

VARIANT CYSTIC FIBROSIS PHENOTYPES IN THE ABSENCE OF *CFTR* MUTATIONS

JOSHUA D. GROMAN, M.S., MICHELLE E. MEYER, M.S., ROBERT W. WILMOTT, M.D., PAMELA L. ZEITLIN, M.D., PH.D., AND GARRY R. CUTTING, M.D.

**ABSTRACT**

**Background** Cystic fibrosis is a life-limiting autosomal recessive disorder with a highly variable clinical presentation. The classic form involves characteristic findings in the respiratory tract, gastrointestinal tract, male reproductive tract, and sweat glands and is caused by loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Nonclassic forms of cystic fibrosis have been associated with mutations that reduce but do not eliminate the function of the *CFTR* protein. We assessed whether alteration in *CFTR* function is responsible for the entire spectrum of variant cystic fibrosis phenotypes.

**Methods** Extensive genetic analysis of the *CFTR* gene was performed in 74 patients with nonclassic cystic fibrosis who had been referred by 34 medical centers. We evaluated two families that each included a proband without identified mutations and a sibling with nonclassic cystic fibrosis to determine whether there was linkage to the *CFTR* locus and to measure the extent of *CFTR* function in the sweat gland and nasal epithelium.

**Results** Of the 74 patients studied, 29 had two mutations in the *CFTR* gene, 15 had one mutation, and 30 had no mutations. A final genotype of two mutations was more common among patients who had been referred after screening for common cystic fibrosis-causing mutations identified one mutation than among those who had been referred after screening had identified no such mutations (26 of 34 patients vs. 3 of 40 patients,  $P < 0.001$ ). Comparison of clinical features and sweat chloride concentrations revealed no significant differences among patients with two, one, or no *CFTR* mutations. Haplotype analysis in the two families revealed no linkage to *CFTR*. Although each of the affected siblings had elevated sweat chloride concentrations, measurements of cyclic AMP-mediated ion and fluid transport in the sweat gland and nasal epithelium demonstrated the presence of functional *CFTR*.

**Conclusions** Factors other than mutations in the *CFTR* gene can produce phenotypes clinically indistinguishable from nonclassic cystic fibrosis caused by *CFTR* dysfunction. (N Engl J Med 2002;347:401-7.)

Copyright © 2002 Massachusetts Medical Society.

CYSTIC FIBROSIS has been recognized as a distinct clinical entity for more than 60 years.<sup>1</sup> The observation that affected patients have excessive salt loss<sup>2</sup> was a major milestone in the diagnosis of cystic fibrosis, leading to the development of the sweat test.<sup>3</sup> The advent of this biochemical test facilitated the diagnosis in patients with a wide range of phenotypes, including older children and adults who had evidence of clinical disease in only a subgroup of the organ systems involved in classic cystic fibrosis. These forms are termed “nonclassic cystic fibrosis” and account for at least 10 percent of cases.<sup>4-6</sup> Genetic-linkage studies<sup>7-11</sup> mapped both classic and nonclassic forms to a single locus on chromosome 7. Subsequently, mutations causing loss of function of the cystic fibrosis transmembrane conductance regulator (*CFTR*) protein were identified in each *CFTR* gene in patients with classic cystic fibrosis, whereas mutations that reduced but did not eliminate *CFTR* function were identified in patients with nonclassic forms.<sup>12,13</sup>

To further our understanding of the role of *CFTR* dysfunction in the development of the cystic fibrosis phenotype, we extensively analyzed *CFTR* genes in 74 patients with nonclassic cystic fibrosis who were referred for confirmatory genetic diagnosis. Each patient had had either one or no mutations identified on screening for common cystic fibrosis-causing alleles. We also studied linkage to the *CFTR* locus and *CFTR* function in two families that each included two siblings with nonclassic cystic fibrosis.

**METHODS****Patient Population**

All studies were approved by the Johns Hopkins Joint Committee on Clinical Investigation, and written informed consent was obtained from all participants or their parents. Seventy-four patients were referred to the Cystic Fibrosis Foundation Genotyping Center from 1998 to 2002 by 34 centers that specialize in the care of patients with cystic fibrosis. Patients with a nonclassic phenotype were enrolled if they had either one mutation or none (but

From the McKusick-Nathans Institute of Genetic Medicine and Cystic Fibrosis Foundation Genotyping Center (J.D.G., M.E.M., G.R.C.), the Predoctoral Training Program in Human Genetics (J.D.G.), and the Department of Pediatrics (P.L.Z., G.R.C.), Johns Hopkins University School of Medicine, Baltimore; and the Department of Pediatrics, Saint Louis University School of Medicine, St. Louis (R.W.W.). Address reprint requests to Dr. Cutting at Childrens Medical Surgical Center 9-125, 600 N. Wolfe St., Baltimore, MD 21287, or at gcutting@jhmi.edu.

not two mutations) identified by a screening test that can detect at least 80 percent of common cystic fibrosis-causing alleles.<sup>4</sup> A nonclassic phenotype was defined by the presence of two or three (but not all four) of the following: sweat-gland dysfunction (as evidenced by a sweat chloride concentration of more than 40 mmol per liter in a sample of at least 100 mg of sweat induced by means of pilocarpine iontophoresis<sup>3</sup>); cystic fibrosis-related respiratory disease, defined by the presence of sputum cultures positive for *Pseudomonas aeruginosa*, bronchiectasis, chronic productive cough, documented pneumonia, reactive airway disease, abnormal results on pulmonary-function tests, chronic sinusitis, and nasal polyposis, alone or in combination; malformation of the vas deferens on the basis of semen analysis, physical examination, or both; and gastrointestinal disease, as defined by fecal-fat values in excess of 7 g per day during a 72-hour period, rectal prolapse, or recurrent episodes of pancreatitis. Gastrointestinal disease was diagnosed on the basis of the finding of reduced malabsorption and steatorrhea after pancreatic enzyme supplementation in two patients and by the finding of reduced immunoreactive serum trypsinogen levels and clinical evidence of malabsorption in one patient.

### CFTR Genotyping

Point mutations make up approximately 98.5 percent of reported mutations of the *CFTR* gene, and genomic rearrangements account for the remaining 1.5 percent of mutations.<sup>14</sup> Thus, to detect mutations in *CFTR* that were not identified by the standard screening test, we used DNA sequencing optimized for the detection of heterozygous point mutations. For each patient, 31 polymerase-chain-reaction (PCR) products were generated from genomic DNA covering all 27 exons of the gene and 2 intronic mutations known to cause abnormal splicing (3849+10kbC→T and 1811+1.6kbA→G). Bidirectional dye-primer labeled-DNA sequencing was performed on each amplicon (Applied Biosystems), and the resulting sequences were analyzed by automated capillary electrophoresis (ABI-310 or ABI-3100 Genetic Analyzer, Applied Biosystems). All exonic and bordering intronic sequences (25 bp before each exon and 15 bp after each exon) were analyzed independently by two reviewers who were unaware of subjects' identities, using Factura software (Applied Biosystems) and the Sequencher analysis program (Gene Codes). The chromatographs were visually inspected if mutations were not identified by the preceding analyses. Samples from 41 of the 45 patients who did not have two mutations identified after this analysis underwent bidirectional sequencing of the *CFTR* basal promoter (290 bp upstream of the translation starting site) to identify potential regulatory mutations.

Southern blotting was performed to detect rearrangements in genomic DNA in 25 of the 45 patients who did not have two detectable mutations after DNA sequencing. Total genomic DNA was digested with *Sst*I, *Nhe*I, *Kpn*I, or *Bgl*II; separated on 0.5 percent agarose gels; transferred to nitrocellulose filters; and hybridized with probes randomly labeled with phosphorus-32. Probes were derived from the pBQ4.7 plasmid containing *CFTR* complementary DNA (cDNA).

To determine the validity of our sequencing method, we also evaluated nine subjects whose genotypes had previously been determined (five normal subjects, two carriers of cystic fibrosis, and two patients with classic cystic fibrosis) in a blinded fashion and seven unrelated patients with classic cystic fibrosis (four had one previously identified common cystic fibrosis allele and three had no common cystic fibrosis alleles).

### Family Studies

Haplotype studies were performed in two families (Families 1 and 2) that each included two affected siblings to determine whether the *CFTR* locus was linked to the disease phenotype. Five microsatellite markers (D7S523, D7S486, IVS17bTA, D7S480, and D7S490) in a 5-cM region encompassing the *CFTR* locus were

typed.<sup>15</sup> Markers were amplified by PCR with one unlabeled primer and one labeled primer (6-FAM [6-carboxyfluorescein]). Sizes were estimated against an internal standard (TAMRA-500 [6-carboxytetramethylrhodamine]) with capillary electrophoresis (ABI-310, Applied Biosystems) and GeneScan software (Applied Biosystems).

### CFTR Functional Studies

Sweat rates for each of the four affected siblings in Families 1 and 2 were calculated at the Johns Hopkins Hospital.<sup>16</sup> Sweat rates were measured in the forearms of each subject over a 20-minute period after the subcutaneous injection of 0.3 ml of Ringer's solution containing isoproterenol (50  $\mu$ mol per liter) and aminophylline (5 mmol per liter to increase intracellular cyclic AMP), and atropine sulfate (140  $\mu$ mol per liter) to block cholinergic stimulation. We also compared results to the mean ( $\pm$ SD) values in 30 healthy volunteers and in 30 patients with cystic fibrosis.<sup>16</sup>

The nasal potential difference was measured in the two affected siblings in each family at Children's Hospital Medical Center in Cincinnati and Children's Hospital of the University of Alabama at Birmingham with the use of procedures described by Knowles et al.<sup>17</sup> Mean ( $\pm$ SD) values were calculated in 10 healthy control subjects and 10 patients with cystic fibrosis who were evaluated at Johns Hopkins Hospital.<sup>18</sup> These values were similar to those previously reported<sup>17</sup> and to values recorded for healthy controls and patients with cystic fibrosis at the centers in Cincinnati and Birmingham.

### Analysis of CFTR Transcripts

Epithelial cells from the inferior nasal turbinates were collected with a brush (CytoSoft, Medical Packaging) from Subject II-2 in Family 1 and Subjects II-2 and II-4 in Family 2. RNA was extracted with the use of RNeasy B (Tel-Test) according to the manufacturer's directions, and cDNA was synthesized with the use of reverse-transcriptase (ProSTAR Ultra HF RT-PCR system, Stratagene). *CFTR* cDNA was amplified with the use of five sets of *CFTR*-specific primers and sized with the use of agarose-gel electrophoresis to check for splice variants. The entire *CFTR* cDNA from each patient was sequenced in both directions to identify mutations introduced after transcription and to confirm the presence of polymorphic variants in the cDNA.

### Statistical Analysis

Chi-square tests were used to compare the frequencies of recurrent cough and wheezing, pseudomonas infection, nasal polyposis, steatorrhea, and congenital absence of the vas deferens according to the genotype. The sweat chloride concentrations, the forced expiratory volume in one second (FEV<sub>1</sub>), age at diagnosis, and age at referral were compared according to genotype with the use of one-way analysis of variance. The JMP statistical package (version 3.2.2, SAS Institute) was used for all statistical analyses.

## RESULTS

The clinical presentation, sweat chloride concentrations, and the number of mutations identified by screening for common *CFTR* mutations at referral are shown in Table 1 for the 74 patients with nonclassic cystic fibrosis. Approximately half the patients had elevated sweat chloride concentrations ( $\geq$ 40 mmol per liter) and clinical manifestations limited to the respiratory tract, and about one third had clinical symptoms in more than one organ system. There was no difference in the number of organ systems involved or in the mean sweat chloride concentrations between

patients with one mutation and those with no mutations at referral (Table 1).

The nine blinded samples used to validate the sequencing assays were correctly genotyped, and a *CFTR* mutation was identified on each of the 14 chromosomes from the seven patients with classic cystic fibrosis. A total of 73 mutant alleles were discovered on 148 chromosomes from patients with nonclassic cystic fibrosis (Table 2). Thirty-four of the mutant alleles were previously detected by common mutation screens, and the remaining 39 were detected by DNA-sequence analysis (Table 2). Two novel missense mutations at evolutionarily conserved sites and a novel splice-altering mutation were found (Table 2). No promoter mutations or genomic rearrangements were identified.

We were initially surprised by the low rate of detection of mutations. However, when we reexamined the patients' genotypic distribution, we realized that the majority of patients had either two mutations (29 patients) or no mutations (30 patients) (Table 3), and only 15 patients had a single *CFTR* mutation. Moreover, the rates of detection were significantly higher among patients who were referred after the identification of a common cystic fibrosis-causing mutation than among those in whom no common cystic fibrosis-causing mutation had been identified (Table 3). We also reviewed the clinical features of each patient in an attempt to find phenotypic differences between those with *CFTR* mutations and those without such mutations. This analysis revealed that patients with one or more *CFTR* mutations could not be distinguished clinically from those with no *CFTR* mutations

TABLE 1. CHARACTERISTICS OF THE 74 PATIENTS AT REFERRAL.

SWEAT CHLORIDE CONCENTRATION AND No. OF MUTATIONS IDENTIFIED*	ORGAN SYSTEM INVOLVED†			
	RESPIRATORY TRACT	GASTRO-INTESTINAL TRACT	GENITO-URINARY TRACT	>1 ORGAN SYSTEM
	no. of patients			
<40 mmol/liter				
1 Mutation	0	0	0	5
0 Mutations	0	0	0	6
40–60 mmol/liter				
1 Mutation	9	4	1	4
0 Mutations	7	0	0	4
>60 mmol/liter				
1 Mutation	7	1	1	2
0 Mutations	16	4	0	3

\*Patients were screened for common mutations.

†Evidence of organ-system involvement is defined in the Methods section.

TABLE 2. MUTATIONS IDENTIFIED BY SCREENING FOR COMMON MUTATIONS AND DNA-SEQUENCE ANALYSIS IN PATIENTS WITH NONCLASSIC CYSTIC FIBROSIS.

MUTATION IDENTIFIED BY SCREENING FOR COMMON MUTATIONS	MUTATION IDENTIFIED BY DNA SEQUENCING	No. OF PATIENTS
ΔF508	5T*	3
ΔF508	D1152H	2
ΔF508	2789+2insA	2
ΔF508	R117C	2
ΔF508	D110H	1
ΔF508	2789+5G→A	1
ΔF508	P205S	1
ΔF508	L967S	1
ΔF508	I1027T	1
ΔF508	L206W	1
ΔF508	T1053I and 5T	1
ΔF508	V920M and 5T	1
ΔF508	R1070W	1
ΔF508	D579G	1
ΔF508	P67L	1
ΔF508	2811G→T††	1
G85E	F191V†	1
R117H	G103X and 5T	1
I148T	I556V	1
G542X	R1162L	1
W1282X	D1152H	1
None	L138ins and 3272-26 A→G	1
None	G463D† and 5T	1
None	F693L and 5T	1
ΔF508	None	6
G551D	None	1
W1282X	None	1
None	5T	4
None	2307insA	1
None	L997F	1
None	V520I	1
None	None	30

\*5T refers to a mutation in the noncoding sequence of thymidines in intron 8 that results in five thymidines and thus to a decreased proportion of normal *CFTR* messenger RNA.<sup>19</sup>

†This is a novel mutation.

‡This mutation creates a novel splice-site donor, and the mutation was confirmed by reverse-transcriptase-PCR of RNA from nasal epithelium (data not shown).

(Table 4). Furthermore, the degree of multiorgan-system involvement in the sweat gland, gastrointestinal tract, and respiratory system was similar among patients with zero, one, or two *CFTR* mutations (Table 4).

Two patients without identified mutations had a sibling who had been given a diagnosis of nonclassic cystic fibrosis (Fig. 1 and Table 5). Sequence analysis of DNA from these siblings revealed one mutation (5T) in Subject II-4 in Family 2 and no mutations

**TABLE 3. MUTATION-DETECTION RATES AND FINAL GENOTYPES FOR PATIENTS IN WHOM SCREENING FOR COMMON CYSTIC FIBROSIS-CAUSING MUTATIONS IDENTIFIED ONE MUTATION OR NO MUTATIONS.**

GENOTYPE AT REFERRAL	FINAL GENOTYPE			MUTATION-DETECTION RATE*
	2	1	0	
	MUTATIONS MUTATION MUTATIONS			
	no. of patients			percent
1 Common <i>CFTR</i> mutation	26	8	—	76 (26 of 34 chromosomes)
0 Common <i>CFTR</i> mutations	3	7	30	16 (13 of 80 chromosomes)

\*The rate was based on the number of mutations detected by DNA sequencing of alleles that were not identified as having mutations by screening for common mutations ( $P < 0.001$ , 1 df, by the chi-square test).

**TABLE 4. CLINICAL PHENOTYPES ACCORDING TO THE FINAL GENOTYPE.**

VARIABLE	FINAL GENOTYPE			P VALUE
	2 <i>CFTR</i> MUTATIONS	1 <i>CFTR</i> MUTATION	0 <i>CFTR</i> MUTATIONS	
No. of organ systems involved (no. of patients/total no.)*				0.82†
2	23/29	13/15	25/30	
3	6/29	2/15	5/30	
Sweat chloride concentration‡				0.33§
Mean $\pm$ SE (mmol/liter)	62.42 $\pm$ 4.0	55.41 $\pm$ 5.6	54.40 $\pm$ 3.9	
No. of patients	29	15	30	
FEV <sub>1</sub> ¶				0.47§
Mean $\pm$ SE (% of predicted value)	80.69 $\pm$ 5.4	91.00 $\pm$ 6.6	86.70 $\pm$ 5.4	
No. of patients	21	14	21	
Recurrent cough and wheezing (no. of patients/total no.)	21/29	11/15	25/30	0.56†
Pseudomonas infection (no. of patients/total no.)	9/29	3/15	3/30	0.12†
Nasal polyposis (no. of patients/total no.)	5/29	4/15	5/30	0.71†
Documented steatorrhea (no. of patients/total no.)**	2/13	2/7	9/18	0.12†
Congenital absence of vas deferens (no. of patients/total no.)††	6/7	2/5	1/3	0.14†
Age at referral				0.06§
Mean $\pm$ SE (yr)	24.64 $\pm$ 2.7	16.27 $\pm$ 3.8	16.06 $\pm$ 2.7	
No. of patients	29	15	30	
Age at diagnosis				0.30§
Mean $\pm$ SE (yr)	16.88 $\pm$ 2.7	12.86 $\pm$ 3.7	10.90 $\pm$ 2.8	
No. of patients	25	13	23	

\*Organ systems include sweat gland, gastrointestinal, genitourinary, and respiratory systems.

†A chi-square test with 2 df was used.

‡Values obtained with pilocarpine iontophoresis; there were two or more measurements for all but eight patients.

§One-way analysis of variance was used.

¶Values were unavailable for 11 patients who were younger than six years old and for 7 adult patients.

||*Pseudomonas aeruginosa* infection was documented on the basis of positive sputum cultures.

\*\*Tests for steatorrhea were performed only in patients with clinical evidence of malabsorption.

††Congenital absence of the vas deferens was determined by semen analysis, scrotal examination, or both. Not all male patients were examined.

in Subject II-2 in Family 1. We then performed a linkage test using DNA markers encompassing the *CFTR* gene and demonstrated that the affected siblings in Families 1 and 2 had inherited different haplotypes encompassing the *CFTR* gene (Fig. 1). In Family 2, an unaffected sibling (Subject II-3) inher-

ited the same haplotype as an affected sibling (Subject II-4) (Fig. 1). Thus, linkage analysis suggested that the *CFTR* gene was not responsible for the nonclassic phenotype in these families.

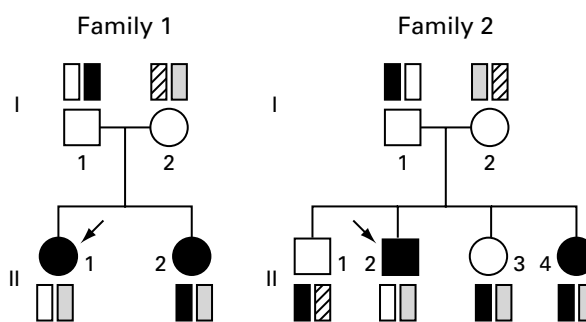
Our next step was to determine whether *CFTR* was functional in these patients. Each of the four siblings

had elevated mean sweat chloride concentrations, consistent with the presence of an electrolyte-transport defect in the duct of the sweat gland (Table 5). However, cyclic AMP-mediated sweat production, which is absent in patients whose cystic fibrosis is due to *CFTR* dysfunction, was in the normal range in all four siblings. Furthermore, measurement of ion and fluid transport across respiratory epithelium of the nasal turbinate revealed values distinct from those for patients with two *CFTR* mutations. Interestingly, both siblings in Family 1 had reduced responses to the sodium-channel blocker amiloride. Finally, *CFTR* cDNA amplified from nasal epithelium of Subject II-2 in Family 1 and Subjects II-2 and II-4 in Family 2 revealed no evidence of aberrant splicing, and sequencing of the cDNA failed to reveal alterations in RNA (Table 5). Furthermore, each of the polymorphisms detected in the coding sequence of genomic DNA from Subjects II-2 and II-4 in Family 2 were also seen in the cDNA sequence, confirming that RNA transcripts from both *CFTR* genes were present in these subjects.

#### DISCUSSION

Although classic cystic fibrosis is a monogenic disorder resulting from sequence variants in the *CFTR* gene,<sup>4,7,11</sup> mutations in *CFTR* have also been found in patients with nonclassic cystic fibrosis phenotypes.<sup>12,13,19,20</sup> The identification of mutations in *CFTR* in a large fraction of our patients confirms the involvement of this gene in the nonclassic phenotype. Indeed, each of the 29 patients with two identified *CFTR* mutations had at least one mutation that was predicted to be associated with residual *CFTR* function, consistent with the hypothesis that nonclassic cystic fibrosis can be caused by at least one mild mutation.<sup>21</sup> Those with only one *CFTR* mutation may harbor a second mutation that was not detectable with the methods that we used. Alternatively, a single *CFTR* mutation in combination with specific alleles of other genes or an unfavorable environment may produce a nonclassic phenotype. This possibility is supported by numerous studies indicating that persons with a single mutation in *CFTR* are at higher risk than persons in the general population for a clinical feature of cystic fibrosis.<sup>22-27</sup>

A surprising result of our study was the large proportion of patients with symptoms of cystic fibrosis who did not have *CFTR* mutations. Although some mutations may have been missed, several lines of evidence suggest that undetected mutations would not explain our findings. First, the analytic validity of the DNA-sequencing technique for the detection of mutations in the heterozygous state was 100 percent in our blinded sample from nine subjects and in samples from the seven subjects with classic cystic fibrosis. We



**Figure 1.** Pedigree of Two Families in Which *CFTR* Was Not Linked to the Nonclassic Phenotype.

Bars indicate the haplotypes. Square symbols indicate male family members, circles female family members, and solid symbols family members with clinical features of nonclassic cystic fibrosis. Arrows indicate the probands in each family.

observed the same level of sensitivity when analyzing 35 carriers of  $\beta$ -thalassemia, and 51 carriers of X-linked adrenoleukodystrophy.<sup>28</sup> Second, if mutations were missed, we would expect these unidentified alleles to be distributed equally between patients who were referred with a common cystic fibrosis-causing mutation and those who did not have such a mutation. Instead, the rates of detection of mutations were five times as high in patients with a common mutation. Finally, the nonclassic phenotype was not linked to the *CFTR* gene in Families 1 and 2, and the sweat rates and nasal potential-difference values were consistent with normal *CFTR* function.

Taken together, these data suggest that factors other than *CFTR* dysfunction can cause the nonclassic phenotype. A previous report described a patient with a nonclassic phenotype in whom no *CFTR* mutations could be found.<sup>29</sup> Although we cannot rule out the intriguing possibility of an environmental cause, our identification of affected siblings with similar clinical and electrophysiological features suggests that a genetic basis is likely. Of course, the ultimate proof of a genetic cause is the identification of the causative gene or genes.

Patients with *CFTR* mutations could not be distinguished from those without *CFTR* mutations on the basis of the sweat chloride concentration or clinical presentation, highlighting the need for tests that differentiate patients with *CFTR* dysfunction from those with cystic fibrosis from other causes. Screening for common *CFTR* mutations can be a valuable tool in evaluating the role of *CFTR* in the nonclassic phenotype. Our data suggest that the presence of a single common mutation predicts the presence of a second disease-causing mutation and that the absence of a

**TABLE 5.** CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF SUBJECTS II-1 AND II-2 OF FAMILY 1 AND SUBJECTS II-2 AND II-4 OF FAMILY 2.

CHARACTERISTIC	FAMILY 1		FAMILY 2	
	SUBJECT II-1	SUBJECT II-2	SUBJECT II-2	SUBJECT II-4
<b>Clinical</b>				
Current age (yr)	9	12	10	14
Age at diagnosis (yr)	7	10	9	13
Pulmonary symptoms	Chronic cough, dyspnea, orthopnea, sinusitis	Chronic cough, wheezing	Recurrent cough	None
FEV <sub>1</sub> (% of predicted)	120	100	106	94
Forced vital capacity (% of predicted)	85	89	108	90
Steatorrhea*	No (2.3)	No (2.1)	Borderline (6.0)	Yes (9.0)
Other	Nasal polyposis, abdominal pain, rectal prolapse	Abdominal pain	<i>Staphylococcus aureus</i> in sputum culture	—
<b>Biochemical and molecular</b>				
Sweat chloride concentration (mmol/liter)†				
Mean	42	50	75	64
Range	26–58	38–64	73–78	52–73
Cyclic AMP–mediated sweat rate (mg/20 min)‡	2.4	3.8	2.3	2.6
Nasal potential difference (mV)§				
At base line	–12.5	–14.3	–22.0	–22.0
After amiloride	2.3	2.0	9.0	7.0
After amiloride, low-chloride buffer, and isoproterenol	–13.3	–7.3	–28.0	–22.0
Transcript analysis¶	NA	Normal	Normal	Normal

\*Results of 72-hour fecal-fat tests (expressed as grams per day) are provided in parentheses. Steatorrhea was defined by a value of more than 7.0 g per day, and borderline status was defined by a value between 6.0 and 7.0 g per day.

†Values were obtained with pilocarpine iontophoresis.

‡The mean (±SD) sweat rate was 4.0±2.2 mg per 20 minutes in 30 healthy controls and 1.3±0.8 mg per 20 minutes in 30 patients with cystic fibrosis.

§The mean base-line value was –18±7.9 mV in 10 healthy controls and –36.3±5.2 mV in 10 patients with cystic fibrosis. The mean value after the administration of amiloride was 8.3±5.9 and 20.2±5.4, respectively. The mean value after the administration of amiloride, low-chloride buffer, and isoproterenol was –15.8±6.0 and 5.2±2.0, respectively.

¶No splice variants or disease-causing mutations were detected in *CFTR* complementary DNA from nasal epithelium. NA indicates that RNA was not available for analysis.

common mutation predicts normal *CFTR* status. There were, however, several exceptions to these generalizations (Table 3), suggesting that great care must be taken to ensure that extensive genotypic analysis is conducted before the diagnosis of nonclassic cystic fibrosis without *CFTR* mutations is made. Methods of measuring *CFTR* function other than pilocarpine iontophoresis (e.g., with the use of nasal potential-difference values and cyclic AMP–mediated sweat rate) may also be helpful in distinguishing *CFTR* dysfunction from other causes of the nonclassic phenotype.

The recognition and characterization of different causes of cystic fibrosis should facilitate the discovery of new pathways important for disease pathophysiology. This strategy may be particularly informative for the study of cystic fibrosis, since there is considerable controversy regarding the exact mechanism by which defective *CFTR* causes disease.<sup>30–32</sup> Of particular interest is whether the defective pathways in these patients involve *CFTR* or act independently of *CFTR*. Further study of our patients may reveal additional pathways contributing to cystic fibrosis phe-

notypes, and these pathways may ultimately prove more amenable to therapeutic intervention than *CFTR* itself.

Supported by grants from the Cystic Fibrosis Foundation and the National Institutes of Health (to Dr. Cutting) and by a Clinical Research Center Grant from the National Institutes of Health (RR00052, to Dr. Zeitlin).

*We are indebted to J.P. Clancy and V. Kociela for assistance with nasal potential-difference measurements; to L. Brass-Ernst and S. Watts for assistance with the calculation of the sweat rates; to M. Boyle, C. Merlo, R. Enke, and M. Couch for assistance with nasal brushings; to C. Yurk, J. Kirk, and the Johns Hopkins DNA Diagnostic Laboratory for technical assistance; to B. Karczeski for assistance with patient enrollment and the collection of clinical data; to P. Campbell, B. Rosenstein, T. Holtzman, W. Guggino, R. McWilliams, and A. Hamosh for their critique of the manuscript; to the clinicians and staff of the cystic fibrosis centers who referred patients; and most of all, to the patients and families for their willingness to participate in this study.*

## REFERENCES

- Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease: a clinical and pathologic study. *Am J Dis Child* 1938;56:344–99.
- di Sant'Agnese PA, Darling RC, Perera GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas: clinical significance and relationship to the disease. *Pediatrics* 1953;12:549–63.

3. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics* 1959;23:545-9.
4. Welsh MJ, Ramsey BW, Accurso F, Cutting GR. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic & molecular bases of inherited disease*. 8th ed. Vol. 3. New York: McGraw-Hill, 2001: 5121-88.
5. Cystic Fibrosis Foundation Patient Registry annual data report 1999. Bethesda, Md.: Cystic Fibrosis Foundation, September 2000.
6. Cutting GR. Cystic fibrosis. In: Rimoin DL, Connor JM, Peyeritz RE, eds. *Emery and Rimoin's principles and practice of medical genetics*. 3rd ed. Vol. 2. New York: Churchill-Livingstone, 1997:2685-717.
7. Tsui LC, Buchwald M, Barker D, et al. Cystic fibrosis locus defined by a genetically linked polymorphic DNA marker. *Science* 1985;230:1054-7.
8. Tsui LC, Buchwald M. No evidence for genetic heterogeneity in cystic fibrosis. *Am J Hum Genet* 1988;42:184.
9. McConkie-Rosell A, Chen Y-T, Harris D, et al. Mild cystic fibrosis linked to chromosome 7q22 markers with an uncommon haplotype. *Ann Intern Med* 1989;111:797-801.
10. Kerem BS, Buchanan JA, Durie P, et al. DNA marker haplotype association with pancreatic sufficiency in cystic fibrosis. *Am J Hum Genet* 1989;44:827-34.
11. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066-73. [Erratum, *Science* 1989;245:1437.]
12. Kristidis P, Bozon D, Corey M, et al. Genetic determination of exocrine pancreatic function in cystic fibrosis. *Am J Hum Genet* 1992;50:1178-84.
13. Highsmith WE, Burch LH, Zhou Z, et al. A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. *N Engl J Med* 1994;331:974-80.
14. Cystic fibrosis mutation data base. Toronto: Hospital for Sick Children, 2001. (Accessed July 15, 2002, at <http://www.genet.sickkids.on.ca>.)
15. Dreesen JC, Jacobs LJ, Bras M, et al. Multiplex PCR of polymorphic markers flanking the *CFTR* gene: a general approach for preimplantation genetic diagnosis of cystic fibrosis. *Mol Hum Reprod* 2000;6:391-6.
16. Callen A, Diener-West M, Zeitlin PL, Rubenstein RC. A simplified cyclic adenosine monophosphate-mediated sweat rate test for quantitative measure of cystic fibrosis transmembrane regulator (*CFTR*) function. *J Pediatr* 2000;137:849-55.
17. Knowles MR, Paradiso AM, Boucher RC. In vivo nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. *Hum Gene Ther* 1995;6:445-55.
18. Rubenstein RC, Zeitlin PL. A pilot clinical trial of oral sodium-4-phenylbutyrate (Buphenyl) in  $\Delta F508$  homozygous cystic fibrosis patients. *Am J Respir Crit Care Med* 1998;157:484-90.
19. Noone PG, Pue CA, Zhou Z, et al. Lung disease associated with the IVS8 5T allele of the *CFTR* gene. *Am J Respir Crit Care Med* 2000;162: 1919-24.
20. Gan K-H, Veeze HJ, van den Ouweland AMW, et al. A cystic fibrosis mutation associated with mild lung disease. *N Engl J Med* 1995;333:95-9.
21. Zielenski J. Genotype and phenotype in cystic fibrosis. *Respiration* 2000;67:117-33.
22. Mak V, Zielenski J, Tsui LC, et al. Proportion of cystic fibrosis gene mutations not detected by routine testing in men with obstructive azoospermia. *JAMA* 1999;281:2217-24.
23. Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 1998;339:653-8.
24. Miller PW, Hamosh A, Macek M Jr, et al. Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 1996;59:45-51.
25. Marchand E, Verellen-Dumoulin C, Mairesse M, et al. Frequency of cystic fibrosis transmembrane conductance regulator gene mutations and 5T allele in patients with allergic bronchopulmonary aspergillosis. *Chest* 2001;119:762-7.
26. Wang XJ, Moylan B, Leopold DA, et al. Mutation in the gene responsible for cystic fibrosis and predisposition to chronic rhinosinusitis in the general population. *JAMA* 2000;284:1814-9.
27. Bronsveld I, Bijman J, Mekus F, Ballmann M, Veeze HJ, Tümmler B. Clinical presentation of exclusive cystic fibrosis lung disease. *Thorax* 1999; 54:278-81.
28. Boehm CD, Cutting GR, Lachtermacher MB, Moser HW, Chong SS. Accurate DNA-based diagnostic and carrier testing for X-linked adrenoleukodystrophy. *Mol Genet Metab* 1999;66:128-36.
29. Mekus F, Ballmann M, Bronsveld I, et al. Cystic-fibrosis-like disease unrelated to the cystic fibrosis transmembrane conductance regulator. *Hum Genet* 1998;102:582-6.
30. Guggino WB. Cystic fibrosis and the salt controversy. *Cell* 1999;96: 607-10.
31. Wine JJ. The genesis of cystic fibrosis lung disease. *J Clin Invest* 1999; 103:309-12.
32. Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant *CFTR*-dependent  $\text{HCO}_3^-$  transport in mutations associated with cystic fibrosis. *Nature* 2001;410:94-7.

Copyright © 2002 Massachusetts Medical Society.

---

#### IMAGES IN CLINICAL MEDICINE

---

The *Journal* welcomes consideration of new submissions for Images in Clinical Medicine. Instructions for authors and procedures for submissions can be found on the *Journal's* Web site at <http://www.nejm.org>. At the discretion of the editor, images that are accepted for publication may appear in the print version of the *Journal*, the electronic version, or both.

---