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W. Edward Highsmith, Lauranell H. Burch, Zhaoqing Zhou, John C. Olsen ...+8 more authors

Institutions: University of North Carolina at Chapel Hill, Cincinnati Children's Hospital Medical Center, Duke University, North Shore-LIJ Health System ...+1 more institutions

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A NOVEL MUTATION IN THE CYSTIC FIBROSIS GENE IN PATIENTS WITH PULMONARY DISEASE BUT NORMAL SWEAT CHLORIDE CONCENTRATIONS

W. Edward Highsmith, Ph.D., Lauranell H. Burch, M.S., Zhaoqing Zhou, Ph.D., John C. Olsen, Ph.D., Thomas E. Boat, M.D., Alexander Spock, M.D., Jack D. Gorvoy, M.D., Lynne Quittell, M.D., Kenneth J. Friedman, Lawrence M. Silverman, Ph.D., Richard C. Boucher, M.D., and Michael R. Knowles, M.D.

Abstract *Background.* Many patients with chronic pulmonary disease similar to that seen in cystic fibrosis have normal (or nondiagnostic) sweat chloride values. It has been difficult to make the diagnosis of cystic fibrosis in these patients because no associated mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene has been identified.

Methods. We evaluated 23 patients with pulmonary disease characteristic of cystic fibrosis but with sweat chloride concentrations in the normal range. Mutations in the *CFTR* gene were sought by direct sequencing of polymerase chain reaction–amplified nasal epithelial messenger RNA and by testing the functioning of affected epithelium.

Results. A cytidine phosphate guanosine dinucleotide C-to-T point mutation in intron 19 of the *CFTR* gene, termed 3849+10 kb C to T, was identified in 13 patients from eight unrelated families. This mutation was found in patients from three different ethnic groups with three different extended haplotypes. The mutation leads to the creation of a partially active splice site in intron 19 and to the insertion into most *CFTR* transcripts of a new 84-base-pair "exon," containing an in-frame stop codon, between exons 19 and 20. Normally spliced transcripts were also detected at a level approximately 8 percent of that found in normal subjects. This mutation is associated with abnormal nasal epithelial and sweat acinar epithelial function.

Conclusions. We have identified a point mutation in intron 19 of *CFTR* and abnormal epithelial function in patients who have cystic fibrosis–like lung disease but normal sweat chloride values. The identification of this mutation indicates that this syndrome is a form of cystic fibrosis. Screening for the mutation should prove diagnostically useful in this population of patients. (N Engl J Med 1994; 331:974-80.)

CYSTIC fibrosis is an autosomal recessive genetic disorder that reflects mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene,[1-3] which codes for a cyclic AMP (cAMP)–regulated chloride channel.[4,5] The disorder has a broad range of severity but has traditionally been manifested by chronic pulmonary disease, pancreatic exocrine insufficiency, elevated concentrations of chloride in sweat, and identified mutations, most commonly ΔF_{508}.[3,6]

There is a group of patients, however, who have chronic lung disease similar to that seen in cystic fibrosis but who have normal sweat chloride values.[6] These patients have no common mutation in the *CFTR* gene detectable by conventional genetic analysis of genomic DNA and thus present a diagnostic problem. To evaluate the diagnosis of cystic fibrosis in such patients, we characterized the physiologic functioning of epithelium in affected organs and sought mutations in the *CFTR* gene by direct sequencing of messenger RNA (mRNA) transcripts obtained from nasal epithelium and amplified by the polymerase chain reaction (PCR).

From the Applied Technology Genetics Corp., Malvern, Pa. (W.E.H.); the Department of Medicine (L.H.B., Z.Z., J.C.O., R.C.B., M.R.K.) and the Division of Molecular Pathology (Z.Z., K.J.F., L.M.S.), University of North Carolina, Chapel Hill; Children's Hospital Medical Center, Cincinnati (T.E.B.); the Department of Pediatrics, Duke University, Durham, N.C. (A.S.); Schneider Children's Hospital–Long Island Jewish Medical Center, New Hyde Park, N.Y. (J.D.G.); and the Pediatric Pulmonary Division, Columbia–Presbyterian Medical Center, New York (L.Q.). Address reprint requests to Dr. Knowles at the Division of Pulmonary Diseases, 724 Burnett-Womack Bldg., CB 7020, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7020.

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Methods

Patients

We evaluated 23 patients in 13 families who had disease resembling cystic fibrosis (obstructive airways disease and characteristic microbiologic contents of sputum, i.e., *Staphylococcus aureus, Pseudomonas aeruginosa,* or both) but who had sweat chloride values[7] in the nondiagnostic or normal range[8] (<60 mmol per liter in patients older than 15 years and <40 mmol per liter in patients 15 years old or younger, calculated as the median of at least three determinations). Pancreatic exocrine function was assessed by the measurement of stool fat during a 72-hour period (normal, <7 g per day per 100 g of fat ingested) or by clinical features and the measurement of serum vitamin E (normal, 5 to 20 mg per milliliter of solution), serum trypsinogen (normal, 10 to 57 ng per milliliter of solution), or both. Each patient was screened for 29 different mutations of *CFTR*.[9] The study protocol was approved by the institutional review board of the University of North Carolina, and informed consent was obtained.

Identification and Characterization of the Mutation in Intron 19

Total RNA was prepared by the method of Chirgwin et al.[10] or the Ultraspect system (Biotecx, Houston) from nasal epithelial cells (5×10^5) obtained by scraping the inferior turbinates with a Rhinoprobe.[11] For the sequence analysis, reverse transcription was performed with *CFTR*-specific primers and avian moloney virus reverse transcriptase (Red Module, Invitrogen, San Diego, Calif.). The complementary DNA (cDNA) was amplified (for 35 cycles) to yield segments of cDNA of approximately 1 kilobase (kb). Each first-round PCR product was reamplified (for 35 cycles) with nested primers, resulting in overlapping products of 400 to 600 base pairs (bp). The second-round products were purified on low-melting-temperature agarose gels; single-stranded DNA was prepared by asymmetric PCR[12] and purified by two rounds of spin dialysis (Centricon 100, Amicon, Beverly, Mass.). Dideoxy sequencing was done with Sequenase (United States Biochemical, Cleveland).

The region of *CFTR* cDNA spanning exon 18 to exon 20 was amplified with a primer complementary to bases 3552 to 3596 (exon 18) and a primer complementary to the junction of exons 20 and 21 (bases 3991 to 4009). After electrophoresis in composite gels consist-

ing of 3 percent NuSieve agarose and 1 percent Seakem agarose (FMC Bioproducts, Rockland, Me.), the amplified fragment was detected by Southern hybridization with ^{32}P-labeled oligonucleotide complementary to bases 3600 through 3619. *Eco*RI digests of phage clones (TE24II and TE28), which together span intron 19, were analyzed by the Southern blot assay with an insert-bearing PCR product (542 bp). The primers (forward primer, 5'TTGACTTGTCATCTTGATTTC3'; reverse primer, 5'CATTTTAATACTGCAACAGAT3') derived from the insert sequence (as described in the next section) were used to amplify DNA from a plasmid subclone (pTE24II-6) to provide sequences in regions flanking the 84-bp insert. In sequencing key regions of intron 19, primers were used to amplify a 437-bp PCR product from genomic DNA (forward primer 4712, 5'AGGCTTCTCAGTGATCTGTTG3'; reverse primer 4713, 5'GAATCATTCAGTGGGTATAAGCAG3').

Quantitation of *CFTR* mRNA

Total RNA was prepared from nasal epithelium with the Ultraspect system (Biotecx). Two micrograms of total RNA was used to synthesize cDNA (in 20-μl reactions) with reverse transcriptase (Super Script, Life Technologies, Gaithersburg, Md.). After 20-fold dilution in water, 10-μl aliquots of cDNA were included in the 50-μl PCR reactions. The normal *CFTR* sequence was amplified with primer 051193-1 and a primer complementary to the junction of exons 19 and 20 (bases 3843 to 3867). Amplification of γ-actin sequences was performed in parallel with primers HAG3 and HAG4.[13] *CFTR* and γ-actin sequences were amplified by 25 cycles of PCR (94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 1 minute) with the rapid-initiation technique (HotStart AmpliWax paraffin beads, Perkin-Elmer, Norwalk, Conn.). Plasmids carrying the *CFTR* sequence (pCFTR474) or the γ-actin sequence (pHF1)[14] were amplified in parallel; the initial copy number of each plasmid was increased serially (10-fold, from 10 to 10^5). Amplification products were analyzed by Southern blot hybridization with ^{32}P-labeled oligonucleotides complementary to *CFTR* (bases 3600 to 3619) or γ-actin[15] (bases 1072 to 1096). The DNA fragments were quantitated by phosphor-screen autoradiography (PhosphorImager, Molecular Dynamics, Sunnyvale, Calif.).

Haplotype Determination

Extended haplotypes were constructed from an analysis of PCR-amplified DNA for the diallelic markers XV.2c and KM-19[16] and the marker loci J44, GATT, M470, and TUB18.[17]

Sweat-Gland and Nasal Epithelial Function

Sweat ductal function was assessed by measurement of sweat chloride[7] and by measurement of the potential difference (PD), or voltage, across the epithelium of the sweat duct.[18,19] Sweat acinar epithelial chloride secretion mediated by cAMP was assessed by measuring sweat formation after an intradermal injection of isoproterenol, aminophylline, and atropine.[20-22] We tested the nasal PD and the response to sequential superfusion with amiloride (10^{-4} M), chloride-free amiloride (in which the chloride is replaced by gluconate), and a combination of isoproterenol (10^{-5} M) and chloride-free amiloride.[23-25] The chloride-diffusion PD (an index of basal chloride permeability) indicates the change with chloride-free superfusion; values reported here are corrected (by adding −9 mV) for the change in voltage caused by the replacement of chloride by gluconate. The change in PD with isoproterenol correlates with the cAMP-activated permeability of chloride; a negative value indicates chloride secretion.

Results

Clinical Phenotype of Patients

The patients referred for study had obstructive lung disease, chest radiographs consistent with cystic fibrosis, and characteristic microbiologic features of sputum — that is, mucoid *P. aeruginosa*, *S. aureus*, or both. Thirteen patients from eight families (Table 1) had at least one copy of the mutation in intron 19, as described below. Except for Patient 8A, these patients tended to have lung disease similar to or milder than that expected for their age as compared with a large population of patients with cystic fibrosis who were homozygous for ΔF_{508}.[27] Six of these study patients had never been hospitalized for their lung disease. Their sweat chloride values were nondiagnostic of cystic fibrosis, and the median value on multiple sweat tests was within the normal range. Most of the patients (10 of 13) had preservation of pancreatic exocrine function. One adult man (Patient 6A) who underwent fertility testing had an undeveloped vas deferens and obstructive azoospermia.

Sweat-Gland and Nasal Epithelial Function

Eight patients underwent physiologic assessment of epithelial function. Sweat ductal PD measurements for all eight were normal (Table 2), in a manner consistent with effective sweat chloride absorption and normal sweat chloride concentrations. In contrast, the assessment of sweat acinar epithelial chloride secretory function revealed that none of these patients secreted chloride in response to the β-agonist isoproterenol, a pattern typical of classic cystic fibrosis. The patients' nasal bioelectric properties were also abnormal, similar to those seen in patients homozygous for ΔF_{508} (Table 2): the base-line PD and amiloride-sensitive PD were greater than normal, and the chloride diffusion PD (an index of chloride permeability) and the cAMP-mediated chloride secretory response to isoproterenol were smaller than normal.

Genetic Analysis of Patients

The initial studies were of a 20-year-old woman (Patient 1 in Table 1). She was homozygous for the B haplotype[16] and was the product of consanguineous mating (her paternal grandparents were first cousins, as were her parents). No mutation was identified when the coding region of her *CFTR* gene was sequenced. However, PCR amplification of cDNA spanning from exon 18 to exon 20 revealed two products that hybridized to an oligonucleotide complementary to exon 19 (Fig. 1A). The smaller product (474 bp), found in both normal subjects and the sample from the homozygous patient, corresponded to the normal *CFTR* sequence. The larger species (558 bp), found only in the sample from the patient, contained an 84-bp insert between exons 19 and 20 that included an in-frame TAA stop codon (Fig. 1B). Hybridization of the relevant PCR product to *Eco*RI digests of phage clones TE24II and TE28 revealed, in addition to the expected bands overlapping exons 19 and 20, a 6.0-kb fragment common to both clones (Fig. 1C). This fragment begins approximately 10 kb downstream of exon 19. Sequence analysis of DNA flanking the 84-bp insert from the 6.0-kb fragment revealed cryptic splice lariat and acceptor sequences 5′ to the insert and a consensus splice-donor site 3′ to the insert, except for the C residue at position 2 (Fig. 1D).

Table 1. Clinical Features and Genotype of Patients with Pulmonary Disease and Normal Sweat Chloride Levels.

Patient No.*	Sex/Age (yr)	Sweat Chloride†	FEV_1‡	Chest Radiograph	Sputum Microbiology	Pancreatic Exocrine Function§	Haplotype¶	Genotype‖	Ethnic Origin**	Comments††
		mmol/liter	*% of predicted*							
1	F/20	16	73	Bronchiectatic upper zones	*S. aureus*	Sufficient	BB	Int19/Int19	Pakistani	Parents, paternal grandparents consanguineous
2	F/30	43	69	Bronchiectatic, diffuse	*S. aureus, P. aeruginosa* (mucoid)	Sufficient	CC	Int19/Int19	Ashkenazi Jewish	Parents probably consanguineous; sister died‡‡
3A	F/23	30	92	Diffuse markings	*S. aureus*	Sufficient	DB	Int/19/ΔF	N. European white	Never hospitalized
3B	M/16	44	65	Diffuse markings	*S. aureus, P. aeruginosa* (mucoid)	Sufficient	DB	Int19/ΔF	N. European white	—
4	F/39	42	45	Bronchiectatic, diffuse	*P. aeruginosa* (mucoid)	Sufficient	Uncertain§§	Int19/ΔF	Ashkenazi Jewish	Sister died¶¶
5	M/12	39	75	Diffuse markings	*S. aureus*	Sufficient	DB	Int19/ΔF	N. European white	Never hospitalized
6A	M/32	58	78	Markings, upper lung zone	*S. aureus*	Sufficient	BB	Int19/ΔF	N. European white	Azoospermic, never hospitalized
6B	F/26	19	85	Markings, upper lung zone	*S. aureus*	Sufficient	BB	Int19/ΔF	N. European white	Never hospitalized
7A	M/29	51	47	Bronchiectatic, diffuse	*S. aureus, P. aeruginosa* (mucoid)	Insufficient	Uncertain§§	Int19/ΔF	Ashkenazi Jewish	—
7B	M/27	34	63	Bronchiectatic, diffuse	*P. aeruginosa* (mucoid)	Insufficient	Uncertain§§	Int19/ΔF	Ashkenazi Jewish	Never hospitalized
7C	M/24	50	56	Bronchiectatic, diffuse	*P. aeruginosa* (mucoid)	Insufficient	Uncertain§§	Int19/ΔF	Ashkenazi Jewish	Never hospitalized
8A	F/13	29	22	Bronchiectatic, diffuse	*P. aeruginosa* (mucoid)	Sufficient	CB	Int19/W1282X	Ashkenazi Jewish	—
8B	F/4	45	Not done	Diffuse markings	Not done	Sufficient	CB	Int19/W1282X	Ashkenazi Jewish	—

*Numbers refer to the patients' families and letters to siblings within a family. Patient 4 was described previously by Davis et al.[26]

†Calculated as the median value on at least 3 tests (range, 3 to 12).

‡FEV_1 denotes forced expiratory volume in one second.

§Sufficiency was determined by the measurement of 72-hour stool fat in Patients 2, 3A, 3B, 4, and 6A and by clinical evaluation and measurement of serum vitamin E, serum trypsinogen, or both in Patients 1, 5, 6B, 7A, 7B, 7C, 8A, and 8B, as described in the Methods section.

¶According to the nomenclature of Beaudet et al.[16]

‖Int19 denotes the mutation in intron 19, and ΔF the ΔF_{508} mutation.

**Refers to the origin of the parent with the chromosome bearing the mutation in intron 19.

††Patients described as never hospitalized were not hospitalized for lung disease.

‡‡The sister died at the age of 30 years; her sweat chloride concentration (the median of 11 tests) was 67 mmol per liter.

§§This haplotype was heterozygous at both marker loci; the haplotype was either BC or AD.

¶¶The sister died at the age of 10 years; her autopsy findings were compatible with cystic fibrosis.

Table 2. Physiologic Studies of Sweat-Gland and Nasal Epithelial Function in Eight Patients with Pulmonary Disease and the Mutation in Intron 19 of *CFTR*.*

Patient No.	Sweat-Duct PD	Sweat Acinar Response to Isoproterenol	Nasal Bioelectric Properties				
			Mean PD	Maximal PD	Amiloride Inhibition	Chloride Diffusion PD	Isoproterenol Augmentation
	mV	*nl/min/gland*	*mV*		*%*	*mV*	
1	−21	0	−39	−50	78	−12	−7
2	−38	0	−51	−58	76	−5	−1
3A	−22	0	−45	−63	84	−7	0
3B	−23	0	−45	−58	75	−13	0
4	−28	0	−56	−66	75	−7	−2
5	ND	0	−46	−59	73	−5	0
6A	−30	0	−45	−54	80	−10	−4
8A	ND	0	ND	−69	ND	ND	ND
Normal range	−10 to −45	0.2 to 3.0	−10 to −40	−10 to −45	<70	−15 to −40	−11 to −28
Cystic fibrosis	−45 to −80	0	−40 to −60	−50 to −80	>70	0 to −10	0 to −6

*The ranges of values indicated for normal subjects and patients with cystic fibrosis are not absolute and are shown only for the purpose of comparison.[18-21,23-25] Values for chloride-diffusion PD were corrected as described in the Methods section. PD denotes potential difference, and ND not done.

The sequence of genomic DNA (Fig. 2) revealed that the inbred patient had a T residue two bases downstream of the 84-bp insert, whereas a C residue was present in the normal subject. The father was heterozygous for the C-to-T mutation at this position. This substitution creates a sequence with strong homology to a splice-donor site (Fig. 1D).

The C-to-T mutation in intron 19 is termed a 3849+10 kb C-to-T mutation (3849 is the number of the last base in exon 19, and 10 kb indicates that the mutation is approximately 10 kb downstream from 3849). The mutation creates a new *Hph*I restriction site, and we used *Hph*I restriction digestion to screen the other patients with cystic fibrosis who had normal sweat

chloride values. Thirteen patients had at least one copy of the mutation in intron 19 (Table 1): two were homozygous for the mutation, nine were compound heterozygotes with ΔF_{508}, and two siblings were compound heterozygotes with W1282X. The mutation in intron 19 was associated with three different ethnic origins (Pakistani, Ashkenazi Jewish, and northern European white) and at least three different extended haplotypes (data not shown).

Correlation of Mutation with Disease

One patient who was homozygous for the mutation in intron 19 (Patient 2 in Table 1) was homozygous for the C haplotype; although consanguinity could not be documented, both parents traced their ancestry to the same Jewish community in Eastern Europe. The pedigree of this patient's family shows the correlation of the disease with the mutation in intron 19 (Fig. 3); neither of two clinically unaffected sisters was homozygous for the mutation. Sixteen siblings or parents of affected persons were genotyped, and the mutation in intron 19 was associated with known cystic fibrosis mutations only in persons who had clinical disease compatible with cystic fibrosis. We screened 137 cystic fibrosis chromosomes with unidentified mutations from patients with cystic fibrosis who had clearly elevated sweat chloride values and 60 non–cystic fibrosis chromosomes derived from obligate carriers. No mutations in intron 19 were found.

Expression of Normally Spliced *CFTR* mRNA

Normally spliced *CFTR* mRNA was detected in the nasal epithelial sample from one patient homozygous for the mutation in intron 19 (Fig. 4A, lanes 1 through 5) relative to the normal sample (Fig. 4A, lanes 6 through 10). The amount of normal *CFTR* mRNA in the sample from the patient was only 8 percent of that in the normal sample (Fig. 4B). Similar results were obtained by an alternative method using quantitative competitive PCR with other samples from this patient. The amount of normal *CFTR* mRNA was only 5 to 10 percent of that in nasal epithelial samples from three normal subjects.

Discussion

Patients with clinical disease compatible with cystic fibrosis who do not have diagnostic sweat chloride values are commonly evaluated at cystic fibrosis centers.[6,26,28,29] It has been difficult to establish firmly the diagnosis of cystic fibrosis in these patients, because testing for specific biologic markers for cystic fibrosis[18,23,24] is not widely available and only one mutation in *CFTR* (G551S), found infrequently in such patients, has been associated with this syndrome.[22]

We identified a change in a single base pair in intron 19, termed a 3849+10 kb C-to-T mutation, in 13 patients who had this syndrome of cystic fibrosis–like lung disease and normal sweat chloride values. The C-to-T mutation in intron 19 leads to novel alternative splicing through the partial activation of a cryptic splice site and the insertion into most *CFTR* transcripts of a new 84-bp "exon," complete with an in-frame stop codon between exons 19 and 20 (Fig. 1B). The C residue at position 2 of the cryptic (nonfunctional) splice-donor site in normal genomic DNA (Fig. 1D) is part of a cytidine phosphate guanosine dinucleotide, which is the site of frequent point muta-

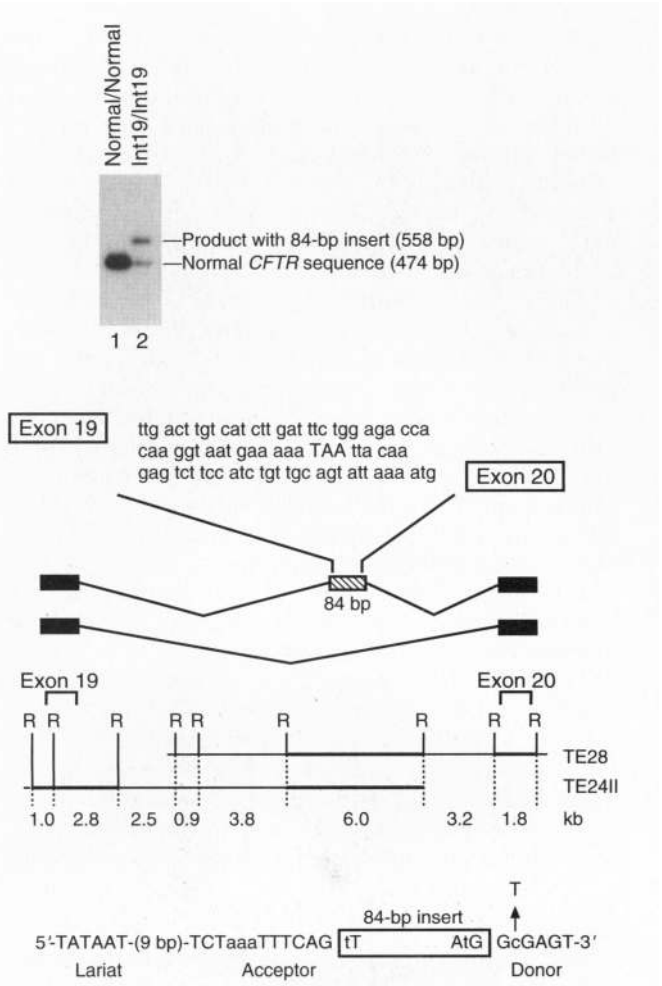

Figure 1. Identification and Characterization of the Mutation in Intron 19.

Panel A shows an autoradiograph of PCR products from the region spanning exon 18 to exon 20 of *CFTR* cDNA from a normal subject (lane 1) and a patient with cystic fibrosis who is homozygous for the mutation in intron 19 (lane 2). The 474-bp product is the normal *CFTR* sequence, and the 558-bp product contains the 84-bp insert from intron 19. Panel B shows the sequence of the 84-base insert; the in-frame stop codon TAA appears in capital letters. The diagram shows alternative splicing (above) and normal splicing (below); the hatched box represents the 84-bp insert, and the solid boxes represent exon 19 and exon 20. Panel C shows an *Eco*RI restriction map of *CFTR* intron 19 and a schematic drawing of Southern blot analysis of *Eco*RI-digested phage clones TE24II and TE28, with an insert-bearing PCR product used as the hybridization probe; the heavy lines indicate hybridization to the probe. R denotes *Eco*RI recognition site. Panel D shows sequences flanking the 84-bp insert in intron 19. The flanking regions demonstrate homology to splice lariat, acceptor, and donor sites; the bases matching the consensus sequence are capitalized. The index patient had a C-to-T mutation in the second base 3′ of the insert sequence (arrow).

tions.[30] No other sequence variant was found in the coding region of a patient who was homozygous for the mutation in intron 19. This mutation was not found in 60 normal chromosomes or in 137 cystic fibrosis chromosomes associated with elevated sweat chloride values. These findings, along with the correlation of this change of a base pair with the disease, demonstrates that these 13 patients have a form of cystic fibrosis.

The molecular nature of the mutation in intron 19 is compatible with the exon-shuffling theory,[31] which proposes that point mutations that create or destroy splice sites can cause the insertion or deletion of clusters of amino acids ("exons"), thereby increasing the rate at which proteins can evolve. A similar intronic mutation has been described in the small second intervening sequence of the beta-globin gene.[32] Such splice mutations are not easily detected with genomic DNA, and an approach using mRNA offers a better opportunity to identify them. Once they are identified, the presence of new unique restriction sites permits rapid diagnosis based on DNA analysis.

The 3849+10 kb C-to-T mutation is found in patients with sufficient sweat-duct epithelial chloride absorption to produce normal sweat. Patients with the mutation in intron 19 also appear to have milder pulmonary disease than do patients homozygous for ΔF_{508}, although the varying severity of pulmonary disease among patients with the same genotype[27] precludes a definitive analysis in our small and prospectively selected population. In this small group there was no obvious difference in clinical phenotype between patients homozygous for the mutation in intron 19 and those heterozygous for the mutation.

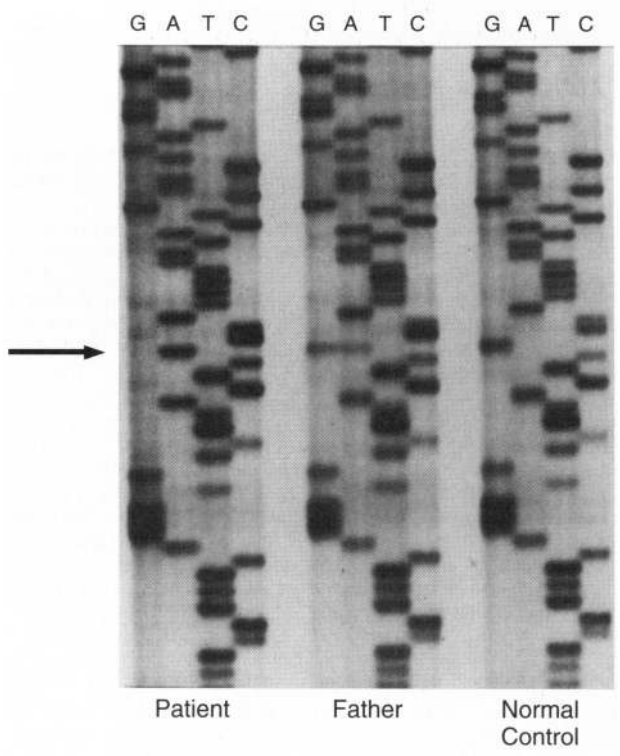

Figure 2. Sequence of the PCR Product from Intron 19 in the Index Patient, Her Father, and a Normal Control.

The patient is homozygous for a G-to-A mutation (arrow) on the antisense strand. Her father is a heterozygous carrier.

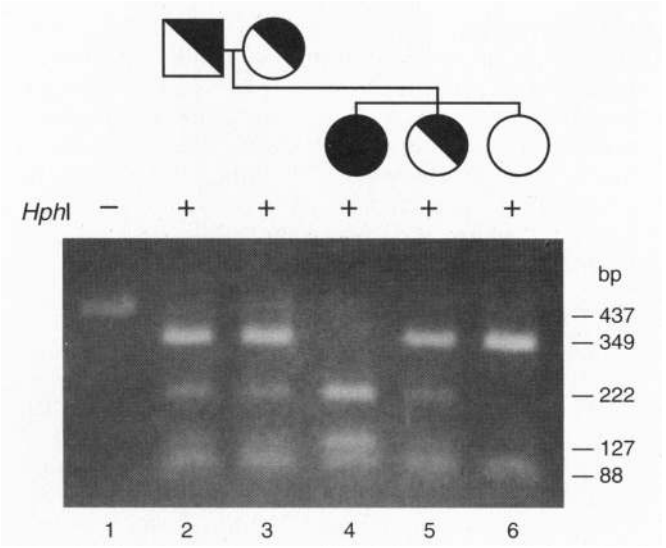

Figure 3. Detection of the 3849+10 kb C-to-T Mutation by PCR and *Hph*I Digestion.

The figure shows the pedigree of a patient (Patient 2 in Table 1) who is homozygous for the 3849+10 kb C-to-T mutation. The square denotes a male family member, circles female family members, the solid symbol a homozygous family member, the open symbol a normal family member, and half-solid symbols heterozygous family members. DNA from each family member was amplified with primers 4712 and 4713 and digested with *Hph*I. The normal allele has one *Hph*I site, yielding bands of 349 and 88 bp (the noncarrier sister, lane 6). The C-to-T mutation creates a new *Hph*I restriction site, and the mutant allele shows bands of 222, 127, and 88 bp (lane 4). Plus and minus signs indicate whether samples were treated with *Hph*I. Lane 1 shows a sample of undigested DNA from the patient.

A clue to the molecular basis of the variant phenotype was provided by the analysis of mRNA transcripts expressed in these patients. No full-length CFTR protein is expected to be translated from the mRNA containing the 84-bp insert with the in-frame stop codon, but mRNA without the insert (occurring at approximately 8 percent of the normal level in respiratory epithelium) would be associated with the synthesis of normal CFTR protein. Whereas these patients have lung disease, Chu et al. have reported that some people with in-frame splice skipping of exon 9 have no lung disease and only 8 to 12 percent of normal *CFTR* transcripts (i.e., those containing exon 9) in bronchial epithelium.[33] The apparent difference between these two studies is probably due to technical and molecular considerations.

Modulation of the clinical phenotype associated with the reduced production of a normal protein has been reported. Mutations in the regulatory elements of the beta-globin gene leading to reduced amounts of normal beta chain (i.e., beta-thalassemia) are associated with milder disease than are mutations leading to

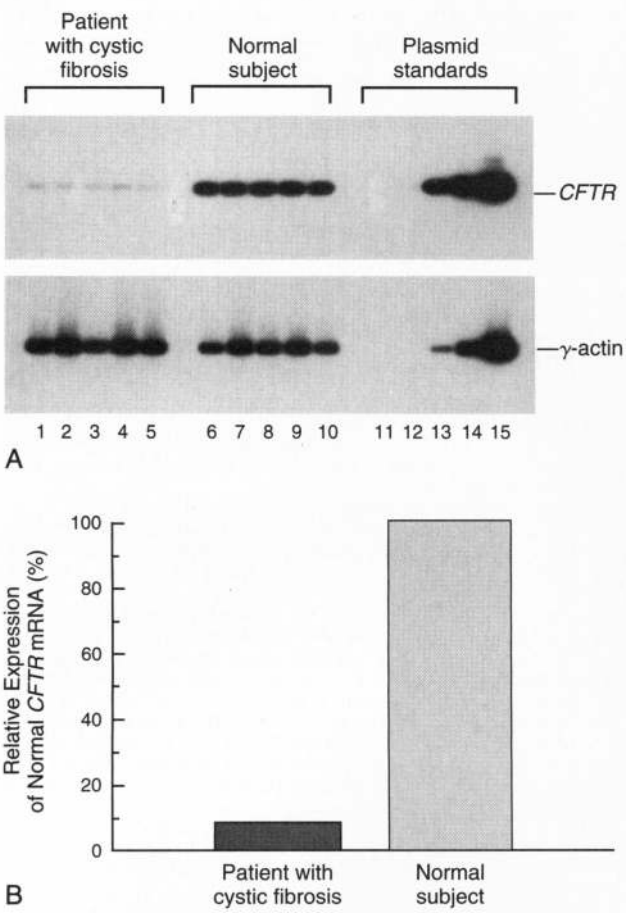

Figure 4. Quantitation of Normally Spliced *CFTR* mRNA Expressed in Nasal Epithelial Cells from a Patient with the Intron 19 Mutation.

Panel A shows an autoradiograph of a Southern blot assay of PCR products from parallel reactions of *CFTR* cDNA and γ-actin cDNA from a patient with cystic fibrosis who was homozygous for the point mutation in intron 19 (lanes 1 through 5), a normal subject (lanes 6 through 10), and plasmid standards with sequential 10-fold increases (from 10 to 10^5) in the initial target copy numbers (lanes 11 through 15). Panel B shows the relative expression of normal *CFTR* mRNA in the sample from the patient who was homozygous for the mutation in intron 19, as compared with the sample from the normal subject. The level of expression was determined by a quantitative analysis of the hybridization in Panel A using phosphor-screen autoradiography. *CFTR* expression was standardized to γ-actin expression.

nonfunctional or absent beta chains.[34] With respect to these patients with cystic fibrosis–like lung disease and normal sweat chloride values, the differences in disease expression at the organ level, such as abnormal airway epithelial function as compared with normal sweat ductal function, may reflect quantitative differences in the requirement for functional CFTR protein, differences in tissue-specific RNA splicing, or both.

The identification of a mutation in the *CFTR* gene in patients with normal sweat chloride concentrations has several implications for diagnosis and treatment. First, consideration of the diagnosis of cystic fibrosis must extend to patients with suppurative pulmonary disease and normal sweat chloride values. The mutation in intron 19 may be relatively common, because it was seen in three ethnic groups and on three different extended haplotypes. Furthermore, this mutation has been noted on 104 chromosomes reported to the Cystic Fibrosis Gene Analysis Consortium since our initial report,[35] and it accounts for about 4 percent of the mutations in Ashkenazi patients with cystic fibrosis in Israel.[36] Whereas this mutation is common in patients with cystic fibrosis and normal sweat chloride values, it has also been reported in patients with borderline or raised sweat chloride concentrations.[37,38]

Second, patients with the mutation in intron 19 have low levels of normal *CFTR* mRNA. Future ability to quantitate the expression of CFTR protein in patients with cystic fibrosis and this mutation will provide information about the levels of *CFTR* expression needed to achieve a normal phenotype in a variety of affected epithelial sites. This information may provide a better understanding of *CFTR* function and serve as a guide for protein or gene therapy.

Finally, increased understanding of *CFTR* gene regulation, mRNA splicing, and protein expression may give patients with the mutation in intron 19 therapeutic alternatives: the up-regulation of *CFTR* gene expression, the inhibition of alternative splicing, and the stabilization of CFTR protein would presumably all improve the defense mechanisms of the lung.

Note added in proof: Since submitting this manuscript, we have identified 6 of approximately 45 men known to have this mutation who do not have obstructive azoospermia according to semen analysis or who have fathered children, or both, including one of our patients (Patient 7A in Table 1)[37] (and Bowman M, Cutting G, Dreyfus D, McCoy K, Gelfand E, Bethel R: personal communication). This is higher than the expected prevalence of fertility (<1 percent) in men with other mutations in *CFTR*.

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