 10	−23 ± 10	−9 ± 9
CBAVD-0	6	33 ± 3	22 ± 9	−25 ± 7	12 ± 4	−8 ± 6	−22 ± 10	−9 ± 8
CBAVD-1	18	37 ± 6	44 ± 23	−28 ± 11	19 ± 7	−5 ± 5	−12 ± 7	+7 ± 10
CBAVD-2	36	34 ± 5	54 ± 23	−35 ± 9	23 ± 10	−2 ± 3	−8 ± 7	+15 ± 11
CF-PS	24	32 ± 10	73 ± 21	−44 ± 13	27 ± 12	+3 ± 5	+2 ± 5	+28 ± 13
CF-PI	26	20 ± 9	102 ± 14	−54 ± 9	36 ± 8	+3 ± 4	+4 ± 5	+40 ± 7

Definition of abbreviations: Amil = amiloride; CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CF-PI = pancreatic-insufficient cystic fibrosis; CF-PS = pancreatic-sufficient cystic fibrosis; Iso = isoproterenol; PD = potential difference.

Values are mean ± SD.

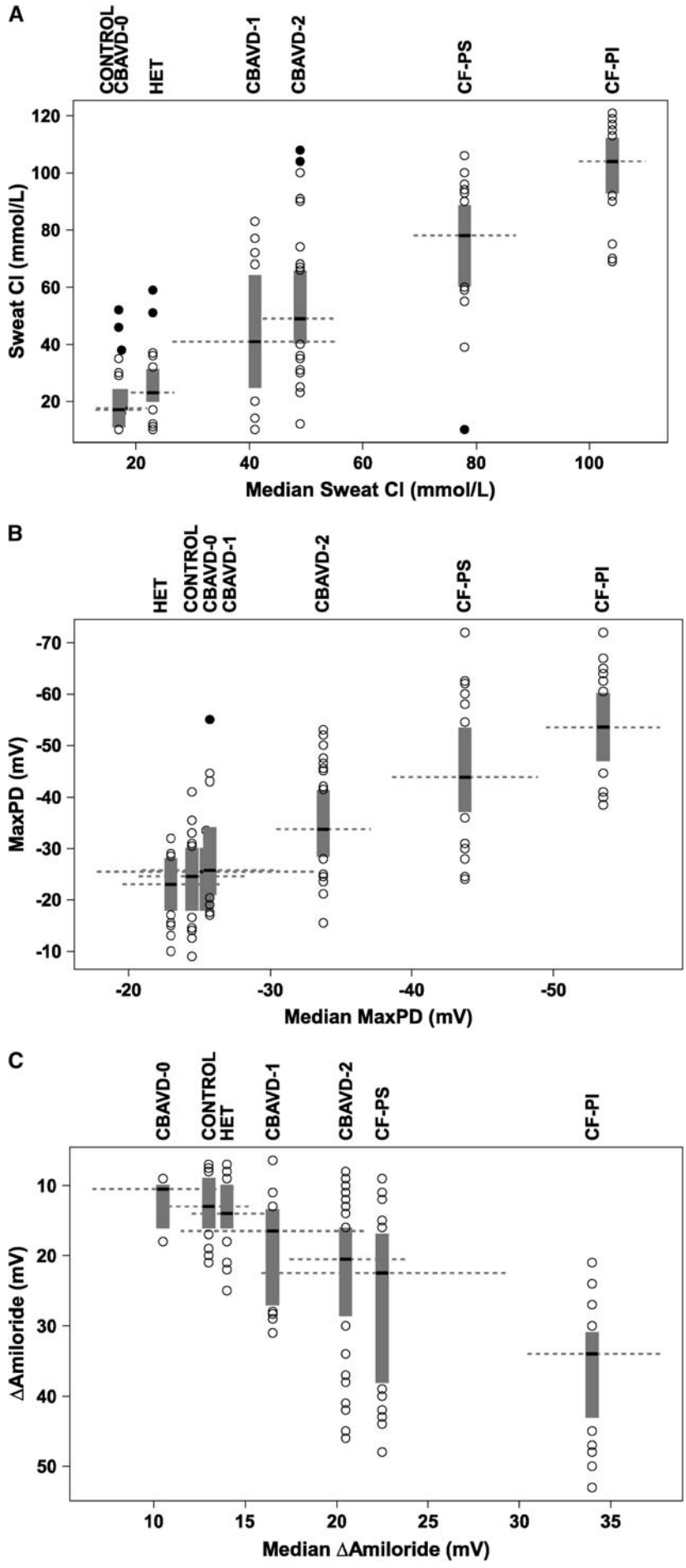

Figure 1. The range and variability of each measurement, and the relationship of each group with one another, are demonstrated by arranging *box plots* for each group along the *x axis* according to the value of the median. Each *box plot* represents values within the 25th to 75th percentiles (interquartile range). Values outside the 25th and 75th percentile are represented by *circles*. Outliers, values that are more than 1.5 × interquartile range above and below the 75th and 25th percentiles, respectively, are represented by *solid circles*. *Horizontal dashed lines* depict the 95% confidence intervals for the medians of each group. (*A*) Sweat chloride, (*B*) maximum basal potential difference (MaxPD), (*C*) amiloride inhibition of sodium transport, (*D*) chloride-free and isoproterenol–stimulated chloride diffusion potential. *See* text for interpretation of results. The reference range for the control subjects is based on 25 healthy men with no identified CFTR gene mutations. CBAVD = congenital bilateral absence of the vas deferens; CF-PI = pancreatic-insufficient cystic fibrosis; CF-PS = pancreatic-sufficient cystic fibrosis; HET = heterozygotes.

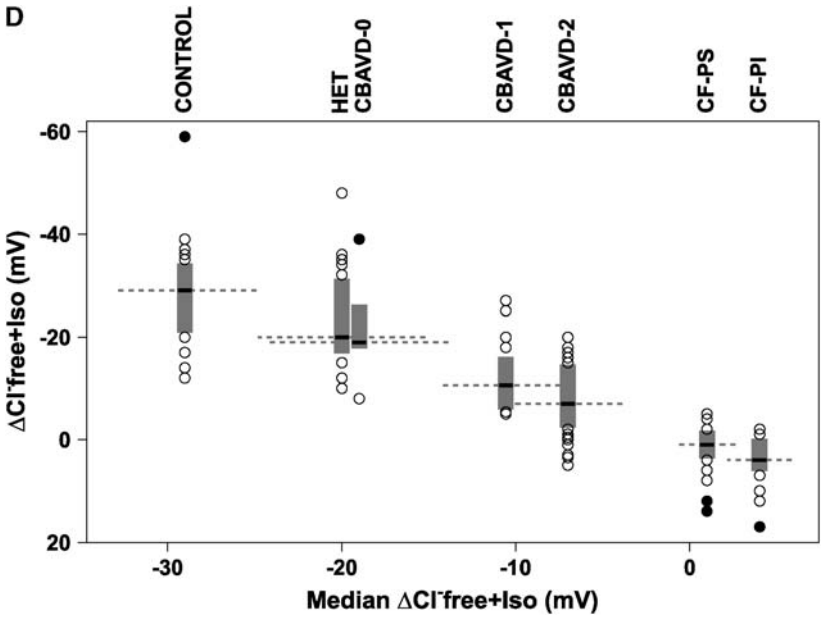

Figure 1. (*Continued*).

a wide spectrum of values. All sweat chloride and nasal PD measurements for patients in the CBAVD-0 group were normal (*bottom left quadrant*). In contrast, a large number of patients in the CBAVD-1 and CBAVD-2 groups fell within the abnormal range for sweat chloride alone (n = 8), nasal PD alone (n = 17), or sweat chloride and nasal PD (n = 9). This figure also illustrates differences in the ion channel measurements between the CF-PS and CF-PI patient groups. Sweat chloride and PD measurements in all the CF-PI patients were abnormal (*right upper quadrant*), whereas values for the CF-PS patients tended to be lower and merged with those obtained in the CBAVD-1 and CBAVD-2 subgroups.

Among several patients with outlying results, a healthy man had a borderline sweat chloride result (52 mmol/L), but no CFTR gene mutations were identified and nasal PD measurements were normal. A second sweat test also showed a borderline result (52 mmol/L). A CF-PS patient who carried the 3849+10kbC→T mutation on one allele had a normal sweat chloride result but the nasal PD measurement was within our established range for CF disease.

As shown in Table 4, all patients with a prior diagnosis of CF fulfilled the diagnostic criteria for CF on the basis of one or more diagnostic tests (33). Sweat chloride testing alone would have detected all CF-PI patients, but there was a false-negative rate of 21% in the CF-PS group. Nasal PD testing alone confirmed the diagnosis of CF in all CF-PS and CF-PI patients. Nasal PD was normal in all healthy control subjects (with and without CFTR gene mutations) and obligate heterozygotes. On the basis of the same criteria, 57% of the men with CBAVD would be considered to carry a diagnosis of CF. None of the patients in the CBAVD-0 group carried a diagnosis of CF, but at least one test supported a diagnosis of CF in 56 and 67% of

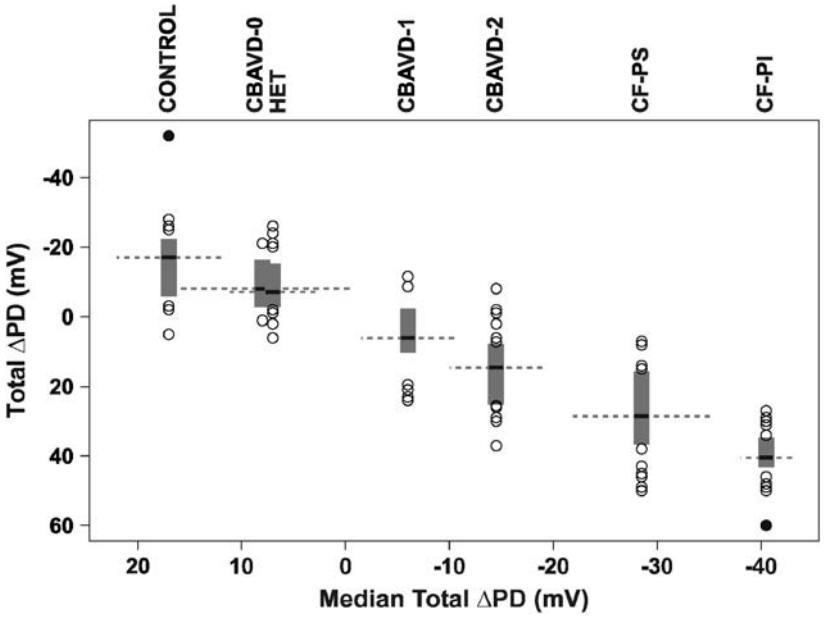

Figure 2. The combined effects of sodium and chloride diffusion potential (ΔAmil + Cl^--free + Iso) are demonstrated. The values on the *x axis* represent the change in potential difference (ΔPD) from the basal measurement immediately before commencing perfusion with amiloride to the final plateau that is achieved after perfusion with the chloride-free solution and isoproterenol. The range and variability of each measurement and the relationship of each group with one another are shown as in Figure 1. The patient groups show a continuum of values, which appear to reflect the combined consequences of sodium and chloride transport. The reference range for the control subjects is based on 25 healthy men without identified CFTR gene mutations.

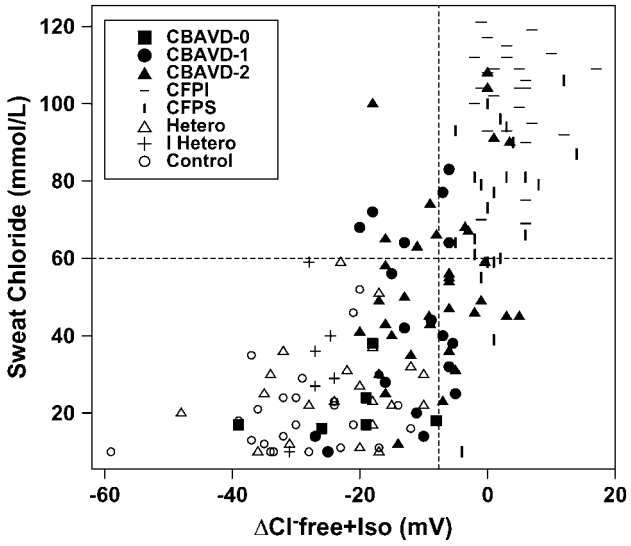

Figure 3. Individual sweat chloride measurements are plotted against stimulated nasal chloride conductance (ΔCl^--free + Iso). The reference limits for both tests are illustrated to identify patients with: normal sweat chloride and nasal PD values (*bottom left quadrant*), abnormal sweat chloride and nasal PD (*top right quadrant*), normal sweat test but abnormal nasal PD (*bottom right quadrant*), and abnormal sweat test but normal nasal PD (*top left quadrant*). The incidental heterozygotes (I Hetero) are distinguished from the control subjects without identified CFTR gene mutations. *See* text for details.

patients in the CBAVD-1 and CBAVD-2 groups, respectively. On the basis of stringent criteria for designating CF-causing mutations (Table 4), genotyping alone carried the lowest diagnostic sensitivity. This conclusion remained unchanged when additional mutations that have been subsequently designated as CF disease causing were included (Table 1)

DISCUSSION

This comprehensive, prospective study of healthy males and men with a variety of CFTR-associated disorders demonstrates a wide spectrum of CFTR-mediated abnormalities of transepithelial transport, which shows a relation to the number and functional severity of CFTR gene mutations. Our investigations were limited to men, because in comparison with other CF-associated conditions, such as idiopathic pancreatitis and chronic sinusitis, men with CBAVD carry by far the highest frequency of mutations in the CFTR gene. The men with CBAVD showed a wide range of transepithelial measurements, which correlated with the number of identified CFTR mutations and overlapped with those in the healthy control subjects and obligate heterozygotes at one extreme, and those in the CF-PS and CF-PI patients on the other.

Osborne and colleagues (37) found normal basal PD in nine patients with CBAVD, but some showed an abnormal response to perfusion with low chloride. Stimulated chloride diffusion potential was not done and extensive mutation analysis was not evaluated. Pradal and coworkers (38) identified abnormal chloride diffusion potential in 3 of 12 patients with CBAVD but extensive CFTR gene mutation analysis was not performed. Dohle and colleagues (39) examined electrophysiologic function in rectal biopsies of 21 men with CBAVD. However, CFTR genotyping was limited to the 10 most common CF-causing mutations and the 5T variant. More recently, Hirtz and colleagues (40) evaluated CFTR-mediated chloride secretion in rectal biopsies from patients with CF with a spectrum of mutations. Although these investigators did not ascertain patients with CF-associated phenotypes, such as CBAVD, they did demonstrate residual CFTR- mediated chloride channel function in patients with mild mutations.

Extensive mutation analysis fails to identify mutations in 4% of patients with a conventional diagnosis of CF (29). The limitations of this methodology, which is shared by DNA sequencing, include an inability to detect mutations in the noncoding and promoter regions of CFTR. Although it is possible that some men with CBAVD carry undetected mutations, we found that 90% of men with CBAVD carried a CFTR gene mutation on one or two alleles. This is consistent with previous reports (11–17), including our own (18–20). A wide variety of mutations were represented in the men with CBAVD; 37 of 120 (31%) alleles were from the 24 CF-causing mutations listed in the consensus document and 35 were identified at least once in the CF group. However, there were differences in the nature and distribution of mutations between patients with CBAVD and the conventionally diagnosed patients with CF. All but one patient in the CF-PI group with identified mutations carried severe CF-causing mutations on both alleles. The single exception carried the splice mutation 2789+5 G→A, which has been associated with a mild phenotype (35). In contrast, all but two males in the CBAVD-2 group and all men in the CF-PS group with identified mutations on both alleles carried at least one mild CFTR mutation. Only 2 of 36 (6%) of the CBAVD-2 patients carried published CF-causing mutations on both alleles, whereas 47% of the CF-PS

TABLE 4. DIAGNOSIS OF CYSTIC FIBROSIS BY DISEASE-CAUSING MUTATIONS, SWEAT CHLORIDE, AND NASAL POTENTIAL DIFFERENCE

Group	n	Two Published CF Disease–causing Mutations,* n (%)	Sweat Chloride > 60 mmol/L, n (%)	ΔCl^--free + Iso < −7.65 mV, n (%)	Any Test, n (%)
Control	31	0 (0)	0 (0)	0 (0)	0 (0)
CF heterozygote	21	0 (0)	0 (0)	0 (0)	0 (0)
All CBAVD	60	2 (3)	17 (28)	26 (43)	34 (57)
CBAVD-0	6	0 (0)	0 (0)	0 (0)	0 (0)
CBAVD-1	18	0 (0)	6 (33)	7 (39)	10 (56)
CBAVD-2	36	2 (6)	11 (31)	19 (53)	24 (67)
CF-PS	24	9 (38)	19 (79)	24 (100)	24 (100)
CF-PI	26	20 (77)	26 (100)	26 (100)	26 (100)

Definition of abbreviations: Amil = amiloride; CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CF-PI = pancreatic-insufficient cystic fibrosis; CF-PS = pancreatic-sufficient cystic fibrosis; Iso = isoproterenol.

* Based on the 1998 consensus statement on the diagnosis of CF (33).

men with identified mutations on both alleles carried two proven CF-causing mutations (33). The differing distribution of "mild" mutations between the CF-PS and CBAVD patients probably accounts for the distinguishable, but overlapping ion transport measurements in these groups. For example, the 7T variant, which allows more efficient splicing than 5T, was associated with the mild missense R117H mutation in all men with CBAVD, whereas R117H was associated with the less-efficient 5T splice variant in all the CF-PS patients (41). Although the number of subjects was too small to permit statistical analysis, the number of TG repeats adjacent to the 5T splice variant did appear to influence the phenotypic penetrance (31, 32). Although the single control and the obligate heterozygote both carried TG11 with 5T, all but two patients with CBAVD and the patient with CF with the PS phenotype carried TG12, TG13, or TG14 on the same allele as the 5T polythymidine variant. Similarly, other mild CFTR mutations identified in this study are likely to have variable degrees of transepithelial transport. Taken together, this probably accounts for the fact that genotypes associated with men with CBAVD are rarely observed in conventionally diagnosed patients with CF with the PS phenotype.

Of all the organs that are pathologically affected by reduced functional CFTR, the male reproductive tract is the most sensitive to very minor deficiencies of function. This is likely due to a number of factors, including the length, tortuosity, and narrow lumen of the vas deferens; the high concentration of intraluminal macromolecules; and dependence on CFTR for ion transport and fluid secretion (42). The fact that most CBAVD patients carrying mutations on both alleles show no disease or mild manifestations in other CF-affected organs adds indirect support for the exquisite sensitivity of the male genital tract to minor derangements of CFTR function (43–48). CBAVD is the severest form of obstructive abnormalities involving the male reproductive tract. Milder forms of obstructive azoospermia, such as epididymal obstruction and even oligospermia, are also associated with a higher than expected frequency of CFTR gene mutations and polymorphisms. However, mutations are hardly ever found on both alleles. Patients with primary testicular failure do not have a higher frequency of CFTR gene mutations (48). Even the polymorphism M740V in exon 10, which is known to produce less functional CFTR, may be sufficient to predispose patients to a mild form of obstructive azoospermia (45). In such circumstances, other factors, including polymorphisms in the noncoding regions of CFTR, modifier genes elsewhere in the human genome, or possibly extraneous environmental factors, may contribute to disease pathogenesis.

Nasal PD has been advocated as a diagnostic tool for patients with CF with unusual manifestations and a method for assessing new therapies directed at correcting CFTR function (26, 49). We provide evidence that men with CBAVD and CF-PS have increased amiloride-sensitive sodium transport. Although this effect could be due to absence of a parallel anion shunt, this observation indirectly suggests that mild or moderate reductions in functional CFTR could influence amiloride-dependent sodium transport (50). The functional consequences of the various CFTR gene mutations on both sodium and CFTR-mediated chloride transport are illustrated in Figure 2, which shows a sequential continuum of values among the various patient groups.

The diagnosis of CF requires the presence of at least one characteristic manifestation of CF, plus CF-causing mutations on each allele, or evidence of abnormal chloride diffusion potential in the sweat gland or the nasal epithelium (33). On the basis of these criteria, 34 of 60 (57%) men with CBAVD in this study were predicted to carry a diagnosis of CF by at least one diagnostic test. CF was confirmed in all the patients with CF and excluded in all healthy control subjects and obligate heterozygotes. However, the results of the nasal PD method should be interpreted with caution because this technique has neither been standardized nor validated for clinical use. For this reason, we opted to use a conservative measure of abnormal chloride permeability for establishing a diagnosis of CF. In this regard, only 9 of 26 men with CBAVD who met the criteria for a CF diagnosis on the basis of abnormal nasal PD had an abnormal sweat test alone. Furthermore, the overlapping measurements between the men with CBAVD and the healthy control and disease control reference groups underscore the challenges of definitively excluding or establishing a diagnosis of CF disease. Longitudinal monitoring of patients in whom conflicting nasal PD and sweat chloride results are obtained will allow further refinements to the diagnostic criteria.

This study also demonstrates the poor sensitivity of CFTR genotyping alone as a diagnostic tool of CF disease when limited to the mutations listed in the 1998 consensus document (33). Only two men with CBAVD and 38% of the CF-PS patients met the diagnostic criteria for CF on the basis of the 24 published CF disease–causing mutations. This general observation remains unchanged with the addition of mutations that have been designated as "CF disease causing" by anecdotal observations since the consensus document was published.

To conclude, CFTR-mediated transepithelial transport is closely correlated with the number and severity of CFTR gene mutations. Furthermore, the overlapping spectra of measurements in men with CBAVD range from values observed in healthy control subjects and obligate heterozygotes at one extreme to those observed in men with CF-PS and CF-PI at the other. The poor sensitivity of genotyping as well as the overlapping transepithelial transport measurements illustrate the difficulties of establishing or excluding a definitive diagnosis of CF among subjects with CFTR-associated phenotypes. Finally, these observations underscore the importance of offering appropriate genetic counseling to men with obstructive azoospermia, as well as extensive evaluation for evidence of CF disease of both partners, before embarking on epididymal sperm aspiration for *in vitro* fertilization.

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